Uttar Pradesh Rajarshi Tandon
Open University

PGBCH-103

Bioenergetics and Metabolism

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BLOCK

1

Bioenergetics

Curriculum Design Committee

Dr. (Prof.) Ashutosh Gupta, **Chairman**
School of Science, UPRTOU, Prayagraj

Prof. Prof. Umesh Nath Tripathi **Member**
Department of chemistry
Deen Dayal Upadhyaya Gorakhpur University, Gorakhpur

Prof. S.I. Rizvi **Member**
Department of Biochemistry
University of Allahabad, Prayagraj

Prof. Dinesh Yadav **Member**
Department of Biotechnology
Deen Dayal Upadhyaya Gorakhpur University, Gorakhpur

Prof. Sharad Kumar Mishra **Member**
Department of Biotechnology
Deen Dayal Upadhyaya Gorakhpur University, Gorakhpur

Dr. Ravindra Pratap Singh **Member**
Academic Consultant (Biochemistry)
School of Science, UPRTOU, Prayagraj

Dr. Dharmveer Singh **Course Coordinator**
Academic Consultant (Biochemistry)
School of Science, UPRTOU, Prayagraj

Course Preparation Committee

Dr. Sanjeev Kumar Maurya Associate Professor Department of biotechnology, Invertis University, Bareilly	**Author**	**Block-1&2 (Unit: 1- 4)**
Dr. Shashank Upadhaya Asst. Professor Department of biotechnology, Invertis University, Bareilly	**Author**	**Block-3 (Unit: 5, 6)**
Dr. V.C. Srivastava Former Associate Professor, Department of Zoology, University of Allahabad, Prayagraj	**Editor**	**(All blocks and units)**
Dr. Dharmveer Singh School of Sciences, UPRTOU, Prayagraj		**(Course Coordinator)**

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COURSE INTRODUCTION

The objective of this course is to cover the process of bioenergetics and metabolism. The discussion of chemical reaction mechanism that underline the biological energy transactions. Transactions of energy occurs within living organisms, for example the chemical mechanisms within cells, organisms require an input of energy. This course covers the different cellular and metabolic process which leads to production and utilization of energy. The course is organized into following blocks:

Block-1 covers the bioenergetics process of the energy transfer in different cellular and metabolic steps.

Block-2 deals the coenzymes and carbohydrate metabolism.

Block-3 describes in brief on metabolism of amino acid and nitrogen compounds.

Introduction

This is the first block on Bioenergetics. It consists of following two units:

Unit-1: This unit covers the general introduction of Bioenergetics. The chemical mechanisms that underlie biological energy transactions have fascinated and challenged biologists for centuries. Taking this in mind, the free energy, standard free energy, determination of ΔG for a reaction, relationship between equilibrium constant and standard free energy is change defined in this unit. The biological standard state and standard free energy is also discussed briefly.

Unit-2: All reactions which involve electron flow are considered oxidation-reduction reactions in this unit, biological oxidation-reduction reactions, redox potentials are discussed in brief. The high energy phosphate compounds-ATP and phosphate group transfer photosynthetic light reactions are discussed briefly.

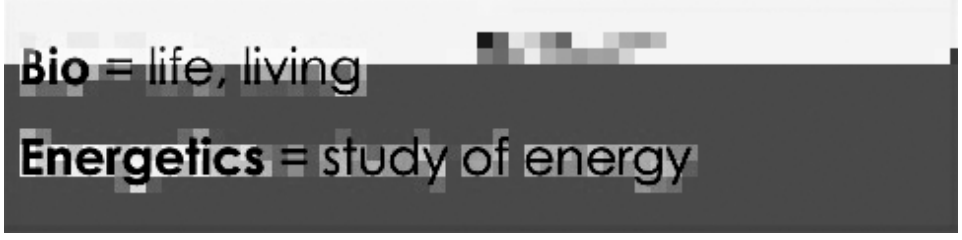
UNIT-1

BIOENERGETICS-I

Structure

1.1. Introduction

Bioenergetics is the branch of biochemistry that focuses on how cells transform energy, often by producing, storing or consuming adenosine triphosphate (ATP). Bioenergetics processes, such as cellular respiration or photosynthesis, are essential to most aspects of cellular metabolism, therefore to life itself. One saving grace of science terminology is that it can almost always be broken down into smaller, less overwhelming pieces. This is true for **bioenergetics**.



how energy is transformed (changed from one form into another) through two major processes: cellular respiration and photosynthesis. For the sake of really understanding what bioenergetics means, let us take a closer (but still brief) look at how energy is transformed in cellular respiration and photosynthesis.

Modern organisms carry out a remarkable variety of energy transductions, conversions of one form of energy to another. They use chemical energy in fuels to bring about the synthesis of complex molecules from simple precursors, producing macromolecules with highly ordered structure. They also convert the chemical energy of various fuels into concentration gradients and electrical gradients, motion, heat, and even, in a few organisms such as fireflies, light. Photosynthetic organisms transduce light energy into all of other forms of energy.

The chemical mechanisms that underlie biological energy transductions have fascinated and challenged biologists for centuries. Antoine Lavoisier, before he lost his head in the French Revolution, recognized that animals somehow transform chemical fuels (foods) into heat and that this process of respiration is essential to life. He observed that' in general, respiration is nothing but a slow combustion of carbon and hydrogen, which is entirely similar to that which occurs in a lighted lamp or candle, and that, from this point of view, animals that respire are true combustible bodies that burn and consume themselves. One may say that this analogy between combustion and respiration has not escaped the notice of the poets, or rather the philosophers of antiquity, and which they had expounded and interpreted. This fire stolen from heaven, this torch of Prometheus, does not only represent an ingenious and poetic idea, it is a faithful picture of the operations of nature, at least for animals that breathe; one may therefore say, with the ancients, that the torch of life lights itself at the moment the infant breathes for the first time, and it does not extinguish itself except at death. Bioenergetics is the quantitative study of the energy transductions that occur in living cells and of the nature and function of the chemical processes underlying these transductions. Although many of the principles of thermodynamics have been introduced in earlier chapters and may be familiar to you, it is worth reviewing the quantitative aspects of these principles.

Objectives

1. Define and use correctly the terms system, closed, open, surroundings, state, energy, temperature, thermal energy, irreversible process, entropy, free energy, electromotive force (emf), Faraday constant, equilibrium constant, acid dissociation constant, standard state, and biochemical standard state.

2. State and appropriately use equations relating the free energy change of reactions, the standard-state free energy change, the equilibrium constant, and the concentrations of reactants and products.

3. Explain qualitatively and quantitatively how unfavorable reactions may occur at the expense of a favorable reaction.

4. Apply the concept of coupled reactions and the thermodynamic additivity of free energy changes to calculate overall free energy changes and shifts in the concentrations of reactants and products.

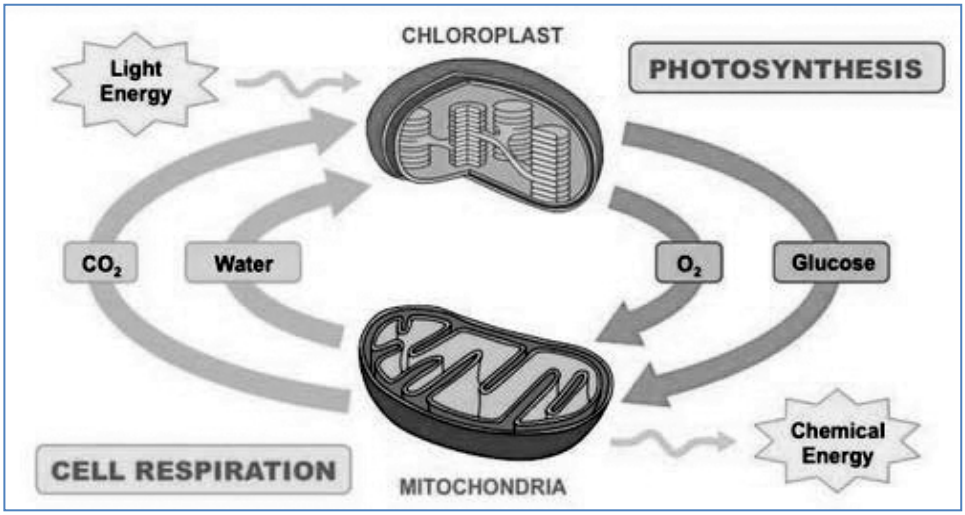
5. Construct balanced reduction–oxidation reactions, using half-reactions, and calculate the resulting changes in free energy and emf.

6. Explain differences between the standard-state convention used by chemists and that used by biochemists, and give reasons for the differences.

7. Recognize and apply correctly common biochemical conventions in writing biochemical reactions.

1.2. Bioenergetics and concept of free energy

Bioenergetics is the branch of biochemistry that focuses on how cells transform energy, often by producing, storing or consuming adenosine triphosphate (ATP). Bioenergetics processes, such as cellular respiration or photosynthesis, are essential to most aspects of cellular metabolism, therefore to life itself. In other words, bioenergetics is the study of energy transfer within the living beings.

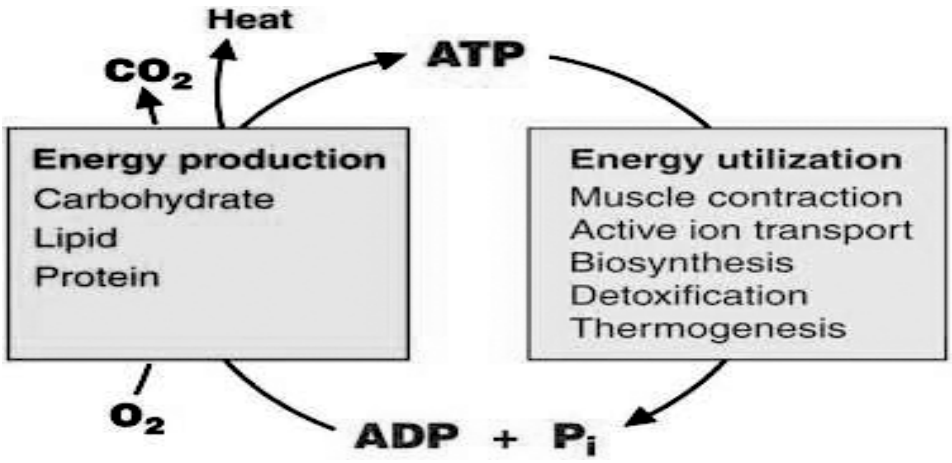


There are two laws of bioenergetics

1. Energy cannot be created or destroyed, but can be changed from one form to another. The main forms of energy within the body are heat, light, mechanical, chemical, “free energy” and entropy (Entropy is a form of energy that cannot be re-used in chemical reactions, and is defined synonymously with increased randomness or disorder).

"Free energy" is referred to as Gibb's free energy, and is abbreviated "ΔG". Typically, during energy transfers there is a "change in energy forms, which is indicated by the "symbol. Thus, a change in Gibb's free energy is expressed as "ΔG".

2. Energy transfer will always proceed in the direction of increased entropy, and the release of "free energy".



All reactions proceed in the direction of: (-↑a) entropy b) a release of free energy (-ΔG, Kcal/Mol). The greater the release of free- ΔG, the more negative the energy during a chemical reaction **(Fig.1.2)**. Chemical reactions that have -ΔG are termed exergonic reactions. By convention, reactions that require free energy input to proceed are termed endergonic reactions, but there are no such reactions in the human body. The free energy not used to do work is expressed as heat. Reactions that have no net change in substrate or product are termed equilibrium reactions, and have no change in free energy (ΔG=0). All reactions are potentially reversible. The directionality and amount of free energy release of a chemical reaction can be modified by altering substrate and product concentrations.

Continuation of life requires continuous chemical reaction. Reactions that reach equilibrium have stopped, can't get out of that state without external change. Consumption of food provides a continued supply of substrates for reactions yielding net negative ΔG. Net negative ΔG ensures that reactions precede in the required direction for continuation of life. Energy made available from breakdown of some compounds, e.g. sugars, fats, amino acids, can be used to drive the synthesis of other molecules, e.g. structural components of cells or compounds such as polysaccharides that store energy for future needs.

1.3. Standard free energy, determination of ΔG for a reaction

When you hear the term "free energy," what do you think of? Most of the time, ATP is the "storage battery" of cells. In order to understand

how energy is captured, we must first understand Gibbs free energy and in doing so, we begin to see the role of energy in determining the directions taken by chemical reactions. Gibbs free energy is “a thermodynamic potential that measures the "useful" or process-initiating work obtainable from an isothermal, isobaric thermodynamic system,” and further points out that it is “the maximum amount of non-expansion work that can be extracted from a closed system; this maximum can be attained only in a completely reversible process.”

Instead, are going to look at the type of free energy that is associated with a particular chemical reaction, and which can provide a measure of how much usable energy is released (or consumed) when that reaction takes place.

we can write out a simple definition of the change in Gibbs free energy as:

$$\Delta G = G(\text{final}) - G(\text{initial})$$

J W Gibbs (late 19th Century) combined 1st and 2nd Laws to express spontaneity of reactions in terms of measurable system parameters.

$$\Delta G = \Delta H - T\Delta S$$

- ΔH: Change in enthalpy: ENTHALPY in biology refers to energy stored in bonds, and the change in enthalpy is the difference in bond energies between the products and the reactants. A negative Δ*H* means heat is released in going from reactants to products, while a positive Δ*H* means heat is absorbed. (This interpretation of Δ*H* assumes constant pressure, which is a reasonable assumption inside a living cell).

- T: Absolute Temperature: Temperature (*T*) determines the relative impacts of the Δ*S* and Δ*H* terms on the overall free energy change of the reaction. (The higher the temperature, the greater the impact of the Δ*S* term relative to the Δ*H* term.) Note that temperature needs to be in Kelvin (K) here for the equation to work properly.

- ΔS is the entropy change of the system during the reaction. If ΔS is positive, the system becomes more disordered during the reaction (for instance, when one large molecule splits into several smaller ones). If ΔS is negative, it means the system becomes more ordered.

- ΔG: change in (Gibbs) Free Energy. Units: J/mol, : a measure of the useful work system can perform, must be – ve for spontaneous reaction.

  - If ΔG is –ve; reaction exergonic i.e. thermodynamically downhill.
  - If ΔG is +ve, reaction endergonic i.e. proceeds in reverse direction.
  - If ΔG is 0: equilibrium; no change.

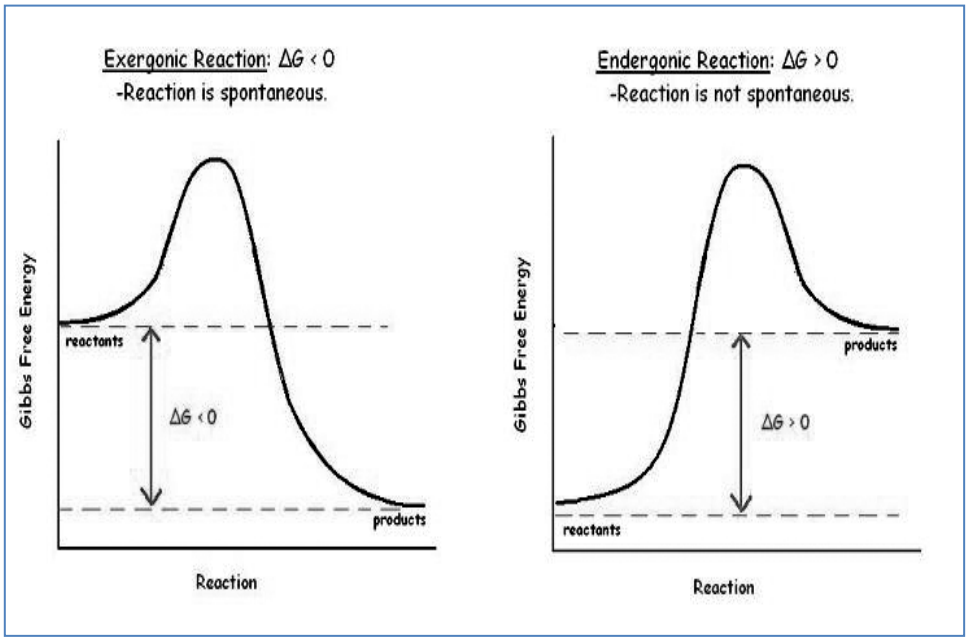


Fig. 1.3: Free energy dynamics in any reaction

For a reaction aA <=> bB (where 'a' and 'b' are integers and A and B are molecules) at pH 7, ΔG can be determined by the following equation,

$$\mathbf{\Delta G} = \mathbf{\Delta} G^{\circ} + RT \ln \frac{[C][D]}{[A][B]}$$

ΔG° = standard free energy change

R = Gas constant = 1.98* 10-3 kcal mol-1 deg-10

T = usually room temperature = 298 K

$$\mathbf{K} = \frac{\mathbf{[C][D]}}{\mathbf{[A][B]}}$$

For multiple substrate reactions, such as aA + cC <=> bB + dD

$$\Delta G = \Delta G^{\circ\prime} + RT \ln [B]^b[D]^d/[A]^a[C]^c$$

In simple terms, if we collect all of the terms of the numerator together and call them products and all of the terms of the denominator together and call them reactants.

$$\Delta G = \Delta G^{\circ} + RT \ln (Products/Reactants)$$

R = 8.314 J K-1 mol-1, the gas constant.

T = 298 K; use this value even for processes expected to occur at 37°, since tabulated data for ΔG are all based on 298 K measurements.

Table 1.1: Energy rich compounds

Table 8.1 : Free energy liberated by some energy-rich compounds under standard conditions	
Energy-rich compound	ΔG° K cal / mole
Adenosine triphosphate (ATP)	–7.3
Guanosine triphosphate (GTP)	–7.3
Uridine triphosphate (UTP)	–7.3
Cytidine triphosphate (CTP)	–7.3
Acetyl phosphate	–10.5
Phosphoenol pyruvate (PEP)	–13.0
Diphospho glyceric acid	–11.8

For example: the reaction catalysed by hexokinase:

Glucose + ATP ----------Glu-6-P + ADP ΔG0 = -16.7 kJ/molΔ

1.4. ATP-ADP energy exchange

If a reaction is endergonic in one direction (e.g., converting products to reactants), then it must be exergonic in the other, and vice versa. As an example, let us consider the synthesis and breakdown of the small molecule adenosine triphosphate (ATP) which is the "energy currency" of the cell.

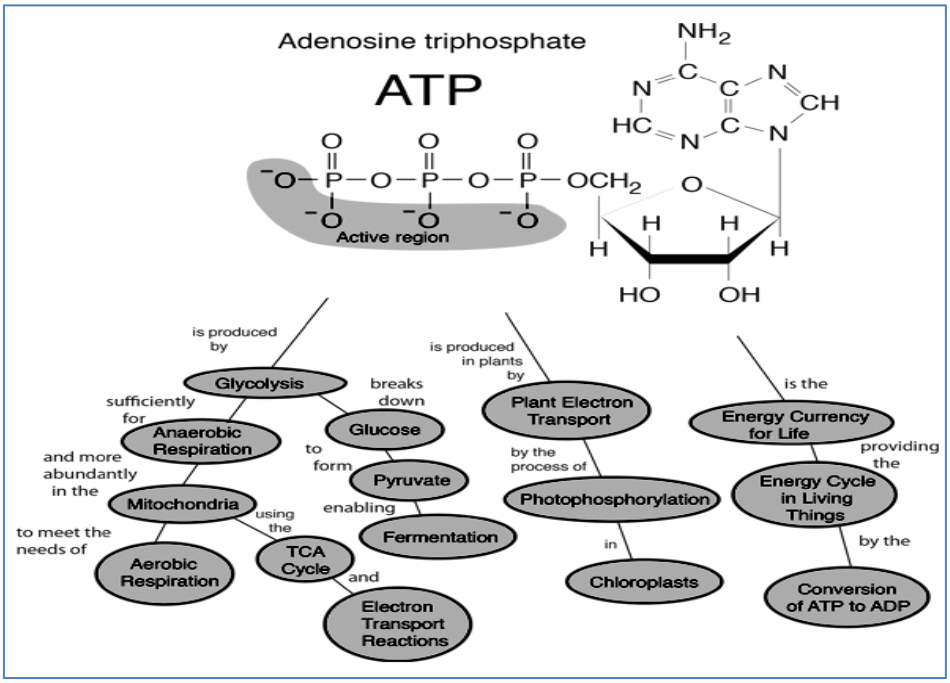
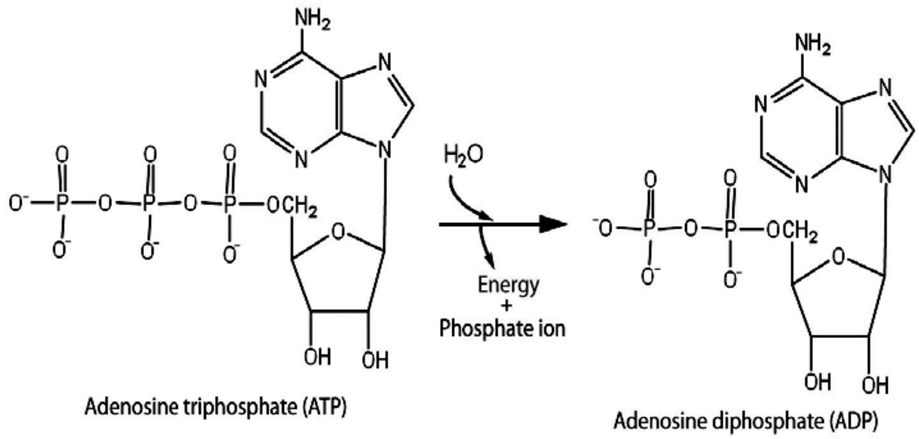


Fig. 1.4: ATP structure and generation

ATP is made from adenosine diphosphate ADP and phosphate according to the following equation:

ADP + Pi-------------ATP+ H_2O

This is an endergonic reaction, with ΔG = +7.3 under standard and conditions concentrations of all reactants and products, i.e. 1M of all reactant and product, 1 atm pressure, 25^0C and pH 7.0. In the cells of our body, the energy needed to make ATP is provided by the breakdown of fuel molecules, such as glucose, or by other reactions that are energy-releasing (exergonic).



The reverse process, the hydrolysis (water-mediated breakdown) of ATP is identical but with the reaction flipped backwards:

ATP+H_2O→ADP+Pi

This is an exergonic reaction, and its ΔG is identical in magnitude and opposite in sign to that of the ATP synthesis reaction (ΔG = -7.3 kcal/mol under standard conditions). This relationship of same magnitude and opposite signs will always apply to the forward and backward reactions of a reversible process.

1.5. Non-standard conditions and chemical equilibrium

You may have noticed that in the above section, a careful mention was made that the ΔG values were calculated for a particular set of conditions known as standard conditions. The standard free energy change (ΔGº') of a chemical reaction is the amount of energy released in the conversion of reactants to products under standard conditions. For biochemical reactions, standard conditions are generally defined as 25^0C, 298 K, 1M concentrations of all reactants and products, 1 atm pressure and pH 7.0.

The conditions inside a cell or organism can be very different from these standard conditions, so ΔG values for biological reactions in

vivo may vary widely from their standard free energy change ($\Delta G^{o'}$) values. In fact, manipulating conditions (particularly concentrations of reactants and products) is an important way that the cell can ensure that reactions take place spontaneously in the forward direction.

1.6. Relationship between equilibrium constant and standard free energy change

If a cell were an isolated system, its chemical reactions would reach equilibrium, which would not be a good thing. If a cell's reaction reached equilibrium, the cell would die because there would be no free energy left to perform the work needed to keep it alive.

When a reaction leaves the standard state because of a change in the ratio of the concentrations of the products to the reactants, the system is described in terms of non-standard-state free energies of reaction. The difference between ΔG° and ΔG for a reaction is important. There is only one value of ΔG^{o} for a reaction at a given temperature, but there are an infinite number of possible values of ΔG.

The figure below shows the relationship between ΔG for the following reaction and the logarithm to the base *e* of the reaction quotient for the reaction between N_2 and H_2 to form NH_3.

$$N_2(g) + 3\ H_2(g) \rightleftharpoons 2\ NH_3(g)$$

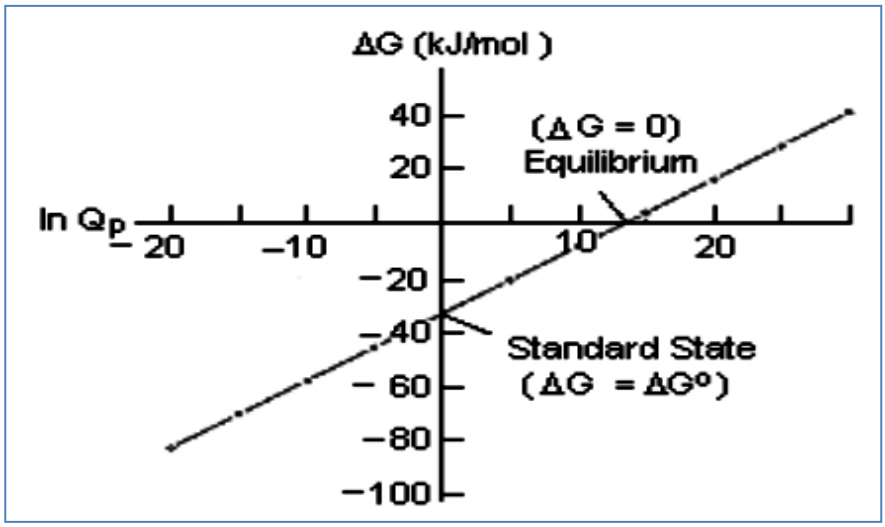
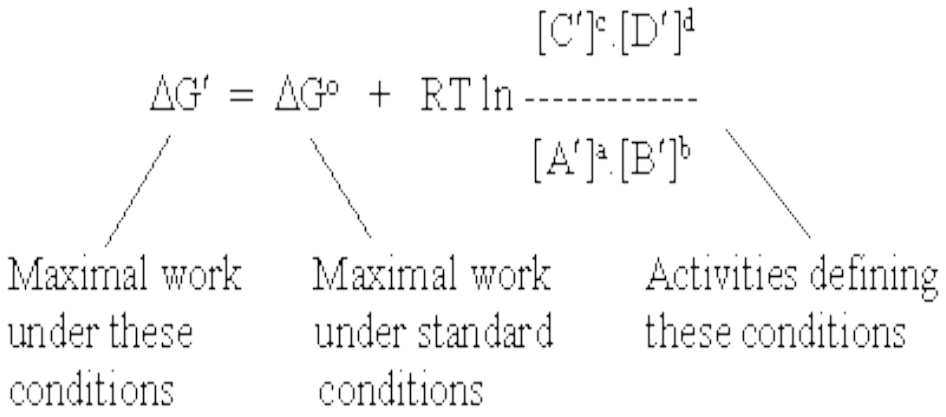


Fig. 1.5: Relationship between equilibrium constant and standard free energy change

Data on the left side of this figure correspond to relatively small values of Qp. They therefore describe systems in which there is far more reactant than product. The sign of ΔG for these systems is negative and the magnitude of ΔG is large. The system is therefore relatively far from equilibrium and the reaction must shift to the right to reach equilibrium.

Data on the far right side of this figure describe systems in which there is more product than reactant. The sign of ΔG is now positive and the magnitude of ΔG is moderately large. The sign of ΔG tells that the reaction would have to shift to the left to reach equilibrium. The magnitude of ΔG tells that one does not need to go to reach equilibrium.



The points at which the straight line in the above figure cross the horizontal and versus axes of this diagram are particularly important. The straight line crosses the vertical axis when the reaction quotient for the system is equal to 1. This point therefore describes the standard-state conditions, and the value of ΔG at this point is equal to the standard-state free energy of reaction, ΔGo.

Cells stay out of equilibrium by manipulating concentrations of reactants and products to keep their metabolic reactions running in the right direction. For instance:

- They may use energy to import reactant molecules (keeping them at a high concentration).
- They may use energy to export product molecules (keeping them at a low concentration).
- They may organize chemical reactions into metabolic pathways, in which one reaction "feeds" the next.

Providing a high concentration of a reactant can "push" a chemical reaction in the direction of products (that is, make it run in the forward direction to reach equilibrium). The same is true of rapidly removing a product, but with the low product concentration "pulling" the reaction forward. In a metabolic pathway, reactions can "push" and "pull" each other because they are linked by shared intermediates: the product of one step is the reactant for the next

A chemical mixture at equilibrium is already in a state of minimal free energy: no free energy is being generated or released. Thus, for a system at equilibrium, we can write under standard conditions. Thus, if the concentrations of reactants and products at equilibrium (i.e., the Keq) are determined, the value of $\Delta G^{\circ\prime}$ can be calculated.

$$0 = \Delta G = \Delta G^{\circ\prime} + RT \ln Q$$

At equilibrium the value of Q is the equilibrium constant Keq, so that

$$\Delta G^{\circ\prime} = -RT \ln K_{eq}$$

Expressed in terms of base 10 logarithms, this equation becomes

$$\Delta G^{\circ\prime} = -2.3RT \log K_{eq}$$

or

$$\Delta G^{\circ\prime} = -2.3\ (1.987)\ (298) \log K_{eq}$$
$$= -1362 \log K_{eq} \qquad (2\text{-}9)$$

Although a chemical equilibrium appears to be unchanging and static, it is actually a dynamic state. The forward and the reverse reactions proceed at exactly the same rate, thereby canceling each other out. As noted earlier, when an enzyme or some other catalyst speeds up a reaction, it also speeds up the reverse reaction; thus equilibrium is reached sooner than it is when the reaction is not catalyzed. However, the equilibrium constant and ΔG° of a reaction are the same in the presence and absence of a catalyst.

1.7. Concentration Gradients generation in cellular metabolic

A cell must often accumulate chemicals, such as glucose and K+ ions, in greater concentrations than exist in its environment. Consequently, the cell must transport these chemicals against a concentration gradient. To find the amount of energy required to transfer 1 mole of a substance from outside the cell to inside the cell, Equation 2-8 relating ΔG to the concentration of reactants and products is used. Because this simple transport reaction does not involve making or breaking covalent bonds and no heat is taken up or released, the ΔG°′ is 0. Thus Equation 2-8 becomes

$$\Delta G = \Delta G^{\circ\prime} + RT \ln Q = RT \ln \frac{C_2}{C_1}$$

where C_2 is the initial concentration of a substance inside the cell and C_1 is its concentration outside the cell. If the ratio of C_2 to C_1 is 10, then at 25 °C, $\Delta G = RT \ln 10 = +1.36$ kcal per mole of substance transported. Such calculations assume that a molecule of a given substance inside a cell is identical with a molecule of that substance outside and that the substance is not sequestered, bound, or chemically changed by the transport.

Since the "uphill" transport of molecules against a concentration gradient ($C_2 > C_1$) has a positive ΔG, it clearly cannot take place spontaneously. To occur, such transport requires the input of cellular

chemical energy, which often is supplied by the hydrolysis of ATP. Conversely, when a substance moves down its concentration gradient ($C_1 > C_2$) in crossing a membrane, ΔG has a negative value and the transport can be coupled to a reaction that has a positive ΔG, say, the movement of another substance uphill across a membrane.

1.8. Free Energy of Enzymes

In the case of an enzyme, ΔG determines the rate of a reaction. Enzymes cannot affect thermodynamics of a reaction, and hence do not affect the equilibrium; additionally, enzymes accelerate the attainment of equilibrium but do not shift their positions. The equilibrium position is a function only of the free-energy difference between reactants and products [Fig. 1.6]. They are however, able to reach the equilibrium point at a far faster rate than without the presence of an enzyme.

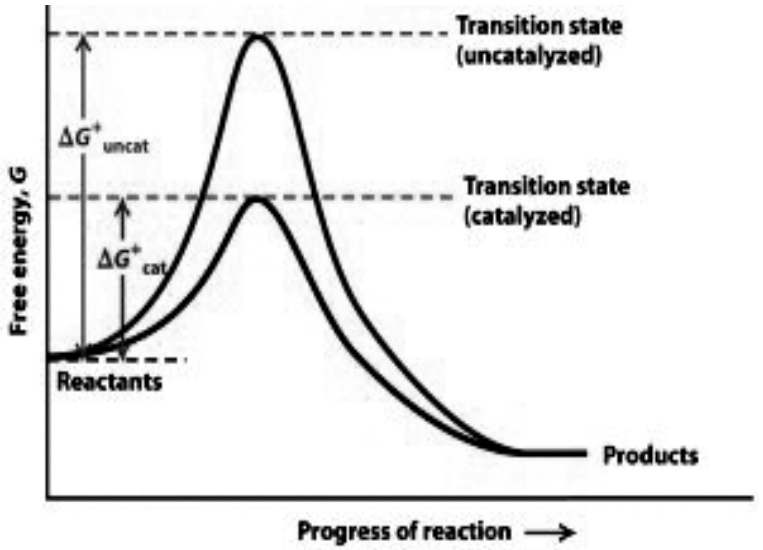


Fig. 1.6: Relationship between free energy and enzymes

For instance, in the presence of an enzyme, products could form within a second. On the other hand, products could take days to form without the presence of the catalyst. In cases, concentration and amount of product formed remains entirely the same or its equilibrium state. The amount of products it has formed has balanced with the amount of substrate.

Enzymes decrease only the free energy of activation- otherwise known as the activation energy. The transition state between a substrate and the product is the point between a reaction where the substrates and products "meet in the middle". At this point, the highest free energy exists for the reaction. The activation energy is the energy it takes for a substrate to reach this transition state.

There are many competing theories of how enzymes actually bind their substrates, and each theory has a different graphic representation of the effect of the enzyme on the free energy of the reaction. In the lock and

key mechanism theory, an enzyme has the pre-existing conformation to bind to a unique substrate. After binding and catalyzing the reaction, the enzyme will release the final products.

1.9. Formation of Double Helix

Double-stranded molecules of nucleic acids form the double helix structure such as DNA and RNA. Formation of double helix is one of the biological process and the principles of thermodynamics are applied to it. In a solution containing single strands, all stands can easily move around, rotate, and disperse in the solution. In addition, forming conformation is easy in the single strands solution. However, when the double helix forms, it cannot displace as easy as two single strands could before. Moreover, it has less possible conformations. Thus, by forming double helix the randomness and entropy decrease.

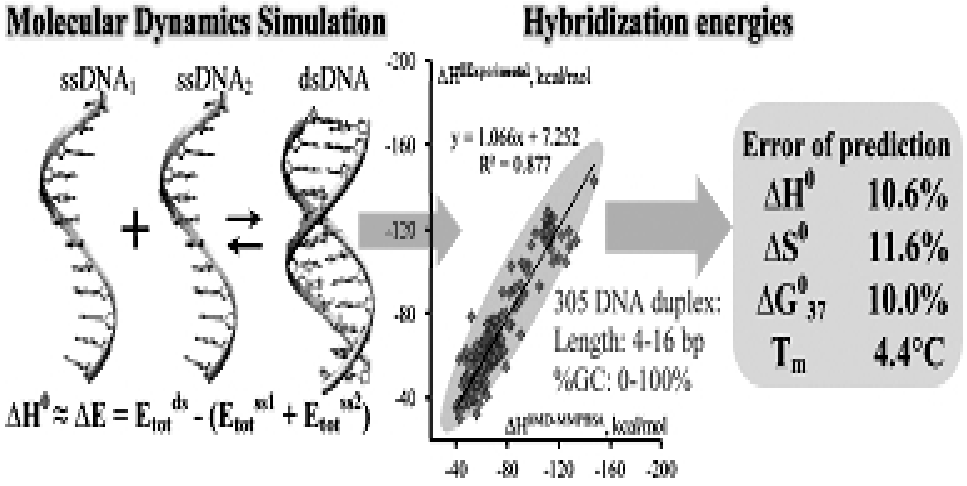


Fig. 1.7: formation of double helix

Due to the Second Law of thermodynamics, significant heat has to be released to the surrounding for the process to be consistent with increasing the entropy of universe. Measuring the changing of temperature of a solution before and after formation of double helix reveals that approximately 250 kJ/mol (60 kcal/mol) heat is released. This large released energy is sufficient to overcome the effects of formation of double helix i.e. increase of order and make universe more disorder.

1.10. Change in coupled reactions

Many chemical reactions are endergonic (i.e., not spontaneous ($\Delta G>0\Delta G>0$)) and require energy to be externally applied. However, these reaction can be coupled to a separate, exergonic (thermodynamically favorable $\Delta G<0\Delta G<0$) reactions that 'drive' the thermodynamically unfavorable one by coupling or 'mechanistically joining' the two reactions, often via a share intermediate. Since Gibbs Energy is a state function, the $\Delta G\Delta G$ values for each half-reaction may be summed, to yield the combined $\Delta G\Delta G$ of the coupled reaction.

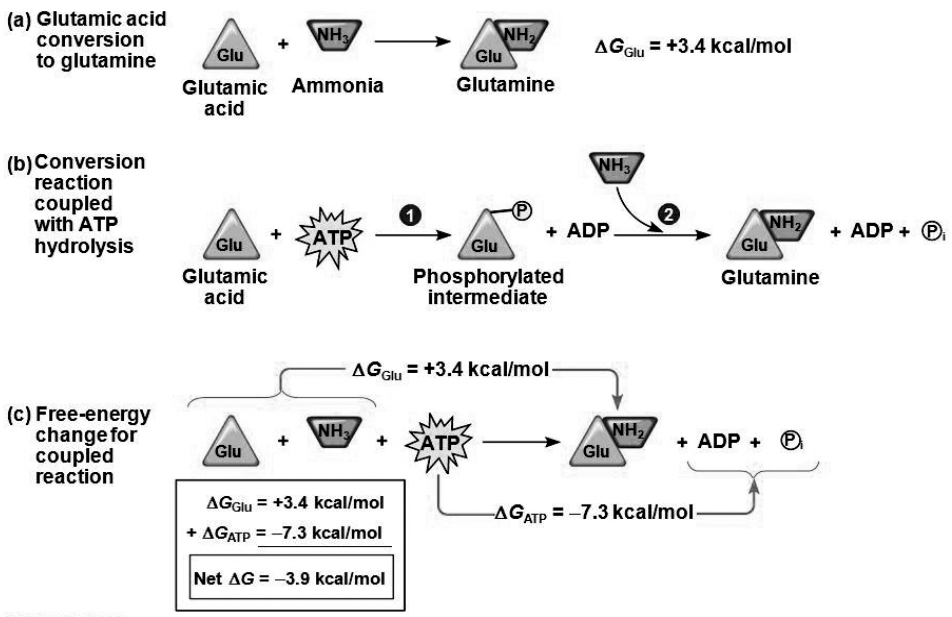


Fig.1.8: Change in coupled reactions

one simple example of the coupling of reaction is the decomposition of calcium carbonate:

$$CaCO3(s) \rightleftharpoons CaO(s) + CO2(g) \qquad \Delta G^{\circ} = 130.40 kJ/mo$$

The strongly positive ΔGΔG for this reaction is reactant-favored. If the temperature is raised above 837 ºC, this reaction becomes spontaneous and favors the products. Now, let us consider a second and completely different reaction that can be coupled to this reaction. The combustion of coal released by burning the coal ΔGo=−394.36kJ/molΔGo=−394.36kJ/mol is greater than the energy required to decompose calcium carbonate (ΔGo=130.40kJ/moΔGo=130.40kJ/mo).

$$C(s) + O2 \rightleftharpoons CO2(g)$$
$$\Delta Go = -394.36 kJ/mol$$

$$CaCO_3(s) + C(s) + O_2 \rightleftharpoons CaO(s) + 2CO_2(g)$$
$$\Delta Go = -263.96 kJ/mol$$

$$CaCO_3(s) + C(s) + O2 \rightleftharpoons CaO(s) + 2CO_2(g)$$
$$\Delta Go = -263.96 kJ/mol$$

and when Hess's Law is applied, the combined reaction is product-favored with $\Delta G^{o} = -263.96 kJ/mol$ $\Delta G^{o} = -263.96 kJ/mol$. This is because the reactant-favored reaction (Equation 19.8.219.8.2) is linked to a strong spontaneous reaction so that both reactions yield products. Notice that the ΔGΔG for the coupled reaction is the sum of the constituent reactions; this is a consequence of Gibbs energy being a state function:

$$\Delta Go = 130.40 kJ/mol + -394.36 kJ/mol = -263.96 kJ/mol$$

For example, the reaction of fermentation in an organism producing lactate as the sole product of glucose metabolism can be written as:

glucose <=> 2 lactate $\Delta Go = -47$ kcal. mol^{-1} (-197 kJ. mol^{-1})

The reaction of glycolysis in the cytoplasm can be written as:

1. glucose + 2 Pi + 2ADP <=> 2 lactate + 2ATP + $2H_2O$ $\Delta Go = -32.4$ kcal. mol^{-}

The difference between these two reactions is:

2 x (ADP + inorganic phosphate (Pi) <=> ATP + H_2O)
2 x 7.3 kcal. mol^{-1}

If the free energy changes for the fermentation reaction and ATP synthesis (-47 kcal.mol^{-1} + 14.6 kcal. mol^{-1}) are assumed one get -32.4 kcal.mol^{-1}, the free energy change from the glycolysis reaction.

One can see that the change in the system represented by the fermentation reaction, with a -□G, is coupled to a change in the surroundings (the change in a separate system represented by the phosphorylation of ADP to ATP), with +□G. When two systems are coupled in this way, it is often convenient to treat them as a single system. In this example, the new system is the reaction represented by the glycolysis equation, with a -□G equal to the sum of values for the two contributing processes.

From this example, it will be apparent that one can, from a thermodynamic perspective, treat metabolic processes in several ways. Individual reactions can be treated as separate systems, or a set of coupled reactions (including the complete set representing the metabolism of the organism as a whole) as a single system. The choice is one of convenience, and the important points are that the system should be carefully defined, the reaction equation balanced in conformity with the Law of conservation of mass, and the energy equation balanced in accordance with the First Law of thermodynamics and the properties of variables of state.

1.11. Summary

ATP can easily release and store energy by breaking and re-forming the bonds between its phosphate groups. This characteristic of ATP makes it exceptionally useful as a basic energy source for all cells. In the process of photosynthesis, plants convert the energy of sunlight into chemical energy stored in the bonds of carbohydrates. - Photosynthetic organisms capture energy from sunlight with pigments. - An electron carrier is a compound that can accept a pair of high-energy electrons and transfer them, along with most of their energy, to another molecule. Photosynthesis uses the energy of sunlight to convert water and carbon dioxide into high-energy sugars and oxygen. - Among the most important factors that affect photosynthesis are temperature, light intensity, and the

availability of water. Organisms get the needed energy from food. Cellular respiration is the process that releases energy from food in the presence of oxygen. Photosynthesis removes carbon dioxide from the atmosphere and cellular respiration puts it back. Photosynthesis releases oxygen into the atmosphere, and cellular respiration uses that oxygen to release energy from food. In the absence of oxygen, fermentation releases energy from food molecules by producing ATP. - For short, quick bursts of energy, the body uses ATP already in muscles as well as ATP made by lactic acid fermentation. For exercise longer than about 90 seconds, cellular respiration is the only way to continue generating a supply of ATP. Bioenergetics, or biochemical thermodynamics, is the study of the energy changes accompanying biochemical reactions. Biologic systems are essentially **isothermic** and use chemical energy to power living processes. The way in which an animal obtains suitable fuel from its food to provide this energy is basic to the understanding of normal nutrition and metabolism. Death from **starvation** occurs when available energy reserves are depleted and certain forms of malnutrition are associated with energy imbalance **(marasmus)**. Thyroid hormones control the **metabolic rate** (rate of energy release), and disease results if they malfunction. Excess storage of surplus energy causes **obesity** an increasingly common disease of Western society which predisposes to many diseases, including cardiovascular disease and diabetes mellitus type 2, and lowers life expectancy.

1.12. Terminal Questions

Q.1. Which organelle is involved in photosynthesis? List and describe the parts of this organelle.

Answer:----------

Q.2. Explain what happens to energy during photosynthesis. In what form does it enter photosynthesis? In what form does it exist during photosynthesis? In what form does it leave photosynthesis? How is this related to the overall goal of photosynthesis?

Answer:----------

Q.3. What is the primary pigment involved in photosynthesis? Why do plants also contain accessory pigments?

Answer:----------

Q.4. What are the products and reactants of cellular respiration? Where does the reaction take place in cells?

Answer:----------

Q.5. How is energy transformed during cellular respiration? In what form does it enter cellular respiration? In what form does it leave cellular respiration? How is this related to the overall goal of cellular respiration?

Answer:----------

Q.6. Compare and contrast photosynthesis and cellular respiration in terms of product, reactant, and energy transformations in each.

Answer:----------

1.13. Further readings

- Bioenergetics, David Nicholls, Academic Press ISBN: 9780123884251 4th Edition
- Bioenergetics, Alexander Lowen Penguin Books, ISBN13: 9780140194715
- Principles of Biochemistry: Lehninger, Nelson and Cox. Student Edition, CBS 1439 Publishers and Distributors, Delhi.
- Fundamentals of Biochemistry: Dr J L Jain, S. Chand and Company
- Textbook of Biochemistry and Human Biology: Talwar and Srivastava. Eastern Economy Edition, Prentice Hall, India.

UNIT-2

BIOENERGETICS –II : OXIDATION-REDUCTION REACTIONS

Structure

2.1. Introduction

Oxidation and reduction reactions occur when electrons are transferred. The molecule that is oxidized looses an electron and the

molecule that is reduced gains the electron that was lost by the oxidized molecule. A redox reaction is the same thing as an oxidation-reduction reaction or just a reaction where reduction and oxidation take place together. The terms redox reaction and oxidation reduction reaction are used interchangeably. Therefore, oxidation and reduction have to occur together. Molecules overall are not partially charged: since charges come in only +1 (protons) and -1 (electrons) in atoms, the charge on a molecule will always be an integer (and any charged molecule will come along with a counter ion of opposite charge). In biological reaction, the source of electrons is a relatively reduced compound such as glucose. As glucose is enzymatically oxidized, electrons are released and flow spontaneously through a series of electron carrier intermediates to another chemical species with a high affinity for electrons, such as O_2.

Objectives

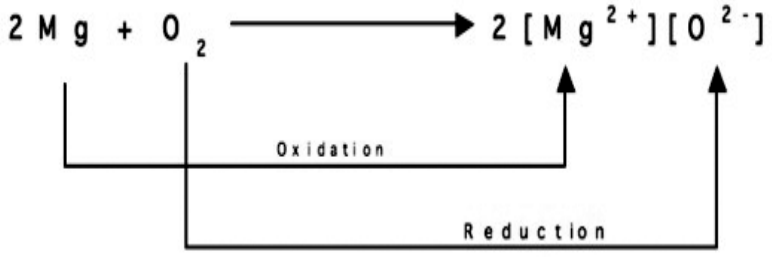
- to learn about biological oxidation and reductions
- to understand about reduction Potentials
- to learn the significance of Adenosine triphosphate (ATP)
- to understand the generating an energy carrier trough ATP

2.2. Biological oxidation-reduction reactions

All reactions which involve electron flow are considered oxidation-reduction reactions. The basic definition can be defined as, one reactant is oxidized (loses electrons), while another is reduced (gains electrons). A couple of basic oxidation-reduction or "redox" examples are given herewith. The reaction of magnesium metal with oxygen, involves the oxidation of magnesium eg.

$$2Mg(s)+O_2(g)\rightarrow 2MgO(s)$$

Since the solid magnesium is oxidized, one expect to see a loss of electrons. Similarly, since oxygen must therefore be reduced, see a gain of electrons is seen.



As the magnesium is oxidized there is a loss of 2 electrons while simultaneously, oxygen gains those two electrons. Another example of a redox reaction is with the two gasses CO_2 and H_2. This redox reaction also

demonstrates the importance of implementing "oxidation numbers" in the methodology of redox reactions, allowing for the determination of which reactant is being reduced and which reactant is being oxidized.

Electrons have a negative charge:

Gaining an electron = loss of charge = reduction

Loosing an electron = gain of charge = oxidation

Nomenclature

Reducer – has the ability to reduce/give electrons to another compound

Oxidizer - has the ability to oxidize/take electrons from another compound

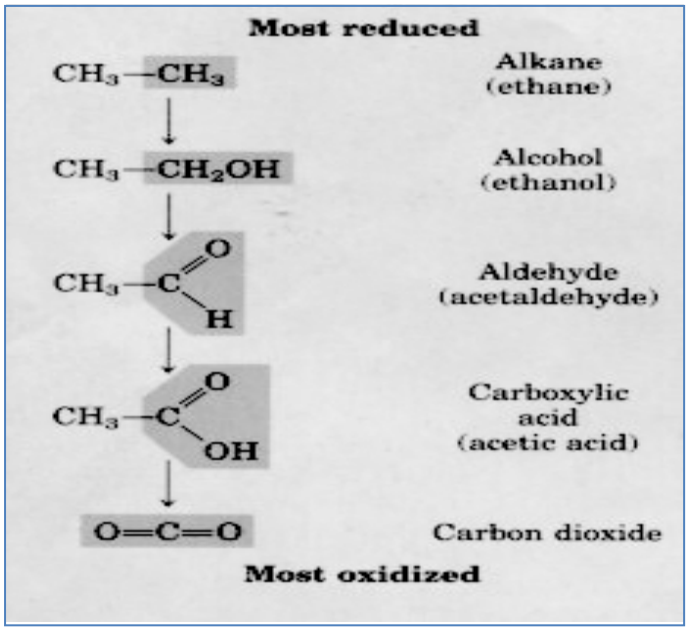
Oxidation and reduction reactions occur simultaneously and one does not occur without the other. Examples of oxidation and reduction reaction in plants and animal are as under.

Photosynthesis: The Use of light energy to fix (i.e. to reduce or incorporate) CO_2 or organic carbon into biomass. ATP is produced by photophosphorylation.	Cellular Respiration: Catabolic processes in which electron donors (reducers) are oxidized using oxygen as the final electron acceptor (oxidizer). ATP is produced by oxidative phosphorylation.

In biological reaction, the source of electrons is a relatively reduced compound such as glucose. As glucose is enzymatically oxidized, electrons are released and flow spontaneously through a series of electron carrier intermediates to another chemical species with a high affinity for electrons, such as O_2. Electron flow is spontaneous and exergonic because O_2 has a higher affinity for electrons than do the intermediates that donate electrons. The resulting electromotive force provides energy to molecular transducers that do biological work. In the mitochondrion, for example, membrane-bound transducers couple electron flow to the production of a transmembrane pH difference, accomplishing osmotic and electrical work.

The proton gradient thus formed has potential emeroy, sometimes called protonmotive force by analogy with electromotive force. Another molecular transducer in the mitochondrial membrane uses the proton-motive force to do chemical work; ATP is synthesized from ADP and Pi as protons flow spontaneously across the membrane. Similarly, membrane-localized transducers in ***E. coli*** convert electro motive to proton motive force, which is then used to power flagella motion for example carbon occurs in living cells in different oxidation states. In the most reduced compounds carbon atoms are rich in electrons and in hydrogen, whereas in the more highly oxidized compounds a carbon atom is bonded to more oxygen and to less hydrogen. In the oxidation of ethane

to ethanol, the compound does not loose hydrogen but one of the carbon atoms does. The hydrogen of the -OH group is, of course, not bonded directly to carbon. In the series of compounds oxidation of a carbon atom is synonymous with its dehydrogenation. When a carbon atom shares an electron pair with another atom such as oxygen, the sharing is unequal, in favor of the more electronegative atom (oxygen). Thus oxidation has the effect of removing electrons from the carbon atom.



Not all biological oxidation-reduction reactions involve oxygen and carbon for example, the conversion of molecular nitrogen into ammonia,

$$6H^+ + 6e^- + N_2 \longrightarrow 2NH_3$$

It represents a reduction of the nitrogen atoms. Electrons are transferred from one molecule to another in one of the four different ways:

1. They may be transferred directly as electrons for example, the Fe^{2+}/Fe^{3+} redox pair can transfer an electron to the Cu^{+}/Cu^{2+} redox pair:

$$Fe^{2+} + Cu^{2+} \rightleftharpoons Fe^{3+} + Cu^{+}$$

2. Electrons may be transferred in the form of hydrogen atoms. Recall that a hydrogen atom consists of a proton (H^+) and a single electron (e^-). In this case one can write the general equation as

$$AH_2 \longleftrightarrow A + 2e^- + 2H^+$$

where AH_2 acts as the hydrogen (or electron) donor. AH_2 and A together constitute a conjugate redox pair, which can reduce another compound B by transfer of hydrogen atoms.

$$AH_2 + B \longleftrightarrow A + BH_2$$

3. Electrons may be transferred from an electron donor to an acceptor in the form of a hydride ion ($:H^-$), which includes two electrons, as in the case of NAD-linked dehydrogenases described below.

4. Electron transfer also takes place when there is a direct combination of an organic reductant with oxygen, to give a product in which the oxygen is covalently incorporated, as in the oxidation of a hydrocarbon to an alcohol:

$$2\ R\text{-}CH_3 + O_2 \longrightarrow 2\ R\text{-}CH_2\text{-}OH$$

In this reaction the hydrocarbon is the electron donor and the oxygen atom is the electron acceptor.

2.2.1. Reduction Potentials

When two conjugate redox pairs are present together in solution, electron transfer from the electron donor of one pair to the electron acceptor of the other may occur spontaneously. The tendency of such a reaction to occur depends upon the relative affinity of the electron acceptor of each redox pair for electrons. The standard reduction potential, Eo, a measure of this affinity, is determined in an experiment such as that described in electrochemists have chosen the half reaction as a standard of reference.

$$H^+ + e^- \longleftrightarrow 2H_2$$

The electrode at which this half reaction occurs is arbitrarily assigned a standard reduction potential of 0.00 V. When this hydrogen electrode is connected through an external circuit to another half cell in which the oxidized and reduced species are both present at standard concentrations (each solute at 1 M, each gas at 1 atm), electrons will tend to flow through the external circuit from the half cell of lower standard reduction potential to the half cell of higher standard reduction potential. By convention, the half cell with the stronger tendency to acquire electrons is assigned a positive value of E_0 (in volts).

The reduction potential of a half cell depends not only upon the chemical species present but also upon their activities, approximated by their concentrations. About a century ago, Walther Nernst derived an equation that relates standard reduction potential (E_0) to reduction potential (E) at any concentration of oxidized and reduced species in the cell.

$$E = E_0 + \frac{RT}{n\mathcal{F}} \ln \frac{[\text{electron acceptor}]}{[\text{electron donor}]} \qquad (13\text{–}6)$$

where R and T have their usual meanings (Table 13-1), n is the number of electrons transferred per molecule, and F is the Faraday constant, 96.48 kJ/V•mol. At 298 K (25 °C), this expression reduces to:

$$E = E_0 + \frac{0.026\ \text{V}}{n} \ln \frac{[\text{electron acceptor}]}{[\text{electron donor}]} \qquad (13\text{–}7)$$

Many half reactions of interest to biochemists involve protons. As in the definition of ΔG°', biochemists define the standard state for oxidation-reduction reactions as pH 7 and express reduction potential as E'_0, the standard reduction potential at pH 7. The values for standard reduction potentials are given in Table 2.1 and used throughout this book for E_0 and are therefore only valid for calculations involving systems at neutral pH. Each value represents the potential difference when the conjugate redox pair at 1 M concentrations at pH 7 is connected with the standard (pH 0) hydrogen electrode. Notice in Table 13-7 that when the conjugate pair $2H^+/H_2$ at pH 7 is connected with the standard hydrogen electrode (pH 0), electrons tend to flow from the pH 7 cell to the standard (pH 0) cell; the measured $\Delta E'_0$ for the $2H^+/H_2$ pair is -0.414 V

Table: 2.1. Standard reduction potential of some biologically important half-reactions (at 25 ˚C, pH 7)

Half-reaction	E'_0(V)
$\frac{1}{2}O_2 + 2H^+ + 2e^- \longrightarrow H_2O$	0.816
$Fe^{3+} + e^- \longrightarrow Fe^{2+}$	0.771
$NO_3^- + 2H^+ + 2e^- \longrightarrow NO_2^- + H_2O$	0.421
Cytochrome *f* (Fe^{3+}) + e^- ⟶ cytochrome *f* (Fe^{2+})	0.365
$Fe(CN)_6^{3-}$ (ferricyanide) + e^- ⟶ $Fe(CN)_6^{4-}$	0.36
$O_2 + 2H^+ + 2e^- \longrightarrow H_2O_2$	0.295
Cytochrome *a* (Fe^{3+}) + e^- ⟶ cytochrome *a* (Fe^{2+})	0.29
Cytochrome *c* (Fe^{3+}) + e^- ⟶ cytochrome *c* (Fe^{2+})	0.254
Cytochrome c_1 (Fe^{3+}) + e^- ⟶ cytochrome c_1 (Fe^{2+})	0.22
Ubiquinone + $2H^+ + 2e^-$ ⟶ ubiquinol + H_2	0.045
Cytochrome *b* (Fe^{3+}) + e^- ⟶ cytochrome *b* (Fe^{2+})	0.077
$Fumarate^{2-} + 2H^+ + 2e^- \longrightarrow succinate^{2-}$	0.031
$2H^+ + 2e^- \longrightarrow H_2$ (at standard conditions, pH 0)	0.000
Crotonyl-CoA + $2H^+ + 2e^-$ ⟶ butyryl-CoA	−0.015
$Oxaloacetate^{2-} + 2H^+ + 2e^- \longrightarrow malate^{2-}$	−0.166
$Pyruvate^- + 2H^+ + 2e^- \longrightarrow lactate^-$	−0.185
Acetaldehyde + $2H^+ + 2e^-$ ⟶ ethanol	−0.197
$FAD + 2H^+ + 2e^- \longrightarrow FADH_2$	−0.219
Glutathione + $2H^+ + 2e^-$ ⟶ 2 reduced glutathione	−0.23
$S + 2H^+ + 2e^- \longrightarrow H_2S$	−0.243
Lipoic acid + $2H^+ + 2e^-$ ⟶ dihydrolipoic acid	−0.29
$NAD^+ + H^+ + 2e^- \longrightarrow NADH$	−0.320
$NADP^+ + H^+ + 2e^- \longrightarrow NADPH$	−0.324
Acetoacetate + $2H^+ + 2e^-$ ⟶ β-hydroxybutyrate	−0.346
α-Ketoglutarate + $CO_2 + 2H^+ + 2e^-$ ⟶ isocitrate	−0.38
$2H^+ + 2e^- \longrightarrow H_2$ (at pH 7)	−0.414
Ferredoxin (Fe^{3+}) + e^- ⟶ ferredoxin (Fe^{2+}) (spinach)	−0.432

Data mostly from Loach, P.A. (1976) In *Handbook of Biochemistry and Molecular Biology*, 3rd edn (Fasman, G.D., ed), *Physical and Chemical Data*, Vol. I, pp. 122–130, CRC Press, Cleveland, OH.

2.2.2. Standard Reduction Potentials Allow the Calculation of Free-Energy Change

The usefulness of reduction potentials stems from the fact that when E has been determined for any two half cells, relative to the standard hydrogen electrode, their reduction potentials relative to each other are

also known. One can therefore predict the direction in which electrons will tend to flow when these two half cells are connected through an external circuit, or when the components of the two half cells are present together in the same solution. Electrons will tend to flow to the half cell with the more positive E, and the strength of that tendency is proportional to the difference in reduction potentials, ΔE.

The energy made available to do work by this spontaneous electron flow (the free-energy change for the oxidation-reduction reaction) is proportional to ΔE.

$\Delta G = -nF\Delta E$, or $\Delta G^{\circ\prime} = -nF\Delta E'_0$

Here n represents the number of electrons transferred in the reaction. With this equation it is possible to calculate the free-energy change for any oxidation-reduction reaction from the values of Eo (found in a table of reduction potentials) and the concentrations of the species involved in the reaction.

Consider the reaction in which acetaldehyde is reduced by the biological electron carrier NADH:

$$\text{Acetaldehyde} + \text{NADH} + H^+ \longrightarrow \text{ethanol} + NAD^+$$

The relevant half reactions and their Eo values (Table 13-7) are:

1) $\text{Acetaldehyde} + 2H^+ + 2e^- \longrightarrow \text{ethanol} \qquad E'_0 = -0.197\ V$

2) $NAD^+ + 2H^+ + 2e^- \longrightarrow NADH + H^+ \qquad E'_0 = -0.320\ V$

For the overall reaction, $\Delta E_0 = -0.197\ V - (-0.320\ V) = 0.123\ V$, and n is 2. Therefore, $\Delta G^{\circ\prime} = -nF\Delta E'_0 = -2(96.5\ kJ/V\cdot mol)(0.123\ V) = -23.7\ kJ/mol$.

This is the free-energy change for the oxidation-reduction reaction when acetaldehyde, ethanol, NAD^+, and NADH are all present at 1 M concentrations. If, instead, acetaldehyde and NADH were present at 1 M, but ethanol and NAD+ were present at 0.1 M, the value for ΔG would be calculated as follows. First, the values of E for both reductants are determined (Eqn 13-7):

$$E_{\text{acetaldehyde}} = E'_0 + \frac{RT}{n\mathcal{F}} \ln \frac{[\text{acetaldehyde}]}{[\text{ethanol}]}$$

$$= -0.197\ V + \frac{0.026\ V}{2} \ln \frac{1.0}{0.1} = -0.167\ V$$

$$E_{\text{NADH}} = E'_0 + \frac{RT}{n\mathcal{F}} \ln \frac{[NAD^+]}{[\text{NADH}]}$$

$$= -0.320\ V + \frac{0.026\ V}{2} \ln \frac{0.1}{1.0} = -0.350\ V$$

Then ΔE is used to calculate ΔG (Eqn 13-8):

$$E = -0.167-(-0.350) = 0.183 \text{ V}$$

$$\begin{aligned} G &= -nF\Delta E \\ &= -2(96.5 \text{kJ/V•mol})(0.183 \text{V}) \\ &= -35.3 \text{kJ/mol} \end{aligned}$$

it is thus possible to calculate the free-energy change for any biological oxidation at any concentrations of the redox pairs.

2.2.3. Cells Oxidize Glucose to Carbon Dioxide in Steps Involving Specialized Electron Carriers

In many organisms, the oxidation of glucose supplies energy for the production of ATP. For the complete oxidation of glucose.

$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O$$

$\Delta G°'$ is -2,840 kJ/mol. This is a much larger change in free energy than that occurring during ATP synthesis (50 to 60 kJ/mol; see Box 13-2). Cells do not convert glucose to CO_2 in a single, very energetic reaction, but rather in a series of reactions, some of which are oxidations. The free-energy change of these oxidation steps is larger than, but of the same order of magnitude as, that required for ATP synthesis from ADP. Electrons removed in these oxidation steps are transferred to coenzymes specialized for carrying electrons, such as NAD^+ and FAD, which are described below.

2.2.4. A Few Types of Cofactors and Proteins Serve as Universal Electron Carriers

Most cells have enzymes to catalyze the oxidation of hundreds of different compounds. These enzymes channel electrons from their substrates into a few types of universal electron carriers. The nucleotides NAD^+, $NADP^+$, FMN, and FAD are water-soluble cofactors that undergo reversible oxidation and reduction in many of the electron transfer reactions of metabolism. Their reduction in catabolic processes results in the conservation of free energy released by substrate oxidation. The nucleotides NAD^+ and $NADP^+$ move readily from one enzyme to another, but the flavin nucleotides FMN and FAD are very tightly bound to the enzymes, called flavoproteins, for which they serve as prosthetic groups. Lipid-soluble quinones such as ubiquinone and plastoquinone act in the nonaqueous environment of membranes, accepting electrons and conserving free energy. Iron-sulfur proteins and cytochromes are proteins with tightly bound prosthetic groups that undergo reversible oxidation and reduction; they, too, serve as electron carriers in many oxidation-reduction reactions.

2.3. Adenosine triphosphate (ATP)

Adenosine triphosphate (ATP) is considered by biologists to be the energy currency of life (Fig. 2.1**)**. It is the high-energy molecule that stores the energy needed to do just about every work done. It is present in the cytoplasm and nucleoplasm of every cell, and essentially all the physiological mechanisms that require energy for operation obtain it directly from the stored ATP. As food in the cells is gradually oxidized, the released energy is used to re-form the ATP so that the cell always maintains a supply of this essential molecule. Karp quotes an estimate that more than 2 x 10^{26} molecules or >160kg of ATP is formed daily in the human body ATP is remarkable for its ability to enter into many coupled reactions, both those to food to extract energy and with the reactions in other physiological processes to provide energy to them. In animal systems, the ATP can be synthesized in the process of glycolysis in which there is a net production of two ATP molecules in a cycle. This glycolysis is a major step in anaerobic respiration. For aerobic respiration the glycolysis is also a source of ATP but the more productive process in the tiny energy factories called mitochondria plays a major role in the production of ATP.

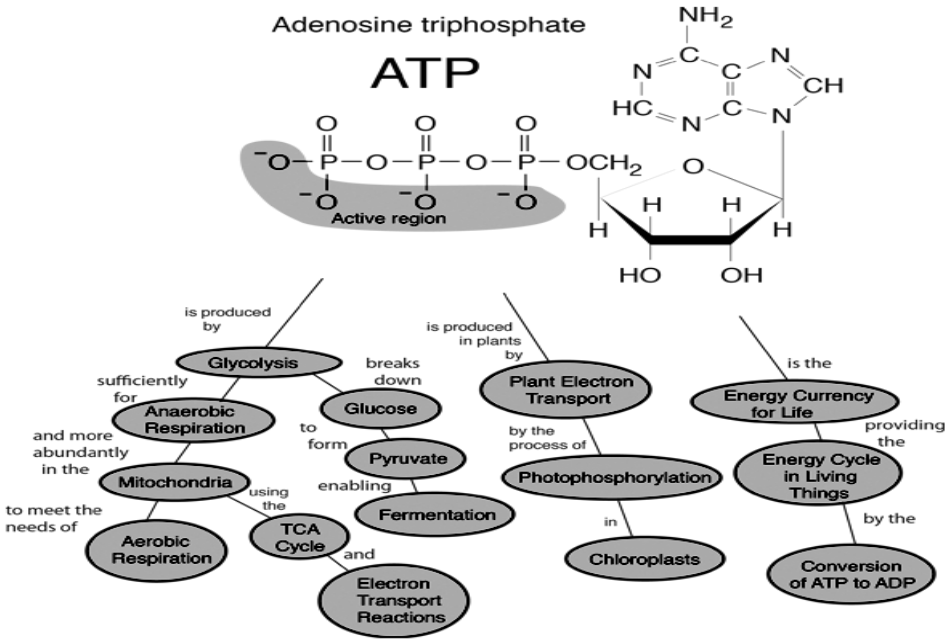


Fig. 2.1: Structure of adenosine triphosphate (ATP)

The structure of ATP has an ordered carbon compound as a backbone, but the part that is really critical is the phosphorous part - the triphosphate. Three phosphorous groups are connected by oxygen to each other, and there are also side oxygens connected to the phosphorous atoms. Under the normal conditions in the body, each of these oxygens has a negative charge, and therefore repel each other. These bunched up negative charges want to escape - to get away from each other, so there is a lot of potential energy.

If one removed just one of these phosphate groups from the end, so that there are just two phosphate groups, the molecule is much happier. This conversion from ATP to ADP is an extremely crucial reaction for the supply of energy for life processes. Just the cutting of one bond with the accompanying rearrangement is sufficient to liberate about 7.3 kilocalories per mole = 30.6 kJ/mol. This is about the same as the energy in a single peanut.

2.3.1.Conversion from ATP to ADP

Adenosine triphosphate (ATP) is the energy currency of life and it provides that energy for most biological processes by being converted to ADP (adenosine diphosphate). Since the basic reaction involves a water molecule,

$$ATP + H_2O \rightarrow ADP + Pi$$

This reaction is commonly referred to as the hydrolysis of ATP.

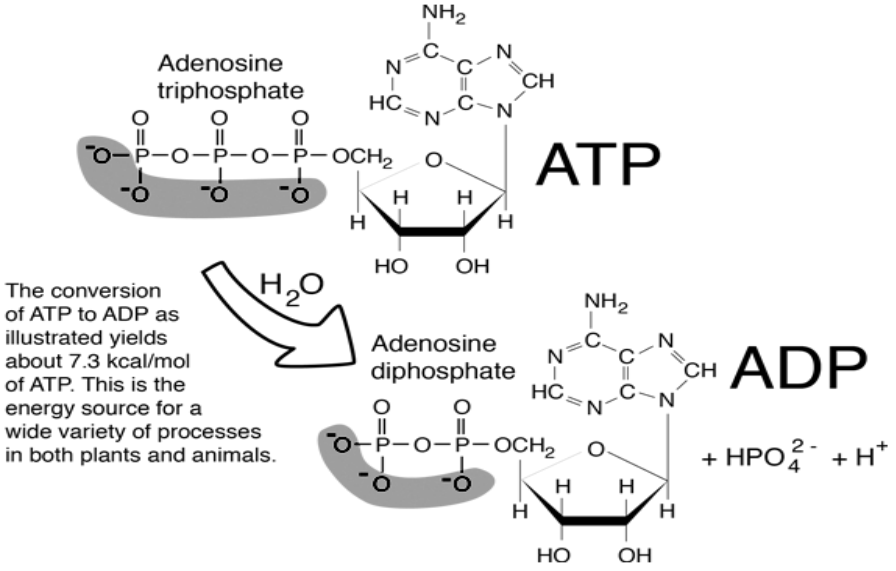


Fig.2.2: Conversion from ATP to ADP

Living things can use ATP like a battery. The ATP can power needed reactions by loosing one of its phosphorous groups to form ADP, but you can use food energy in the mitochondria to convert the ADP back to ATP so that the energy is again available to do needed work. In plants, sunlight energy can be used to convert the less active compound back to the highly energetic form. Animals use the energy from these high energy storage molecules to do what in needed to keep oneself alive, and then they are "recharged" to put them back in the high energy state.

2.3.2. Free Energy from Hydrolysis of ATP

Adenosine triphosphate (ATP) is the energy currency of life and it provides that energy for most biological processes by being converted to

ADP (adenosine diphosphate). Since the basic reaction involves a water molecule,

$$ATP + H_2O \rightarrow ADP + Pi$$

This reaction is commonly referred to as the hydrolysis of ATP. The change in Gibbs free energy in the reaction is used to assess the energy yield of such reactions, and as a general indicator of the spontaneity of reactions. Under standard conditions this change ΔG0' is

$$\Delta G^{0'} = -RT \ln K'_{eq} = -7.3 \frac{kcal}{mol} = -30.5 \frac{kJ}{mol}$$

.

But inside a living cell, typical concentrations of the reactants might be [ATP]=10mM, [ADP]=1mM and [Pi]=10mM. Under those conditions the free energy change is

$$\Delta G = \Delta G^{0'} + 2.303RT \log_{10} \frac{[ADP][P_i]}{[ATP]}$$

$$\Delta G = -7.3 \frac{kcal}{mol} + 1.4 \frac{kcal}{mol} \log_{10} \frac{[10^{-3}][10^{-2}]}{[10^{-2}]}$$

$$\Delta G = -7.3 \frac{kcal}{mol} + 1.4 \frac{kcal}{mol} (-3)$$

$$\Delta G = -11.5 \frac{kcal}{mol} = -46.2 \frac{kJ}{mol}$$

.

Because of the concentrations of ATP and ADP in the cell, the conditions are very favorable for the use of the hydrolysis of ATP as an energy source. In fact, many processes with positive ΔG values can take place when coupled with the hydrolysis of ATP.

2.3.3. Other Triphosphate

Living cells also have other "high-energy" nucleoside triphosphates, such as guanine triphosphate. Between them and ATP, energy can be easily transferred with reactions such as those catalyzed by nucleoside diphosphokinase. Energy is released when hydrolysis of the phosphate-phosphate bonds is carried out. This energy can be used by a variety of enzymes, motor proteins, and transport proteins to carry out the work of the cell. Also, the hydrolysis yields free inorganic phosphate and adenosine diphosphate, which can be broken down further to another phosphate ion and adenosine monophosphate. ATP can also be broken down to adenosine monophosphate directly, with the formation of pyrophosphate. This last reaction has the advantage of being an effectively irreversible process in aqueous solution.

2.4. Photosynthetic light reaction

How can light be used to make food? It is easy to think of light as something that exists and allows living organisms, such as humans, to see, but light is a form of energy. Like all energes, light can travel, change form, and be harnessed to do work. In the case of photosynthesis, light energy is transformed into chemical energy, which autotrophs use to build carbohydrate molecules. However, autotrophs only use a specific component of sunlight. Visible light constitutes only one of many types of electromagnetic radiation, emitted from the sun. The electromagnetic spectrum is the range of all possible wavelengths of radiation. Each wavelength corresponds to a different amount of carried energy.

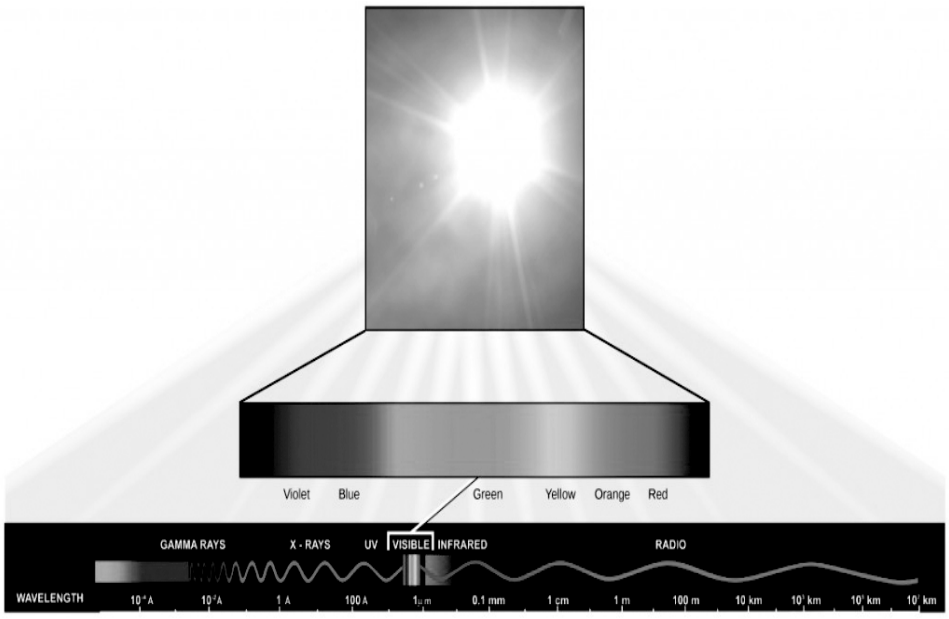


Fig.2.3: Schematic representation of electromagnetic radiation for photosynthetic

The sun emits a broad range of electromagnetic radiation, including X-rays and ultraviolet (UV) rays. The higher-energy waves are dangerous to living things; for example, X-rays and UV rays can be harmful to humans in many ways.

2.4.1. Absorption of Light

Light energy enters the process of photosynthesis when pigments absorb the light. In plants, pigment molecules absorb only visible light for photosynthesis. The visible light seen by humans as white light actually exists in a rainbow of colors. Certain objects, such as a prism or a drop of water, disperse white light to reveal these colors to the human eye.

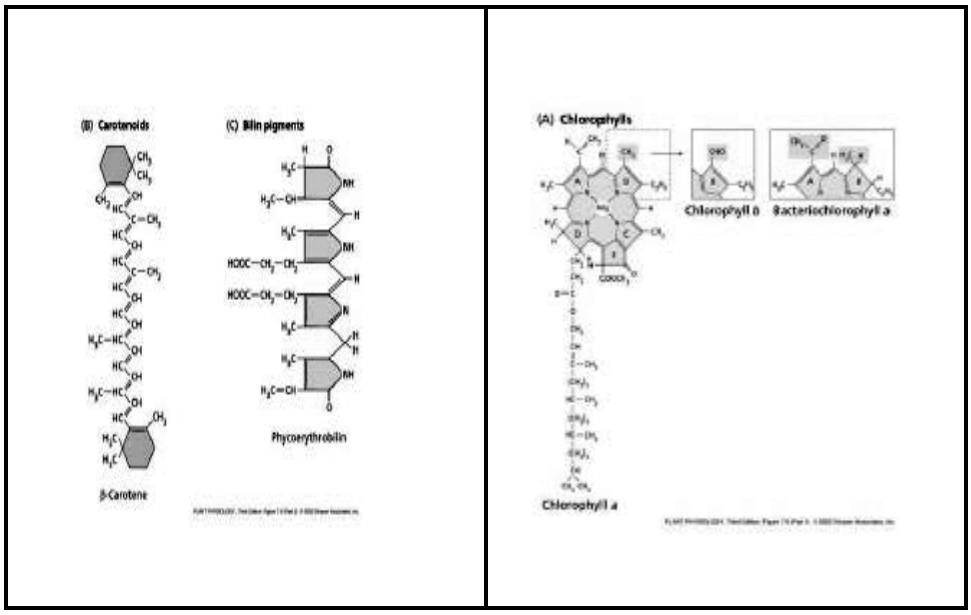


Fig.2.4: Absorption of visible light Chlorophyll molecules

The visible light portion of the electromagnetic spectrum is perceived by the human eye as a rainbow of colors, with violet and blue having shorter wavelengths and, therefore, higher energy. At the other end of the spectrum toward red, the wavelengths are longer and have lower energy.

2.4.2. Understanding Pigments

Different kinds of pigments exist, and each absorbs only certain wavelength (colors) of visible light. Pigments reflect the colour of the wavelengths that they cannot absorb.

All photosynthetic organisms contain a pigment called chlorophyll ***a***, which humans see as the common green colour associated with plants. Chlorophyll ***a*** absorbs wavelengths from either end of the visible spectrum (blue and red), but not from green. Because green is reflected, chlorophyll appears green.

Other pigment types include chlorophyll ***b*** (which absorbs blue and red-orange light) and the carotenoids. Each type of pigment can be identified by the specific pattern of wavelengths it absorbs from visible light, which is its absorption spectrum.

Types of Pigments:

Chlorophyll: a, b and bacteriochlorophylls

Carotenoids: xanthophylls and β-carotene

Many photosynthetic organisms have a mixture of pigments; hence they can absorb energy from a wider range of visible-light wavelengths. Not all photosynthetic organisms have full access to sunlight.

Some organisms grow underwater where light intensity decreases with depth, and certain wavelengths are absorbed by the water. Other organisms grow in competition for light. Plants on the rainforest floor must be able to absorb any bit of light that comes through, because the taller trees block most of the sunlight.

2.4.3. How Light-Dependent Reactions Work

The overall purpose of the light-dependent reactions is to convert light energy into chemical energy. This chemical energy will be used by the Calvin cycle to fuel the assembly of sugar molecules.

The light-dependent reactions begin in a grouping of pigment molecules and proteins called a photosystem (Fig.2.5). Photosystems exist in the membranes of thylakoids. A pigment molecule in the photosystem absorbs one photon, a quantity or "packet" of light energy, at a time.

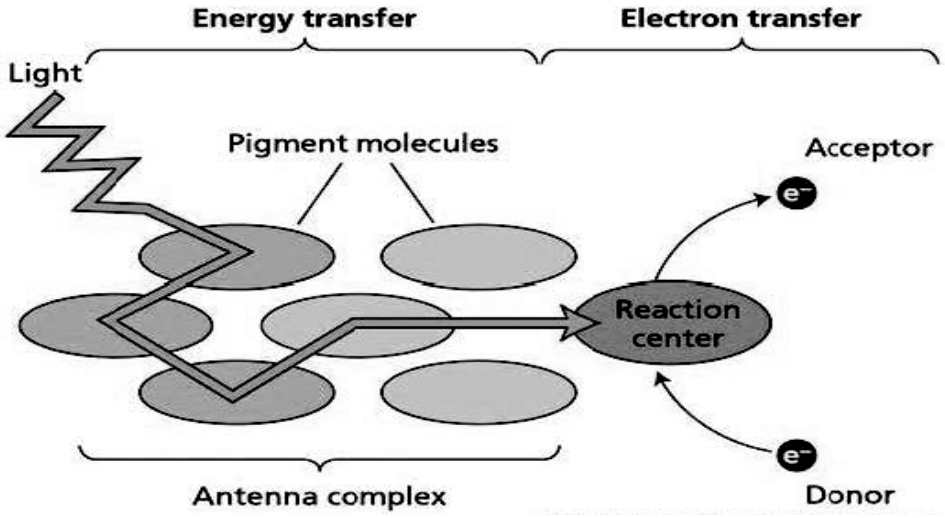


Fig.2.5: Schematic representation of light-dependent reactions to convert light energy into chemical energy

A photon of light energy travels until it reaches a molecule of chlorophyll. The photon causes an electron in the chlorophyll to become "excited." The energy given to the electron allows it to break free from an atom of the chlorophyll molecule. Chlorophyll is therefore said to "donate" an electron.

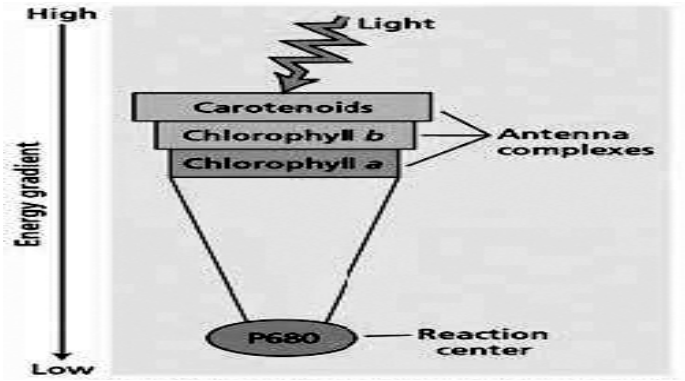


Fig. 2.6: Energy flow in Chlorophyll molecules

To replace the electron in the chlorophyll, a molecule of water is split. This splitting releases an electron and results in the formation of oxygen (O_2) and hydrogen ions (H^+) in the thylakoid space. Technically, each breaking of a water molecule releases a pair of electrons, and therefore can replace two donated electrons.

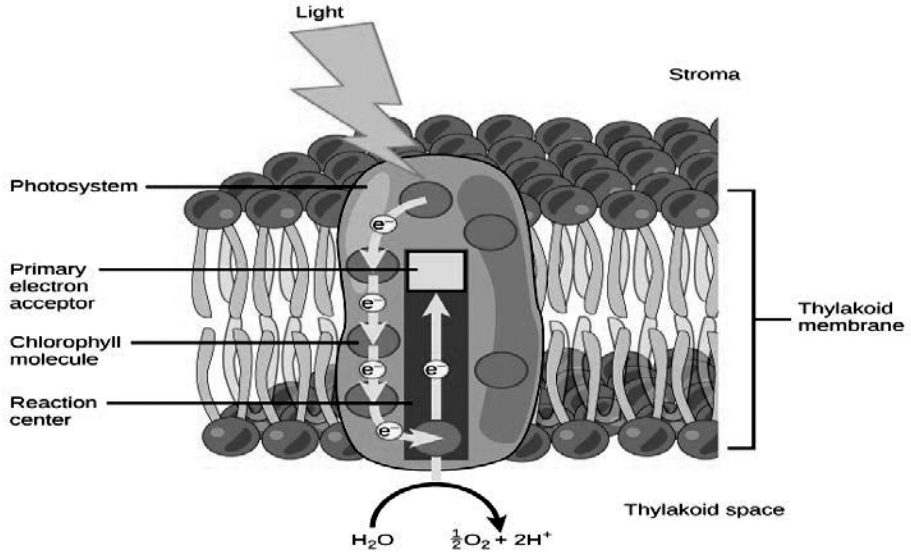


Fig. 2.7: Energy flow in thylakoid membrane

The replacing of the electron enables chlorophyll to respond to another photon. The oxygen molecules produced as byproducts find their way to the surrounding environment. The hydrogen ions play critical roles in the remainder of the light-dependent reactions. Light energy is absorbed by a chlorophyll molecule and is passed along a pathway to other chlorophyll molecules. The energy culminates in a molecule of chlorophyll found in the reaction center. The energy "excites" one of its electrons enough to leave the molecule and be transferred to a nearby primary electron acceptor. A molecule of water splits to release an electron, which is needed to replace the one donated. Oxygen and hydrogen ions are also formed from the splitting of water.

Keep in mind that the purpose of the light-dependent reactions is to convert solar energy into chemical carriers that will be used in the Calvin cycle. In eukaryotes and some prokaryotes, two photosystems exist. The first is called photosystem II, which was named for the order of its discovery rather than for the order of the function.

After the photon hits, Photosystem II transfers the free electron to the first in a series of proteins inside the thylakoid membrane called the electron transport chain. As the electron passes along these proteins, energy from the electron fuels membrane pumps that actively move hydrogen ions against their concentration gradient from the stroma into the thylakoid space. This is quite analogous to the process that occurs in the mitochondrion in which an electron transport chain pumps hydrogen ions from the mitochondrial stroma across the inner membrane and into the intermembrane space, creating an electrochemical gradient. After the

energy is used, the electron is accepted by a pigment molecule in the next photosystem, which is called photosystem I (Figure 2.7). From photosystem II, the electron travels along a series of proteins. This electron transport system uses the energy from the electron to pump hydrogen ions into the interior of the thylakoid. A pigment molecule in photosystem I accepts the electron.

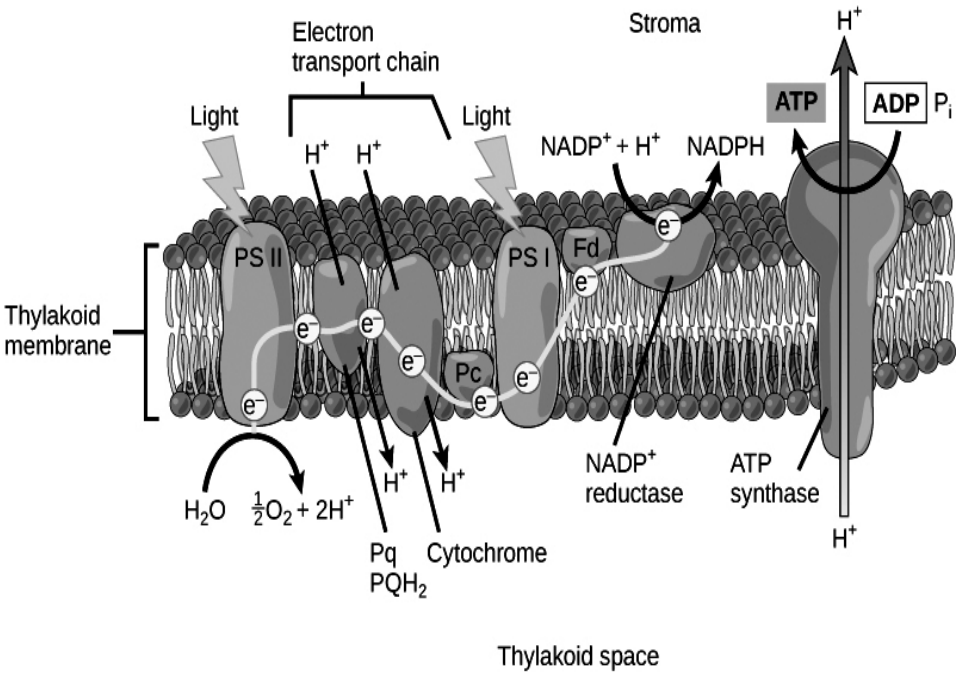


Fig.2.8: ETS in thylakoid

2.5. Generating an Energy Carrier: ATP

In the light-dependent reactions, energy absorbed by sunlight is stored by two types of energy-carrier molecules as ATP and NADPH. The energy that these molecules carry is stored in a bond that holds a single atom to the molecule. For ATP, it is a phosphate atom, and for NADPH, it is a hydrogen atom. Recall that NADH was a similar molecule that carried energy in the mitochondrion from the citric acid cycle to the electron transport chain. When these molecules release energy into the Calvin cycle, they each lose atoms to become the lower-energy molecules ADP and $NADP^+$.

The buildup of hydrogen ions in the thylakoid space forms an electrochemical gradient because of the difference in the concentration of protons (H^+) and the difference in the charge across the membrane that they create. This potential energy is harvested and stored as chemical energy in ATP through chemiosmosis, the movement of hydrogen ions down their electrochemical gradient through the transmembrane enzyme ATP synthase, just as in the mitochondrion.

The hydrogen ions are allowed to pass through the thylakoid membrane through an embedded protein complex called ATP synthase. The same protein generated ATP from ADP in the mitochondrion. The energy generated by the hydrogen ion stream allows ATP synthase to attach a third phosphate to ADP, which forms a molecule of ATP in a process called photophosphorylation. The flow of hydrogen ions through ATP synthase is called chemiosmosis, because the ions move from an area of high to low concentration through a semi-permeable structure.

Here are the basic steps:

- **Light absorption in PSII.** When light is absorbed by one of the many pigments in photosystem II, energy is passed inward from pigment to pigment until it reaches the reaction center. Where, energy is transferred to P680, boosting an electron to a high energy level. The high-energy electron is passed to an acceptor molecule and replaced with an electron from water. This splitting of water releases the \text O_2O2start text, O, end text, start subscript, 2, end subscript one breathes.

- **ATP synthesis.** The high-energy electron travels down an electron transport chain, loosing energy as it goes. Some of the released energy drives pumping of \text H^+H+start text, H, end text, start superscript, plus, end superscript ions from the stroma into the thylakoid interior, building a gradient. (\text H^+H+start text, H, end text, start superscript, plus, end superscript ions from the splitting of water also add to the gradient.) As \text H^+H+start text, H, end text, start superscript, plus, end superscript ions flow down their gradient and into the stroma, they pass through ATP synthase, driving ATP production in a process known as **chemiosmosis**.

- **Light absorption in PSI.** The electron arrives at photosystem I and joins the P700 special pair of chlorophylls in the reaction center. When light energy is absorbed by pigments and passed inward to the reaction center, the electron in P700 is boosted to a very high energy level and transferred to an acceptor molecule. The special pair's missing electron is replaced by a new electron from PSII (arriving via the electron transport chain).

- **NADPH formation.** The high-energy electron travels down a short second leg of the electron transport chain. At the end of the chain, the electron is passed to NADP^++start superscript, plus, end superscript (along with a second electron from the same pathway) to make NADPH.

2.6. Generating another Energy Carrier: NADPH

The remaining function of the light-dependent reaction is to generate the other energy-carrier molecule, NADPH. As the electron from

the electron transport chain arrives at photosystem I, it is re-energized with another photon captured by chlorophyll. The energy from this electron drives the formation of NADPH from $NADP^+$ and a hydrogen ion (H^+). Now that the solar energy is stored in energy carriers, it can be used to make a sugar molecule.

2.7. Summary

Metabolism is central to all cellular life. The cellular and molecular changes occur due to oxidation and reduction processes. The flow of electrons is a vital process that provides the necessary energy for the survival of all organisms. The primary source of energy that drives the electron flow in nearly all of these organisms is the radiant energy of the sun, in the form of electromagnetic radiation or light. The terms redox reaction and oxidation reduction reaction are used interchangeably. A couple of basic oxidation-reduction or "redox" example's are given here. The reaction of magnesium metal with oxygen, involves the oxidation of magnesium eg.

$$2Mg(s)+O_2(g)\rightarrow 2MgO(s)$$

Most cells have enzymes to catalyze the oxidation of hundreds of different compounds. These enzymes channel electrons from their substrates into a few types of universal electron carriers. The nucleotides NAD+, NADP+, FMN, and FAD are water-soluble cofactors that undergo reversible oxidation and reduction in many of the electron transfer reactions of metabolism.

ATP is formed in the human body daily ATP is remarkable for its ability to enter into many coupled reactions, both those to food to extract energy and with the reactions in other physiological processes to provide energy to them.

Many photosynthetic organisms have a mixture of pigments, between them; the organism can absorb energy from a wider range of visible-light wavelengths. The energy culminates in a molecule of chlorophyll found in the reaction center. The energy “excites” one of its electrons enough to leave the molecule and be transferred to a nearby primary electron acceptor, NADPH is the energy-carrier molecule used in metabolic processes.

2.8. Terminal question

Q.1. Define the bioenergetics process in briefly. How the oxidation-reduction process is very useful in metabolism?

Answer:----------

Q.2. Discuss the role of reduction potentials in biological reactions.

Answer:----------

Q.3. How the Photosynthetic light reaction takes place, briefly discuss.

Answer:----------

Q.4. Define the role of ATP in generating energy.

Answer:----------

Q.5. Discuss how light-dependent reactions work.

Answer:----------

Q.6. Write the role of sun light in photosynthesis process.

Answer:----------

2.9. Further readings

- Bioenergetics, David Nicholls, Academic Press ISBN: 9780123884251 4th Edition
- Bioenergetics, Alexander Lowen Penguin Books, ISBN13: 9780140194715
- Principles of Biochemistry: Lehninger, Nelson and Cox. Student Edition, CBS 1439 Publishers and Distributors, Delhi.
- Fundamentals of Biochemistry: Dr J L Jain, S. Chand and Company
- Textbook of Biochemistry and Human Biology: Talwar and Srivastava. Eastern Economy Edition, Prentice Hall, India.

Uttar Pradesh Rajarshi Tandon
Open University

PGBCH-103

Bioenergetics and Metabolism

BLOCK

2

Coenzymes And Carbohydrate Metabolism

Curriculum Design Committee

Dr. (Prof.) Ashutosh Gupta, **Chairman**
School of Science, UPRTOU, Prayagraj

Prof. Prof. Umesh Nath Tripathi **Member**
Department of chemistry
Deen Dayal Upadhyaya Gorakhpur University, Gorakhpur

Prof. S.I. Rizvi **Member**
Department of Biochemistry
University of Allahabad, Prayagraj

Prof. Dinesh Yadav **Member**
Department of Biotechnology
Deen Dayal Upadhyaya Gorakhpur University, Gorakhpur

Prof. Sharad Kumar Mishra **Member**
Department of Biotechnology
Deen Dayal Upadhyaya Gorakhpur University, Gorakhpur

Dr. Ravindra Pratap Singh **Member**
Academic Consultant (Biochemistry)
School of Science, UPRTOU, Prayagraj

Dr. Dharmveer Singh **Course Coordinator**
Academic Consultant (Biochemistry)
School of Science, UPRTOU, Prayagraj

Course Preparation Committee

Dr. Sanjeev Kumar Maurya Associate Professor Department of biotechnology, Invertis University, Bareilly	**Author**	**Block-1&2 (Unit: 1- 4)**
Dr. Shashank Upadhaya Asst. Professor Department of biotechnology, Invertis University, Bareilly	**Author**	**Block-3 (Unit: 5, 6)**
Dr. V.C. Srivastava Former Associate Professor, Department of Zoology, University of Allahabad, Prayagraj	**Editor**	**(All blocks and units)**
Dr. Dharmveer Singh School of Sciences, UPRTOU, Prayagraj		**(Course Coordinator)**

ISBN :

Introduction

This is the second block on Bioenergetics. It consists of following two units:

Unit-3 This unit covers the general introduction of coenzymes and cofactors. The prosthetic group, coenzymes involved in different metabolic pathways is discussed briefly. The classification of coenzymes, role of isozymes, synzyme, pyridoxal phosphate and B12 coenzymes also discuss briefly.

Unit-4 The metabolisms of carbohydrate are discussed in this unit. The process of glycolysis, fermentation and TCA cycle discuss briefly. The electron transport chain, oxidative phosphorylation, gluconeogenesis, glycogenesis, energetic and regulation of metabolic cycles also mentioned in this unit. The metabolism of disaccharides, hormonal regulation of carbohydrate metabolism also contain in this unit.

UNIT-3

COENZYMES AND COFACTORS

Structure

3.1. Introduction

Coenzyme is any of a diverse group of small organic, non-protein, freely diffusing molecules that are loosely associated with and essential for the activity of enzymes, serving as carrier molecules that transfer chemical groups. Coenzymes are sometimes referred to as cosubstrates. These molecules are substrates for enzymes and do not form a permanent part of the enzymes. Well-known coenzymes include adenosine triphosphate (ATP), which transfers phosphate groups; nicotinamide adenine dinucleotide (NADH, NADPH), which transfers hydrogen and electrons; coenzyme A, which transfers acetyl groups; and S-adenosylmethionine, which transfers methyl groups. Coenzymes are

heat-stable, dialyzable non-protein organic molecules and the prosthetic groups of enzymes. Coenzymes typically act as group transfer agents, carrying electrons and chemical groups such as acyl groups, methyl groups, etc., depending on the coenzyme.

Objectives

- To determine the coenzymes and its role in human being
- To understand the nature of coenzymes and their function
- To know the role coenzymes involved in different metabolic pathways
- Understand the nature of Pyridoxal phosphate in metabolism

3.2. Coenzyme overview

A coenzyme is an organic non-protein compound that binds with an enzyme to catalyze a reaction. Coenzymes are often broadly called cofactors, but they are chemically different. A coenzyme cannot function alone, but can be reused several times when paired with an enzyme. Coenzymes participate in enzyme-mediated catalysis in stoichiometry (mole-for-mole) amounts, are modified during the reaction, and may require another enzyme-catalyzed reaction to restore them to their original state their examples include nicotinamide adenine dinucleotide (NAD), which accepts hydrogen (and gives it up in another reaction), and ATP, which gives up phosphate groups while transferring chemical energy (and reacquires phosphate in another reaction). Most of the B vitamins (***see*** vitamin B complex) are coenzymes and are essential in facilitating the transfer of atoms or groups of atoms between molecules in the formation of carbohydrates, fats, and proteins.

3.3. Functions of Coenzymes

An enzyme without a coenzyme is called an ***apoenzyme***. Without coenzymes or cofactors, enzymes cannot catalyze reactions effectively. In fact, the enzyme may not function at all. If reactions cannot occur at the normal catalyzed rate, then an organism will have difficulty in sustaining life.

When an enzyme gains a coenzyme, it then becomes a ***holoenzyme***, or active enzyme. Active enzymes change substrates into the products an organism needs to carry out essential functions, whether chemical or physiological. Coenzymes, like enzymes, can be reused and recycled without changing reaction rate or effectiveness. They attach to a portion of the active site on an enzyme, which enables the catalyzed reaction to occur. When an enzyme is denatured by extreme temperature or pH, the coenzyme can no longer attach to the active site.

Energy Production

One primary function of coenzymes is to help in the production of energy. Specifically, the coenzyme ATP is a major player in moving energy within the cell. ATP's structure has three phosphate groups and when the last one is cleaved off during a process known as hydrolysis, energy is released. ATP is constantly recycled, picking up more phosphate groups that are then broken off once again, replenishing cellular energy.

Transferring Groups

Coenzymes also aid in transferring certain groups of atoms from one molecule to another for example, hydrogen transfer, the movement of hydrogen atoms from one part of a cell or organelle to another, essential to many processes, including the reproduction of ATP molecules. The coenzyme NADH in particular is important in this procedure. When a process called oxidative phosphorylation begins in a cell, the coenzyme NADH transports four hydrogen atoms from one part of the mitochondria to the next, starting the process of refreshing a cell's ATP supplies.

Redox Reactions

Another primary function of coenzymes is to aid in the loss or gain of electrons in redox reactions. During oxidation, a molecule or atom loses electrons. Reduction occurs when a molecule or atom gains electrons. Oxidative phosphorylation is also a good example of redox as well as an illustration of how coenzymes work in tandem. For NADH to be able to transport the hydrogen atoms, the coenzyme donates two electrons to coenzyme Q. NADH then becomes NAD+, entering an oxidized state because it has lost electrons.

Antioxidants

Because many coenzymes are able to capture electrons, they often function as antioxidants. Unbound electrons, also known as free radicals, can harm cells, damaging DNA and even leading to cell death. Antioxidants are able to bind free radicals, preventing such damage from happening. Certain coenzymes, such as CoQ10, are even used as medical interventions. After a cardiac event like a heart attack or heart failure, CoQ10 can be used to limit free radical damage while the tissue of the heart is healing.

3.4. Examples of Coenzymes

Most organisms cannot produce coenzymes naturally in large enough quantities to be effective. Instead, they are introduced in an organism in two ways:

3.4.1. Vitamins

Many coenzymes, though not all, are vitamins or derived from vitamins. If vitamin intake is too low, then an organism will not have the

coenzymes needed to catalyze reactions. Water-soluble vitamins, which include all B complex vitamins and vitamin C, lead to the production of coenzymes. Two of the most important and widespread vitamin-derived coenzymes are nicotinamide adenine dinucleotide (NAD) and coenzyme A.

NAD is derived from vitamin B3 and functions as one of the most important coenzymes in a cell when turned into its two alternate forms. When NAD loses an electron, the low energy coenzyme called NAD+ is formed. When NAD gains an electron, a high-energy coenzyme called NADH is formed.

NAD+ primarily transfers electrons needed for redox reactions, especially those involved in parts of the citric acid cycle (TAC). TAC results in other coenzymes, such as ATP. If an organism has a NAD^+ deficiency, then mitochondria become less functional and provide less energy for cell functions.

When NAD^+ gains electrons through a redox reaction, NADH is formed. NADH, often called coenzyme 1, has numerous functions. In fact, it is considered as the number one coenzyme in the human body because it is necessary for so many different reactions. This coenzyme primarily carries electrons for reactions and produces energy from food, for example, the electron transport chain can only begin with the delivery of electrons from NADH. A lack of NADH causes energy deficits in cells, resulting in widespread fatigue. Additionally, this coenzyme is recognized as the most powerful biological antioxidant for protecting cells against harmful or damaging substances.

Coenzyme A, also known as acetyl-CoA, naturally derives from vitamin B5. This coenzyme has several different functions. First, it is responsible for initiating fatty acid production within cells. Fatty acids form the phospholipid bilayer that comprises the cell membrane, a feature necessary for life. Coenzyme A also initiates the citric acid cycle, resulting in the production of ATP.

3.4.2. Non-Vitamins

Non-vitamin coenzymes typically aid in chemical transfer for enzymes. They ensure physiological functions, like blood clotting and metabolism, occurring in an organism. These coenzymes can be produced from nucleotides such as adenosine, uracil, guanine, or inosine.

Adenosine triphosphate (ATP) is an example of an essential non-vitamin coenzyme. In fact, it is the most widely distributed coenzyme in the human body. It transports substances and supplies energy needed for necessary chemical reactions and muscle contraction. To do this, ATP carries both the phosphate and energy to various locations within a cell. When the phosphate is removed, the energy is also released. This process is the result of the electron transport chain. Without the coenzyme ATP, there would be little energy available at the cellular level and normal life functions could not occur.

Here is an example of the electron transport chain. The vitamin-derived coenzyme NADH begins the process by delivering electrons. ATP is the final resulting product:

3.4.3. Prosthetic group

A prosthetic group is a tightly bound, specific non-polypeptide unit required for the biological function of some proteins. The prosthetic group may be organic (such as a vitamin, sugar, or lipid) or inorganic (such as a metal ion), but is not composed of amino acids. Prosthetic groups are bound tightly to proteins and may even be attached through a covalent bond, as opposed to cosubstrates, which are loosely bound. In enzymes, prosthetic groups are often involved in the active site, playing an important role in the functions of enzymes.

Vitamins are another common prosthetic group. This is one of the reasons why vitamins are required in the human diet. Inorganic prosthetic groups, however, are usually transition metal ions such as iron. The Heme group in hemoglobin is a prosthetic group located in the porphyrin, which is a tetramer of cyclic carbon groups. It contains an organic component called a protoporphyrin made up of four pyrrole rings and an iron atom in the ferrous state (Fe^{2+}). The red color of blood and muscles is attributed to the Heme groups. The difference between a prosthetic group and a cofactor depends on how tightly or loosely bound to the enzyme they are. If tightly connected, the cofactor is referred to as a prosthetic group.

Functions of a Prosthetic Group

Just as prosthetic limbs can help people do a variety of works like walk, run, chop onions or pick up their shoes, prosthetic groups have many functions. They mainly assist proteins, though they are not limited to just helping proteins. Prosthetic groups can act as scaffolding or a tie to help proteins fold in to a 3-D structure (their **conformation**).

They also can help proteins bind other cellular components or act as carriers of electrons or molecules (protons (H^+) and oxygen) to assist a cell in moving electrons or molecules from one place to another. By attaching to a specific group of proteins called enzymes, prosthetic groups can make enzymes active (turn them on) or increase their activity. Prosthetic groups that attach to enzymes are often called **cofactors** or **coenzymes** because they help the enzyme to function. An enzyme with a prosthetic group is a **holoenzyme**, while any protein with a prosthetic group is generally referred to as a **holoprotein**.

Thiamine diphosphate (ThDP) is a prosthetic group in enzymes. In human metabolism, ThDP is the water soluble vitamin B1. Its molecular function is to catalyse the formation and cleavage of bonds between heavy atoms, including C-S, C-N, C-O, but also the chemically challenging C-C bonds. An important step in its mechanism is a carbanion intermediate.

Flavin adenine dinucleotide

Flavin adenine dinucleotide (FAD) is a prosthetic group in enzymes. In human metabolism, FAD is partly biosynthesised from the water soluble vitamin B2 (riboflavin). The cofactor's molecular function comprises both one and two electron transfer reactions and radical reactions. FAD is usually non-covalently bound to the apoprotein, but can also be bound covalently to the protein at one or two positions. Flails can build stable semiquinone radicals under anaerobic conditions. Vitamin B2 is the universal precursor of all flavo-cofactors.

Flavin mononucleotide

The Flavin mononucleotide (FMN) is mostly a prosthetic group but may also act as a coenzyme. An example mechanism, in which it acts as a coenzyme is the mechanism of alkanal monooxygenase (MACiE entry M0132). Like FAD, it is biosynthesised from vitamin B2. FMN is a substructure of FAD. FAD consists of the two nucleotides FMN and adenosine monophosphate, which are covalently bound through a phosphodiester bond. The flavin portion is the catalytically active portion of the cofactor. Hence, the FMN mechanisms are, as expected, similar to the FAD mechanisms.

Nicotinamide-adenine dinucleotide

Nicotinamide-adenine dinucleotide (NAD(P)) is a collective term for two molecules performing the same function. NAD is chemically a regular dinucleotide, whereas NADP has an additional phosphate group bound at the O2' atom of the ribose ring of the adenosine monophosphate portion (see table 3.1). NAD (P) is mostly acting as a coenzyme, but has also been observed as a prosthetic group. Its main catalytic function is to assist in hydride transfers. Therefore the cofactor exists in two states: NAD(P)H/H+ with, and NAD(P)+ without the hydride that the cofactor shuttles.

Phosphopantetheine

Phosphopantetheine (PNS) is a prosthetic group in enzymes. In human metabolism, its biosynthesis depends on the dietary intake of vitamin B5 (pantothenic acid). The function of this prosthetic group is to bind the initial substrate and then to function as an agile arm to bring the growing substrate from one active site of an multi-domain complex to the next e.g. in the fatty-acid synthase, the non-ribosomal peptide synthases and the polyketide synthases.

Coenzyme A

Coenzyme A (CoA) is a coenzyme and, like the phosphopantetheine prosthetic group, its biosynthesis depends on vitamin B5. CoA may be seen as the coenzyme version of phosphopantetheine. CoA is composed of a phosphopantothenic acid portion and an adenosine mononucleotide, connected by a phosphodiester bond. CoA's function in the cell is to solubilise hydrophobic acyl groups, i.e. to transport them

from one enzyme to the next. Its function as a cofactor is the transfer of this acyl group. The cofactor is involved in many enzyme reactions including the citric acid cycle and also in secondary metabolism (e.g. flavonoid biosynthesis.

Pyridoxal 5'-phosphate (PLP) acts as both a coenzyme and a prosthetic group. It has been described as arguably the most versatile organic cofactor. In human metabolism, the cofactor's biosynthesis depends on vitamin B6 (pyridoxal). In amino acid metabolism, PLP catalyses transamination, decarboxylation, racemisation, aldol condensation, α, β-elimination and β, γ-elimination of amino acids, and amine oxidation. PLP covalently binds the substrate and functions as an electrophilic catalyst.

Glutathione

Glutathione (GSH) is a coenzyme that is biosynthesised in human metabolism. The molecule is a tripeptide comprising glutamate, cysteine and glycine, where glutamate and cysteine are linked through a gamma-glutamyl amide linkage. GSH functions as an antioxidant in the cytoplasm in general and in neurons in particular. Its chemical function is to act as an electron donor.

Biotin

The prosthetic group biotin (BTN) is a vitamin for humans (vitamin B7). The molecule is always covalently bound to its partner enzyme. Its main function as a cofactor is to transfer CO_2 from one active site of an enzyme complex to another or to act as a CO_2 carrier between bicarbonate and the acceptor substrate. It may also transfer C2-units. BTN is essential for fatty acid synthesis.

Tetrahydrofolic acid

In human metabolism, the coenzyme tetrahydrofolic acid (THF) is the essential nutrient vitamin B9. The molecule is composed of a pterin ring, a p-aminobenzoic acid and a polyglutamate chain.

Adenosylcobalamin

Adenosylcobalamin (B12) is a prosthetic group, whose source for humans is dietary (vitamin B12, cobalamin). The cofactor assists enzymes with the catalysis of molecular rearrangements, methylations and dehalogenations.

Ascorbic acid

The coenzyme ascorbic acid (ASC) is also known as the water-soluble vitamin C. The molecule is important in the cell's antioxidant defence, particularly via the ascorbate/glutathione cycle. Ascorbic acid is the major water-soluble antioxidant found in body fluids of mammals. It counteracts free or lipid-peroxidation-initiating radicals and it regenerates other antioxidants like Vitamin E.

Menaquinone

The coenzyme menaquinone (MQ) is taken up into human cells and known as vitamin K2. Vitamin K is a collective term for lipid-like naphthoquinone derivatives synthesised only in eubacteria and plants and functioning as electron carriers in energy transduction pathways and as free radical scavengers maintaining intracellular redox homeostasis."

Ubiquinone

The coenzyme ubiquinone is also known as coenzyme Q (CoQ). The molecule is fully biosynthesised in human cells. CoQ has two major functions in the cell: to transport electrons in the respiratory chain and to act as a lipid soluble antioxidant. This molecule is one out of only four fat-soluble antioxidants in nature, the others being carotinoids, oestrogens and tocopherols (vitamin E).

3.5. Coenzymes involved in different metabolic pathways

3.5.1. Coenzymes

In addition to binding their substrates, the active sites of many enzymes bind other small molecules that participate in catalysis. Prosthetic groups are small molecules bound to proteins in which they play critical functional roles, for example, the oxygen carried by myoglobin and hemoglobin is bound to heme, a prosthetic group of these proteins. In many cases metal ions (such as zinc or iron) are bound to enzymes and play central role in the catalytic process. In addition, various low-molecular-weight organic molecules participate in specific types of enzymatic reactions. These molecules are called coenzymes because they work together with enzymes to enhance reaction rates. In contrast to substrates, coenzymes are not irreversibly altered by the reactions in which they are involved. Rather, they are recycled and can participate in multiple enzymatic reactions.

Coenzymes serve as carriers of several types of chemical groups. A prominent example of a coenzyme is nicotinamide adenine dinucleotide (NAD+), which functions as a carrier of electrons in oxidation-reduction reactions. NAD^+ can accept a hydrogen ion (H+) and two electrons (e^-) from one substrate, forming NADH. NADH can then donate these electrons to a second substrate, re-forming NAD^+. Thus, NAD^+ transfers electrons from the first substrate (which becomes oxidized) to the second (which becomes reduced).

Several other coenzymes also act as electron carriers, and still others are involved in the transfer of a variety of additional chemical groups (e.g., carboxyl groups and acyl groups shown in Table. 3.1. The same coenzymes function together with a variety of different enzymes to catalyze the transfer of specific chemical groups between a wide range of substrates. Many coenzymes are closely related to vitamins, which

contribute part or all of the structure of the coenzyme. Vitamins are not required by bacteria such as *E. coli* but are necessary components of the diets of human and other higher animals, which have lost the ability to synthesize these compounds.

Table 3.1: Examples of Coenzymes and Vitamins

Coenzyme	Related vitamin	Chemical reaction
NAD+, NADP+	Niacin	Oxidation-reduction
FAD	Riboflavin (B2)	Oxidation-reduction
Thiamine pyrophosphate	Thiamine (B1)	Aldehyde group transfer
Coenzyme A	Pantothenate	Acyl group transfer
Tetrahydrofolate	Folate	Transfer of one-carbon groups
Biotin	Biotin	Carboxylation
Pyridoxal phosphate	Pyridoxal (B6)	Transamination

Classification of Coenzymes

I. Based on chemical characteristics:

a) *Containing an aromatic hetero ring.*

1. ATP and its relatives.
2. NAD, NADP.
3. FMN, TPP, B_6-PO_4.

b) *Containing a non-aromatic hetero ring. Biotin, lipoic acid.*

c) *No hetero ring:* Sugar phosphate, coenzyme Q.

II. Based on functional characteristics:

A. Group transferring coenzymes:

1. ATP and its relatives.
2. Sugar phosphates.
3. Thiamine pyrophosphate (TPP).
4. CoA.
5. Pyridoxal phosphate (B_6-$P0_4$).
6. Biotin.

III. Hydrogen transferring coenzymes:

1. Nicotinamide adenine dinucleotide (NAD) and Nicotinamide adenine dinucleotide phosphate (NADP).
2. Flavin adenine dinucleotide (FAD) and flavin mono-nucleotide (FMN).
3. Coenzyme Q.

III. Based on nutritional characteristics:

(a) Containing B vitamins:

1. CoA.
2. TPP.
3. NAD & NADP.
4. B_6-PO_4.
5. FMN, FAD.
6. Folic acid coenzyme.
7. B_{12} coenzyme.
8. Biotin.

Function:

1. Their function is usually to accept atoms or groups from a substrate and to transfer them to other molecules.

2. They are less specific than the enzymes and the same coenzyme can act as such in a number of different reactions.

3. The coenzymes are also attached to the protein at a different but adjacent site so as to be in a position to accept the atoms or groups which are removed from the substrate.

4. NAD and NADP coenzymes function as hydrogen acceptors in dehydrogenation reactions.

5. The chief function of CoA is to carry acryl groups and they are used in the oxidative decarboxylation of pyruvic acid and synthesis of fatty acids and acetylation.

6. The function of TPP (co-carboxylase) is to carry 'active aldehyde [(R. CH (OH)] group.

7. The chief function of pyridoxal phosphate (B_6-PO_4) is involved in transamination reactions.

8. The chief function of tetrahydrofolic acid is expressed as a carrier of format and it is used in the synthesis of purines and pyrimidines.

Serum Enzymes	Concentration increased in	Concentration decreased in
1. Lipase	Acute pancreatitis, pancreatic carcinoma.	Liver disease, vitamin A deficiency, diabetes mellitus.
2. Amylase	High intestinal obstruction, acute pancreatitis, parotitis, diabetes.	Liver disease.
3. Trypsin	Acute disease of the pancreas.	–
4. Cholinesterase	Nephrotic syndrome.	Liver disease, malnutrition, acute infectious diseases.
5. Alkaline phosphatase	Rickets, Paget's disease, hyperparathyroidism, obstructive jaundice, metastatic carcinoma, osteoblastic sarcoma, kidney diseases. Alkaline phosphatase, isozymes can distinguish liver lesions from bone lesions in cases of metastatic carcinoma.	–
6. Acid phosphatase	Metastatic prostatic carcinoma.	–
7. SGOT	Myocardial infarction. Slightly elevated in acute liver diseases.	–
8. SGPT	Acute liver diseases. Slightly elevated in cardiac necrosis.	–
9. Lactate dehydrogenase	Myocardial infarction, acute hepatitis, renal tubular necrosis. In certain solid tumors LD_1 and LD_2 are increased.	–
10. Isocitrate dehydrogenase	Liver diseases.	–
11. Creatine phosphokinase (CK or CPK)	Muscular dystrophy, myocardial infarction.	–
12. Glucose-6-phosphate dehydrogenase	Myocardial infarction.	Congenital deficiency causes hemolytic anemia.
13. Ceruloplasmin (Ferroxidase activity)	Cirrhosis, bacterial infection, pregnancy.	Wilson's disease. (hepatolenticular degeneration)
14. Aldolase	Muscular dystrophy, diabetes mellitus, acute infective hepatitis, leukemia, infectious diseases, hemolytic anemia etc.	–
15. Oxytocinase	Normal pregnancy from fourth month. Increasing level shows good fetal prognosis.	Intrauterine fetal death.

3.5.2. Coenzyme A (CoA):

Chemistry

1. It is composed of adenosine triphosphate (ATP), pantothenic acid and β-mercaptoethalamine. So it is the coenzyme form of pantothenic acid, a vitamin.
2. It is a group transferring coenzyme.
3. The reaction group is the sulfhydryl (-SH) group.
4. The acryl group is accepted by the sulfhydril group to form acetyl coenzyme A ($CH_3CoS.CoA$). The acryl coenzyme derivatives are the high energy compounds.

Function

1. Carrier of acryl groups, e.g., acetyl, sccinyl, benzoyl.

2. Some of the pantothenic acids are bound to protein in the form of "acyl carrier protein". This can be regarded as coenzyme A in which the adenine dinucleotide is replaced by protein. '"Acyl carrier protein" chiefly functions in the synthetic processes, e.g., of fatty acids and cholesterol.

3. It is required in the oxidative decarboxylation of pyruvic acid and α-ketoglutaric acid, in the breakdown and synthesis of fatty acids and in the synthesis of cholesterol which is involved in bile acids, bile salts, steroid hormones and vitamin D formation.

4. It is used for conjugation with amino compounds to form N-acetyl compounds and in the formation of hippuric acid (Benzoyl glycine).

5. It is involved in the formation of ketone bodies.

6. It is used in the formation of acetyl choline.

7. It is finally oxidized to CO_2, H_2O and ATP via citric acid cycle.

3.5.3. Isozymes

A great many different enzymes have been found to exist in more than one molecular form as isozymes or isoenzymes. Indeed it is apparent that the phenomenon of isozyme formation is very common throughout in a large number of species, including human. Isoenzymes (isozymes) are multiple forms of the enzyme that have the same catalytic activity. Although they have the same catalytic activity, they are physically distinct and differ in electrophoretic mobility and liability to inhibitors. Iso means the same and isoenzyme means the same enzyme.

Isozymes are usually the result of gene duplication, but can also arise from polyploidisation or nucleic acid hybridization. Over evolutionary time, if the function of the new variant remains identical to the original, then it is likely that one or the other will be lost as mutations accumulate, resulting in a pseudogene. However, if the mutations do not immediately prevent the enzyme from functioning, but instead modify either its function, or its pattern of expression, then the two variants may both be favoured by natural selection and become specialized to different functions. For example, they may be expressed at different stages of development or in different tissues.

Allozymes may result from point mutations or from insertion-deletion (indel) events that affect the coding sequence of the gene. As with any other new mutation, there are three things that may happen to a new allozyme:

- It is most likely that the new allele will be non-functional in which case it will probably result in low fitness and be removed from the population by natural selection.
- Alternatively, if the amino acid residue that is changed is in a relatively unimportant part of the enzyme (e.g., a long way from

the active site), then the mutation may be selectively neutral and subject to genetic drift.

- ❖ In rare cases, the mutation may result in an enzyme that is more efficient, or one that can catalyze a slightly different chemical reaction, in which case the mutation may cause an increase in fitness, and be favored by natural selection.

Example of isoenzymes

Many enzymes are present in isoenzymes form as

1. Lactate dehydrogenase
2. Creatine kinase
3. Acid phosphatase
4. Alkaline phosphatase

Isozymes (and allozymes) are variants of the same enzyme. Unless they are identical in their biochemical properties, for example their substrates and enzyme kinetics, they may be distinguished by a biochemical assay. However, such differences are usually subtle, particularly between *allozymes* which are often neutral variants. This subtlety is to be expected, because two enzymes that differ significantly in their function are unlikely to have been identified as *isozymes*.

While isozymes may be almost identical in function, they may differ in other ways. In particular, amino acid substitutions that change the electric charge of the enzyme are simple to identify by gel electrophoresis, and this forms the basis for the use of isozymes as molecular markers. To identify isozymes, a crude protein extract is made by grinding animal or plant tissue with an extraction buffer, and the components of extract are separated according to their charge by gel electrophoresis. Historically, this has usually been done using gels made from potato starch, but acrylamide gels provide better resolution.

All the proteins from the tissue are present in the gel, so that individual enzymes must be identified using an assay that links their function to a staining reaction. For example, detection can be based on the localised precipitation of soluble indicator dyes such as tetrazolium salts which become insoluble when they are reduced by cofactors such as NAD or NADP, which generated in zones of enzyme activity. This assay method requires that the enzymes are still functional after separation (native gel electrophoresis), and provides the greatest challenge to using isozymes as a laboratory technique.

Isoenzymes differ in kinetics (they have different K_M and V_{max} values).

Application

Isozymes in general can be used to meet the metabolic needs of different tissues and developmental stages. An example of an enzyme with

different isozymes is lactate dehydrogenase (LDH). This enzyme is used to catalyze the synthesis of glucose in its anaerobic metabolism. The isozymes of this enzyme are divided into two forms, the H isozymes and the M isozymes. The H isozymes are expressed more in the heart, whereas the M isozymes are expressed more frequently in the skeletal muscle. Both isozymes have two polypeptide chains, and each isozymes share 75% of the amino acid sequence for the chains. Both isozymes metabolize glucose, but the difference is that the H isozymes have a higher affinity for their substrates than that of M isozyme. Another difference is that the H isozymes functions better in aerobic environments such as the heart, whereas the M isozymes functions better in anaerobic environments such as the muscle, where strenuous activity may deplete the oxygen supplies. For example, when a rat heart is developing, the amount of H and M isozymes in the rat heart tissue begins to change because of the switch from an anaerobic environment to an aerobic one. The H isozyme is shown as squares and the M isozyme is shown as circles. The negative numbers are the days before birth and the positive numbers are the days after birth. The amount of M isozymes decreases dramatically as the rat grows into the adult stage.

Isozymes may also be utilized to diagnose tissue damage such as damaged heart muscle cells during a heart attack or myocardial infarction. When heart muscle cells are damaged, they release the cellular material such as the H isozyme. When taking blood samples, if the H isozymes appear in increased levels, then there is a possibility that the heart cells are damaged.

Another example of an isozyme is hexokinase. The substrate is usually glucose and the product is glucose-6-phosphate. The six-carbon sugar is also known as a hexose. Glucokinase is one isozyme of hexokinase. A kinase is an enzyme which catalyzes the transfer of a phosphoryl group from NTP to NMP. ATP is often used in these types of reactions. Glucokinase is important in metabolism, and regulating carbohydrates in the human body. The difference of the Glucokinase enzyme is that it has a much lower affinity for glucose. Most Glucokinase activity is found in the liver. This is where it catalyzes the conversion of glucose to triglycerides.

Abzyme

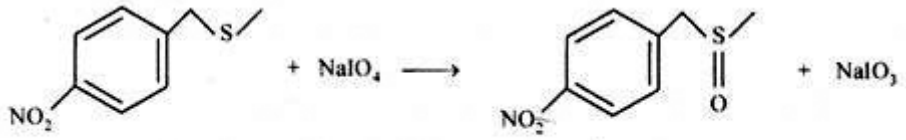
An abzyme is an antibody that expresses catalytic activity. A single molecule of an antibody-enzyme, or abzyme, is capable of catalyzing the destruction of thousands of target molecules. The efficiency of abzyme technology could permit treatments with smaller doses of medicines at lower costs than are possible today. An abzyme is used to lower the activation energy of a reaction allowing for the transition state to be possible and the product to be formed. Abzymes are typically artificially made by having the immune system make antibodies that bind to a molecule that resembles the transition state (Transition State Analogue) of the catalytic process that the researchers want to emulate. Therefore by creating this antibody, now becoming a catalytic antibody

allows for this antibody to act as an abzyme reducing the activation energy of the reaction and allowing for the transition state to occur. Abzymes however do occur naturally in the human body.

The production of the abzymes is based on the following two principles:

1. Enzymes act by binding the transition state of a reactant better than the ground state.
2. Antibodies which bind to specific small molecules can be produced by coupling this small molecule to a protein carrier and using this protein for immunizing experimental animals.

If this small molecule is a transition state analog, then the antibodies that are produced to bind to this molecule will function as enzyme towards the substrate of this reaction. Abzymes are selected from monoclonal antibodies produced by immunizing mice with haptens that mimic the transition state of enzyme catalyzed reactions. For example, 28B4 abzyme catalyzes periodate oxidation of p-nitrotoulene methyl sulphide to sulphoxide, where electrons from the sulfur atom are transferred to the more electronegative oxygen atom.

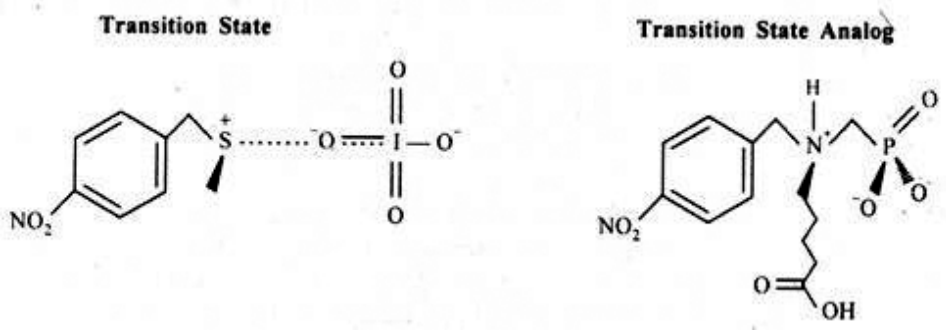


p-nitrotoulene methyl sulphide **p-nitrotoulene methyl sulphoxide**

The rate of this reaction is promoted by enzyme catalysts that stabilize the transition state of this reaction, thereby decreasing the activation energy and allowing for more rapid conversion of substrate to product.

In this case, the transition state is believed to involve a transient positive charge on the sulfur atom and a double negative charge on the periodate ion as shown below:

Transition State **Transition State Analog**



In order to produce abzymes complementary in structure to this transition state, mice were immunized with an aminophosphonic acid hapten as depicted above.

Obviously, its structure mirrors the structure and electrostatic properties of the sulphoxide transition state. Of the hapten-binding monoclonal antibodies produced with this hapten, many were found to catalyze sulphide oxidation but with a wide range of binding affinities and catalytic efficiencies. Abzyme 28B4 binds hapten with high affinity and exhibits a corresponding high degree of catalytic efficiency.

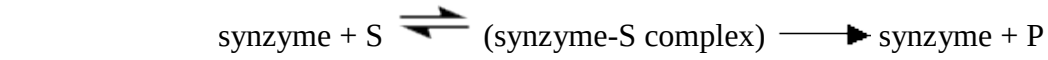
Abzymes have proven to be very important tools in Biotechnology and Medical Pathology in combating dreaded human diseases like AIDS, Cancer etc. One of the promising directions in this field consists of the production of abzymes catalyzing rapid cleavage of hazardous compounds, including toxins and drugs such as cocaine.

3.6. Synzyme

Artificial enzymes

A number of possibilities now exist for the construction of artificial enzymes. These are generally synthetic polymers or oligomers with enzyme-like activities, often called synzymes. They must possess two structural entities, a substrate-binding site and a catalytically effective site. It has been found that producing the facility for substrate binding is relatively straightforward but catalytic sites are somewhat more difficult. Both sites may be designed separately but it appears that, if the synzyme has a binding site for the reaction transition state, this often achieves both functions.

For a one-substrate reaction the reaction sequence is given as under:

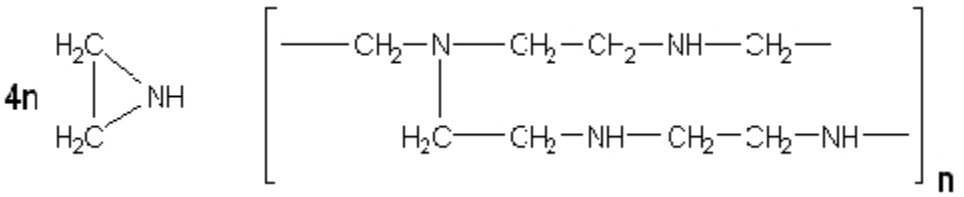


Some synzymes are simply derivatised proteins, although covalently immobilised enzymes are not considered here. An example is the derivatisation of myoglobin, the oxygen carrier in muscle, by attaching $(Ru(NH_3)_5)^{3+}$ to three surface histidine residues. This converts it from an oxygen carrier to an oxidase, oxidising ascorbic acid while reducing molecular oxygen. The synzyme is almost as effective as natural ascorbate oxidases.

It is impossible to design protein synzymes from scratch with any probability of success, as their conformations are not presently predictable from their primary structure. Such proteins will also show the drawbacks of natural enzymes, being sensitive to denaturation, oxidation and hydrolysis. For example, polylysine binds anionic dyes but only 10% as strongly as the natural binding protein, serum albumin, in spite of the many charges and apolar side-chains. Polyglutamic acid, however, shows synzymic properties. It acts as an esterase in much the same fashion as the acid proteases, showing a bell-shaped pH-activity relationship, with optimum activity at about pH 5.3, and Michaelis-Menten kinetics with a K_m of 2 mm and V_{max} of 10^{-4} to 10^{-5} s^{-1} for the hydrolysis of 4-nitrophenyl

acetate. Cyclodextrins (Schardinger dextrins) are naturally occurring toroidal molecules consisting of six, seven, eight, nine or ten a-1, 4-linked D-glucose units joined head-to-tail in a ring (a-, b-, g-, d- and e-cyclodextrins, respectively, they may be synthesised from starch by the cyclomaltodextrin glucanotransferase (EC 2.4.1.19) from *Bacillus macerans*). They differ in the diameter of their cavities (about 0.5-1 nm) but all are about 0.7 nm deep. These form hydrophobic pockets due to the glycosidic oxygen atoms and inwards-facing C-H groups. All the C-6 hydroxyl groups project to one end and all the C-2 and C-3 hydroxyl groups to the other. Their overall characteristic is hydrophilic, being water soluble, but the presence of their hydrophobic pocket enables them to bind hydrophobic molecules of the appropriate size. Synzymic cyclodextrins are usually derivatised in order to introduce catalytically relevant groups. Many such derivatives have been examined. For example, a C-6 hydroxyl group of b-cyclodextrin was covalently derivatised by an activated pyridoxal coenzyme. The resulting synzyme not only acted as a transaminase but also showed stereoselectivity for the L-amino acids. It was not as active as natural transaminases, however.

Polyethyleneimine is formed by polymerising ethyleneimine to give a highly branched hydrophilic three-dimensional matrix. About 25% of the resultant amines are primary, 50% secondary and 25% tertiary.



Ethyleneimine polyethyleneimine

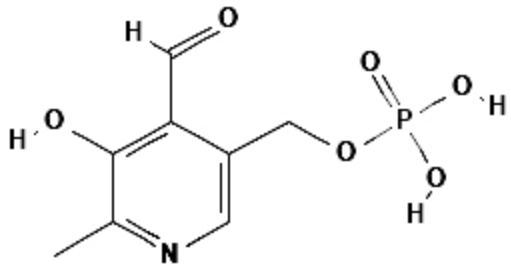
The primary amines may be alkylated to form a number of derivatives. If 40% of them are alkylated with 1-iodododecane to give hydrophobic binding sites and the remainder alkylated with 4(5)-chloromethylimidazole to give general acid-base catalytic sites, the resultant synzyme has 27% of the activity of a-chymotrypsin against 4-nitrophenyl esters. As might be expected from its apparently random structure, it has very low esterase specificity. Other synzymes may be created in a similar manner.

Antibodies to transition state analogues of the required reaction may act as synzymes. For example, phosphonate esters of general formula $(R\text{-}PO_2\text{-}OR')^-$ are stable analogues of the transition state occurring in carboxylic ester hydrolysis. Monoclonal antibodies raised to immunising protein conjugates covalently attached to these phosphonate esters act as esterases. The specificities of these catalytic antibodies (also called abzymes) depends on the structure of the side-chains (i.e., R and R' in $(R\text{-}PO_2\text{-}OR')^-$) of the antigens. The K_m values may be quite low, often in the micromolar region, whereas the V_{max} values are low (below 1 s^{-1}), although still 1000-fold higher than hydrolysis by background hydroxyl ions. A similar strategy may be used to produce synzymes by molecular

'imprinting' of polymers, using the presence of transition state analogues to shape polymerising resins or inactive non-enzymic protein during heat denaturation.

3.7. Pyridoxal phosphate

Pyridoxal 5'-Phosphate is the active form of Vitamin B6 that drives approximately 168 vital enzyme processes in human body. Among these vital roles P5P facilitates a process known as deamination which is responsible for converting essential amino acids into non-essential ones. P5P also helps shuttle certain nutrients such as magnesium across cellular membranes which helps increase absorption rates. Pyridoxal 5'-Phosphate is a compound vital to human health, yet often found in short supply. Learning more about what it is, where one can get it, and how the bodies use it may help you maintain a better overall nutritional balance.



Bioavailable Vitamin B6

The most common active ingredient found in most Vitamin B6 supplements is Pyridoxine Hydrochloride. This compound can be used by the body to help drive the many enzymatic processes attributed to P5P, but it must first be converted. This conversion takes place in the liver almost immediately and releases the several pyridoxine metabolites into the blood stream, with Pyridoxal 5-phosphate being the most preferred. Very limited research on this conversion process suggests that 30 minutes after oral ingestion of pyridoxine, as much as 40% has been diffused into the bloodstream as various metabolites. This illustrates how efficient this conversion process is already in human body. There has been very limited amount of research done on the differences of efficacy of pyridoxal 5`-phosphate vs pyridoxine compounds such as pyridoxine hydrochloride. Some limited data suggests that those suffering from impaired liver function may benefit from taking the active form. One study found that plasma levels of pyridoxal 5`-phosphate were increase nearly 60% after administration of the active P5P form compared to the administration of pyridoxine hydrochloride.

Benefits of Pyridoxal 5`-Phosphate

When considering the benefits of pyridoxal 5`-phosphate, it is best to broadly consider the benefits of all the B6 vitamins. While many health professionals believe P5P to be of superior form, there still isn't a lot of

research to support that notion. P5P is simply a more efficient form of pyridoxine, which avoids the hepatic conversion processes needed to create the active enzyme form. This would likely be beneficial for those with impaired liver function. Ultimately, the case for P5P comes down to it being a bio-optimized form of Vitamin B6 that the body can put to work immediately. Below you are mentioned some of the many benefits of pyridoxal 5`-phosphate.

Reduces Inflammation

Pyridoxial 5`-phosphate levels are inversely correlated to inflammatory markers such as Tumor Necrosis Factor Alpha (TNF-α), Interluken 6 (IL-6) and Interferon Gamma (INF-γ). These markers are commonly used to accurately gauge the level of inflammation in response to certain medical conditions such as IBS, Cancer, Cardiovascular Disease, and Arthritis. Any treatment that effectively lowers these markers likely exhibits an anti-inflammatory action on the cellular level. One study found that levels of these compounds were significantly (*p=0.01*) lower in Rheumatoid Arthritis patients after being given 100mg/day of Vitamin B6. These levels were measured to be inversely correlated to measured serum levels of P5P.

Boosts Immune System

One Taiwanese study considered 50 patients in the Taichung Veteran's General Hospital. These patients were separated into three groups; those receiving no B6 supplementation, those receiving 50mg/day B6, and those receiving 100mg/day B6. After 14 days of treatment, researchers measured a wide array of serum Vitamin B vitamer markers and immune function markers. Both the 50mg/day group and 100mg/day group showed significant increase in serum P5P and immune cell counts such as total lymphocyte count (TLC) and CD3, CD4, CD8, and CD19 cells. This research suggests that Vitamin B6 is an effective treatment for generalized immune improvement.

Helps Support Positive Mood and Relaxation

Some of the more significant researches on Vitamin B and its role in anxiety and hypertension has been conducted with a focus on premenstrual women. One such study investigated the impact of supplementation with magnesium and Vitamin B6 on several mood factors of 126 women. Among those given a magnesium/vitamin B6 supplement, there was a significant decrease in symptoms of depression, water retention, and anxiety. Another such study found supporting results, and noted that the reduction in PMS symptoms were slightly more significant in the group given a magnesium/Vitamin b6 compound than a magnesium compound alone.

Lower Risk of Lung Cancer

Cancer is generally regarded as being a disease seeded by inflammation. Vitamin B6's demonstrated ability to reduce pro-

inflammatory markers makes it a likely candidate in nutritional for the prevention of cancer. One study found that among 1770 participants having donated blood, of which 899 later developed lung cancer, there was a significantly inverse correlation between serum levels of Vitamin B6 and those with cancer. Simply put, lower levels of Vitamin B6 equate to higher chances of lung cancer the lower the level, the higher the chance.

Pyrxidoxal 5`-Phosphate Deficiency

According to the SIB ExPASy Bioformatics Resources Panel (ExPASy) Pyridoxial 5`-Phosphate is a cofactor of 168 unique enzymes. To get some perspective; Magnesium is recorded by ExPASy as being a cofactor for 208 unique enzymes. This paints a picture of P5P being an essential nutrient used by human body for a wide range of processes. With such broad-reaching involvement, it is almost redundant to describe symptoms of a Pyridoxial 5`-phosphate deficiency since they could like be a great many similar to magnesium. Among clinically-studied incidences of patients suffering low levels of P5P, there seems to be a strong relationship between seizures, anemia, irritability, auditory sensitivity, depression, skin issues, and weakened overall immune function. A Vitamin B deficiency is generally regarded as being rare in isolated incidence to other vitamin b deficiencies. This type of deficiency is likely to be present for years without being symptomatic.

B_{12} COENZYMES

B12 Functions

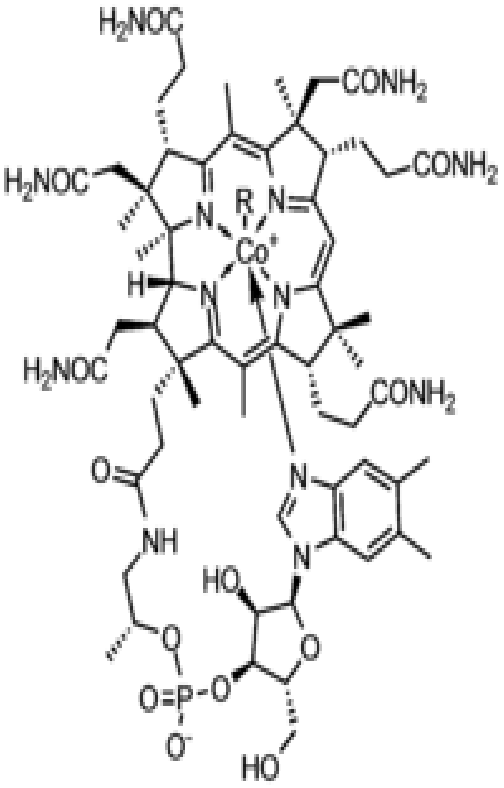
In the cells of mammals, there are two different co-enzyme forms of vitamin B12:

- Methylcobalamin
- Used by the enzyme ***methionine synthase*** to turn homocysteine (HCY) into methionine. Methionine is further converted to the important methyl donor, S-adenosylmethionine (SAM, aka SAMe)
- 5′-deoxyadenosylcobalamin
- Used by the enzyme ***methylmalonyl-CoA mutase*** to convert methylmalonyl-
- CoA to succinyl-CoA
- Used by the enzyme ***leucine aminomutase*** to convert B-leucine into L-leucine and vice-versa

Homocysteine Clearance

Methionine is an essential amino acid provided by the diet. Some methionine is turned into homocysteine. Homocysteine appears to be a nerve and vessel toxin, promoting cardiovascular disease (CVD) at elevated levels. HCY is thought to cause CVD by way of oxidative and vessel wall damage. The body normally turns HCY into other molecules, one of which is back into methionine. If this pathway is blocked, HCY

levels increase. Methylcobalamin (B12) is needed by methionine synthase to convert HCY into methionine. Thus, if someone is B12 deficient, HCY levels will increase.



R = 5'-deoxyadenosyl, Me, OH, CN

Anemia, DNA, and Folate

Traditionally, B12 deficiency, normally resulting from the inablity to absorb B12, was diagnosed by finding abnormally large red blood cells. This sort of anemia has two names:

- Macrocytic anemia occurs when the average volume of the red blood cells, known as the Mean Corpuscular Volume (MCV), is larger than normal

- Megaloblastic anemia occurs when abnormally large red blood cells are observed under a microscope

The vitamin folate (aka folic acid) affects the anemia symptoms of B12 deficiency. Folate is needed to turn uracil into thymidine, an essential building block of DNA. DNA is needed for new red blood cell production and division. B12 is involved in this process because in creating methylcobalamin (used in the HCY to methionine reaction), B12 produces a form of folate needed to make DNA. If there is no B12 available, this form of folate can become depleted (known as the methyl-folate trap) and DNA production slows. See the pathway below.

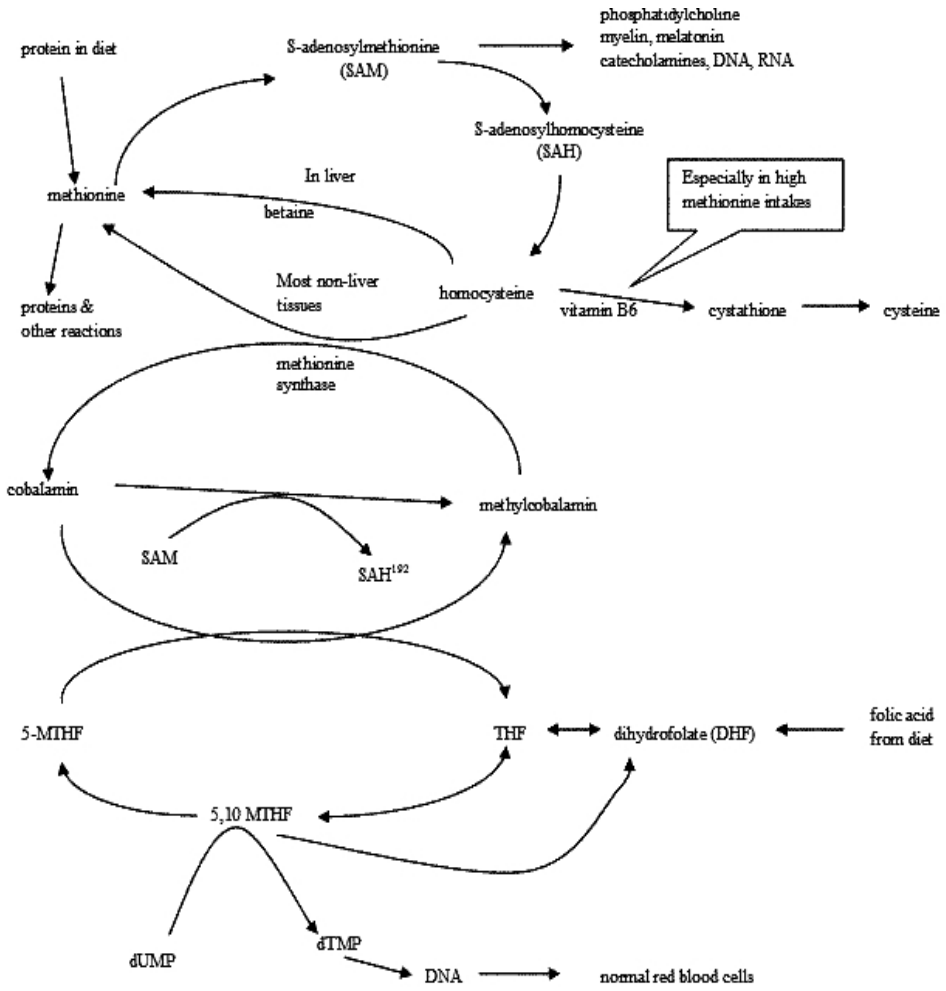


Figure 1: Vitamin B12 Cycle

Only RNA is needed to produce the hemoglobin found in the red blood cells. Unlike DNA, RNA does not require thymidine. Therefore, if there is not adequate folate, the new red blood cells (which start out as large cells called reticulocytes) divide slowly, as they are dependent on DNA for division. At the same time, their hemoglobin is only dependent on RNA and it is produced at a normal rate. This causes formation of large red blood cells known as macrocytes. If enough of these macrocytes accumulate, the result is macrocytic anemia.

If there is large amount of incoming folate from the diet, the body does not need to rely on the regeneration of folate from the B12 cycle. Instead, it can use the extra dietary folate to produce DNA, thus preventing macrocytic anemia. This is why high intake of folate is said to "mask" a B12 deficiency.

To add insult to injury, an iron deficiency (which results in small red blood cells from inadequate hemoglobin synthesis) can counteract the macrocytic cells, making it appear as though the blood cells are normal in the face of multiple nutritional deficiencies.

Intestinal cells are also rapidly dying and being replaced using DNA. A B12 deficiency can make itself worse because it can prevent the production of the intestinal cells needed to absorb B12.

Lack of Anemia Does not Mean that B12 Status is Healthy

Traditionally, the existence of macrocytic anemia was relied on to indicate a B12 deficiency. However, neurological disorders due to B12 deficiency commonly occur in the absence of a macrocytic anemia.

Characteristic features of patients with B12 deficiency but without macrocytic anemia included, sensory loss, inability to move muscles smoothly (ataxia), dementia, and psychiatric disorders.

Table 3.2: B12 levels in neurological patients without macrocytic anemia (pg/ml)

Number of Patients	Serum B12
2	> 200
16	100-200
22	< 100

Methylmalonic Acid (MMA)

The second coenzyme form of B12, adenosylcobalamin, takes part in the conversion of methylmalonyl-CoA to succinyl-CoA. When B12 is not available, methylmalonyl-CoA level increases. Methylmalonyl-CoA is then converted to methylmalonic acid (MMA) which then accumulates in the blood and urine. Since B12 is the only coenzyme required in this pathway, MMA levels are the best indicators of a B12 deficiency.

High MMA levels can also (but rarely) be caused by genetic defects, kidney failure, low blood volume, gut bacteria changes, pregnancy, and thyroid disease

Studies indicate:

- Slightly increasing serum MMA levels from 0.29 to 60 µmol/l may not increase one's risk for neurological problems.
- People with MMA levels above 0.27 µmol/l may have elevated homocysteine which can benefit from B12 therapy.
- People with serum MMA levels above 0.60 µmol/l may have neurological problems that can benefit from B12 therapy.

Because there are few common vegetable sources of the vitamin, vegans must use a supplement or fortified foods for B12 intake or risk serious health consequences. Otherwise, most omnivorous people in developed countries obtain enough vitamin B12 from consuming

animal products, including meat, milk, eggs, and fish. Staple foods, especially those that form part of a vegan diet, are often fortified by having the vitamin added to them. Vitamin B12 supplements are available in single agent or multivitamin tablets; and pharmaceutical preparations may be given by intramuscular injection.

The most common cause of vitamin B12 deficiency in developed countries is impaired absorption due to a loss of gastric intrinsic factor, which must be bound to food-source B12 in order for absorption to occur. Another group affected are those on long term antacid therapy, using proton-pump inhibitors, H2 blockers or other antacids. This condition may be characterised by limb neuropathy or a blood disorder called pernicious anemia, a type of megaloblastic anemia. Folate levels in the individual may affect the course of pathological changes and symptomatology. Deficiency is more likely after age 60, and increases in incidence with advancing age. Dietary deficiency is very rare in developed countries due to access to dietary meat and fortified foods, but children in some regions of developing countries are at particular risk due to increased requirements during growth coupled with lack of access to dietary B12; adults in these regions are also at risk. Other causes of vitamin B12 deficiency are much less frequent.

Dietary recommendations

The U.S. Institute of Medicine (renamed National Academy of Medicine in 2015) updated estimated average requirement (EAR) and recommended dietary allowance (RDA) for vitamin B12 in 1998. The EAR for vitamin B12 for women and men ages 14 and up is 2.0 μg/day; the RDA is 2.4 μg/day. RDA is higher than EAR so as to identify amounts that will cover people with higher than average requirements. RDA for pregnancy equals 2.6 μg/day. RDA for lactation equals 2.8 μg/day. For infants up to 12 months the adequate intake (AI) is 0.4–0.5 μg/day. (AIs are established when there is insufficient information to determine EARs and RDAs.) For children ages 1–13 years the RDA increases with age from 0.9 to 1.8 μg/day. Because 10 to 30 percent of older people may be unable to effectively absorb vitamin B12 naturally occurring in foods, it is advisable for those older than 50 years to meet their RDA mainly by consuming foods fortified with vitamin B12 or a supplement containing vitamin B12. As for safety, tolerable upper intake levels (known as ULs) are set for vitamins and minerals when evidence is sufficient. In the case of vitamin B12 there is no UL, as there is no human data for adverse effects from high doses. Collectively the EARs, RDAs, AIs and ULs are referred to as dietary reference intakes (DRIs).

Sources

Most omnivorous people in developed countries obtain enough vitamin B_{12} from consuming animal products including meat, fish, eggs, and milk.[2][1] Vegan sources in the common food supply are rare.

Bacteria and archaea

B12 is produced in nature by certain bacteria, and archaea. It is synthesized by some bacteria in the gut flora in humans and other animals, but humans cannot absorb this as it is made in the colon, downstream from the small intestine, where the absorption of most nutrients occurs. Ruminants, such as cows and sheep, absorb B_{12} produced by bacteria in their gut. For gut bacteria of ruminants to produce B_{12} the animal must consume sufficient amounts of cobalt. These grazing animals acquire the bacteria that produce vitamin B_{12}.

Animals

Animals store vitamin B_{12} in the liver and muscles and some pass the vitamin into their eggs and milk; meat, liver, eggs and milk are therefore sources of the vitamin for other animals as well as humans. For humans, the bioavailability from eggs is less than 9%, compared to 40% to 60% from fish, fowl and meat. Insects are a source of B_{12} for animals (including other insects and humans).

Food sources with a high concentration of vitamin B_{12}—50 to 99 µg B_{12} per 100 grams of food include clams; liver and other organ meats from lamb, veal, beef, and turkey, mackerel, and crab meat.

Plants and algae

Natural plant and algae sources of vitamin B_{12} include the world's smallest flowering plant Wolffia globosa (Asian watermeal), fermented plant foods (such as tempeh), and seaweeds (such as nori and laver). Many other types of algae are rich in B_{12}, with some species, such as *Porphyra yezoensis*, containing as much cobalamin as liver. Methylcobalamin has been identified in *Chlorella vulgaris*. Since only bacteria and some archea possess the genes and enzymes necessary to synthesize vitamin B_{12}, plant sources obtain the vitamin secondarily from symbiosis with various species of bacteria, or in the case of fermented plant foods, from bacterial fermentation.

Fortified foods

The UK Vegan Society, the Vegetarian Resource Group, and the Physicians Committee for Responsible Medicine, among others, recommend that every vegan who is not consuming adequate B_{12} from fortified foods take supplements.

Foods for which B_{12}-fortified versions are widely available include breakfast cereals, soy products, energy bars, and nutritional yeast.

Parenteral administration

Injection and patches are sometimes used if digestive absorption is impaired,[1] but this course of action may not be necessary with high-potency oral supplements (such as 0.5–1 mg or more) because with large quantities of the vitamin taken orally, even the 1% to 5% of the free crystalline

B_{12} that is absorbed along the entire intestine by passive diffusion, may be sufficient to provide necessary amount.

A person with cobalamin C disease (which results in combined methylmalonic aciduria and homocystinuria) may require treatment with intravenous, intramuscular hydroxocobalamin or transdermal B_{12}, because cyanocobalamin is inadequate in the treatment of cobalamin C disease.

Pseudovitamin-B_{12}

Pseudovitamin-B_{12} refers to B_{12}-like analogues that are biologically inactive in humans and yet found to be present alongside B_{12} in humans, many food sources (including animals), and possibly supplements and fortified foods. Most cyanobacteria, including *Spirulina*, and some algae, such as dried Asakusa-nori (*Porphyra tenera*), have been found to contain mostly pseudovitamin-B_{12} instead of biologically active B_{12}. In one common form of pseudo-B_{12} available to *Salmonella enterica* serovar *Typhimurium*, the α-axial ligand is changed from dimethylbenzimidazole to adenine

3.8. Summary

Coenzymes typically act as group transfer agents, carrying electrons and chemical groups such as acyl groups, methyl groups, etc., depending on the coenzyme. Many of the coenzymes are derived from vitamins which are essential for metabolism, growth, and development. A coenzyme cannot function alone, but can be reused several times when paired with an enzyme. Coenzymes participate in enzyme-mediated catalysis in stoichiometry. Thiamine diphosphate (ThDP) is a prosthetic group in enzymes. In human metabolism, ThDP is the water soluble vitamin B1. In human metabolism, FAD is partly biosynthesised from the water soluble vitamin B2 (riboflavin). The Flavin mononucleotide (FMN) is mostly a prosthetic group but may also act as a coenzyme. Phosphopantetheine (PNS) is a prosthetic group in enzymes. In human metabolism, its biosynthesis depends on the dietary intake of vitamin B5 (pantothenic acid). Coenzyme A (CoA) is a coenzyme and, like the phosphopantetheine prosthetic group, its biosynthesis depends on vitamin B5. Glutathione (GSH) is a coenzyme that is biosynthesised in human metabolism. The prosthetic group biotin (BTN) is a vitamin for humans (vitamin B7). The coenzyme ascorbic acid (ASC) is also known as the water-soluble vitamin C. The coenzyme menaquinone (MQ) is taken up into human cells and known as vitamin K2. Coenzymes serve as carriers of several types of chemical groups. A prominent example of a coenzyme is nicotinamide adenine dinucleotide (NAD+). Isozymes in general can be used to meet the metabolic needs of different tissues and developmental stages. An example of an enzyme with different isozymes is lactate dehydrogenase (LDH). Pyridoxal 5'-Phosphate is the active form of Vitamin B6 that drives approximately 168 vital enzyme processes in human body.

3.9. Terminal question

Q.1. What are coenzymes? Define it function in human body.

Answer: ----------

Q.2. What is Prosthetic group? Discuss briefly.

Answer: ----------

Q.3. Write the functions of a prosthetic group.

Answer: ----------

Q.4. How to coenzymes involved in different metabolic pathways.

Answer: ----------

Q.5. What are isozymes? Discuss briefly.

Answer: ----------

Q.6. Write the application isozymes.

Answer: ----------

3.10. Further readings

- Bioenergetics, David Nicholls, Academic Press ISBN: 9780123884251 4th Edition
- Bioenergetics, Alexander Lowen Penguin Books, ISBN13: 9780140194715
- Principles of Biochemistry: Lehninger, Nelson and Cox. Student Edition, CBS 1439 Publishers and Distributors, Delhi.

- Fundamentals of Biochemistry: Dr J L Jain, S. Chand and Company
- Textbook of Biochemistry and Human Biology: Talwar and Srivastava. Eastern Economy Edition, Prentice Hall, India.

UNIT-4

CARBOHYDRATE METABOLISM

Structure

4.1. Introduction

Carbohydrates are one of the four major classes of biomolecules along with proteins, nucleic acids, and lipids. Carbohydrates are compounds that contain at least three carbon atoms, a number of hydroxyl groups and usually an aldehyde or ketone group. They may contain phosphate, amino or sulfate groups. Carbohydrates serve as energy stores, fuels and metabolic intermediates. Ribose and deoxyribose sugars form part of the structural framework of RNA and DNA. Polysaccharides are structural elements in the cell walls of bacteria and plants.

Carbohydrates are also known as saccharides since many of them have relatively small molecular weight have a sweet taste, although this is not true of those with large molecules. They are widely distributed molecules in both plant and animal tissues. They are indispensable for living organisms, serving as skeletal structures in plants and also in insects and crustaceans. They also occur as food reserves in the storage organs of plants and in the liver and muscles of animals. In addition, they are an important source of energy required for the various metabolic activities of the living organisms; the energy being derived as a result of their oxidation. They also serve to lubricate skeletal joints, to provide adhesion between cells and to confer biological specificity on the surface of animal calls.

Glucose is the most important carbohydrate; most dietary carbohydrate is absorbed into the bloodstream as glucose and other sugars are converted into glucose in the liver. Glucose is the major metabolic fuel of mammals (except ruminants) and a universal fuel of the fetus. It is the precursor for synthesis of all the other carbohydrates in the body, including glycogen for storage; ribose and deoxyribose in nucleic acids; and galactose in lactose of milk, in glycolipids, and in combination with protein in glycoproteins and proteoglycans. Diseases associated with carbohydrate metabolism include diabetes mellitus, galactosemia, glycogen storage diseases, and lactose intolerance.

Objectives

- Briefly to learn the function of glycolysis during aerobic respiration and their reactants and products, and state the total number and the net number of ATP produced by substrate-level phosphorylation during glycolysis.
- During aerobic respiration, to learn state what happens to the 2 NADH produced during glycolysis and during aerobic respiration, what happens to the two molecules of pyruvate produced during glycolysis.
- To learn about fermentation and the mechanism for ATP generation during fermentation, the function of glycolysis during fermentation and the reactants and products.
- To compare the maximum yield of ATP from one molecule of glucose for aerobic respiration and for fermentation.
- To enlist the steps of the Krebs (or citric acid) cycle, and to describe the function of the electron transport chain during aerobic respiration, and the chemiosmotic theory of generation of ATP as a result of an electron transport chain.
- To compare where the electron transport chain occurs in prokaryotic cells in eukaryotic cells, and what is meant by proton motive force. State the function of ATP synthases in chemiosmosis.
- To state the final electron acceptor and the end product formed at the end of aerobic respiration, and understand the significance of regulation of carbohydrate metabolism by hormones to maintain the body homeostasis
- To appreciate the regulatory mechanisms that plays an important role in maintaining the blood glucose level and to understand the responses of the body to different energy utilization conditions.

4.2. Glycolysis

Glycolysis is the first step in the breakdown of glucose to extract energy for cellular metabolism. Glycolysis consists of an energy-requiring

phase followed by an energy-releasing phase. Glycolysis, which translates to "splitting sugars", is the process of releasing energy within sugars. In glycolysis, a six-carbon sugar known as glucose is split into two molecules of a three-carbon sugar called pyruvate. This multistep process yields two ATP molecules containing free energy, two pyruvate molecules, two high energy electron-carrying molecules of NADH and two molecules of water.

- **Glycolysis** is the process of breaking down glucose.
- Glycolysis can take place with or without oxygen.
- Glycolysis produces two molecules of **pyruvate**, two molecules of **ATP**, two molecules of **NADH**, and two molecules of **water**.
- Glycolysis takes place in the **cytoplasm**.
- There are 10 enzymes involved in breaking down the sugar. The 10 steps of glycolysis are organized by the order in which specific enzymes act upon the system.

Glycolysis can occur with or without oxygen. In the presence of oxygen, glycolysis is the first stage of cellular respiration. In the absence of oxygen, glycolysis allows cells to make small amounts of ATP through a process of fermentation.

4.2.1. Phases of Glycolysis

Glycolysis is usually split into 2 phases:

- **Energy investment phase** – requires two ATP molecules to produce a very high energy intermediate (reactions 1-3).
- **Energy pay out phase** – The intermediate is broken down into 3 carbon molecules producing four ATP molecules and two NADH molecules (reactions 4-10)

The molecules required to start this process are two ATP molecules, two NAD+ molecules and a glucose molecule. The net products of glycolysis are two NADH molecules, two ATP molecules and two pyruvate molecules**.** Glycolysis takes place in the cytosol of the cell's cytoplasm. A net of two ATP molecules are produced through glycolysis (two are used during the process and four are produced. Learn more about the 10 steps of glycolysis below.

Step 1

The enzyme **hexokinase** phosphorylates a phosphate group to glucose in a cell's cytoplasm. In the process, a phosphate group from ATP is transferred to glucose producing glucose 6-phosphate or G6P. One molecule of ATP is consumed during this phase.

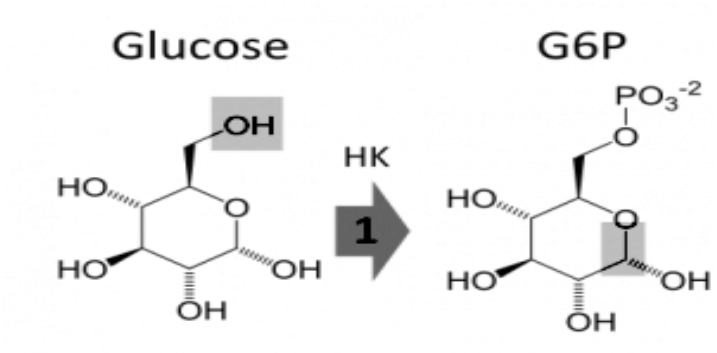


Fig. 4.1

Step 2

The enzyme phosphoglucomutase is omerizes G6P into its isomer fructose 6-phosphate or F6P. Isomers have the same molecular formula but different atomic arrangements.

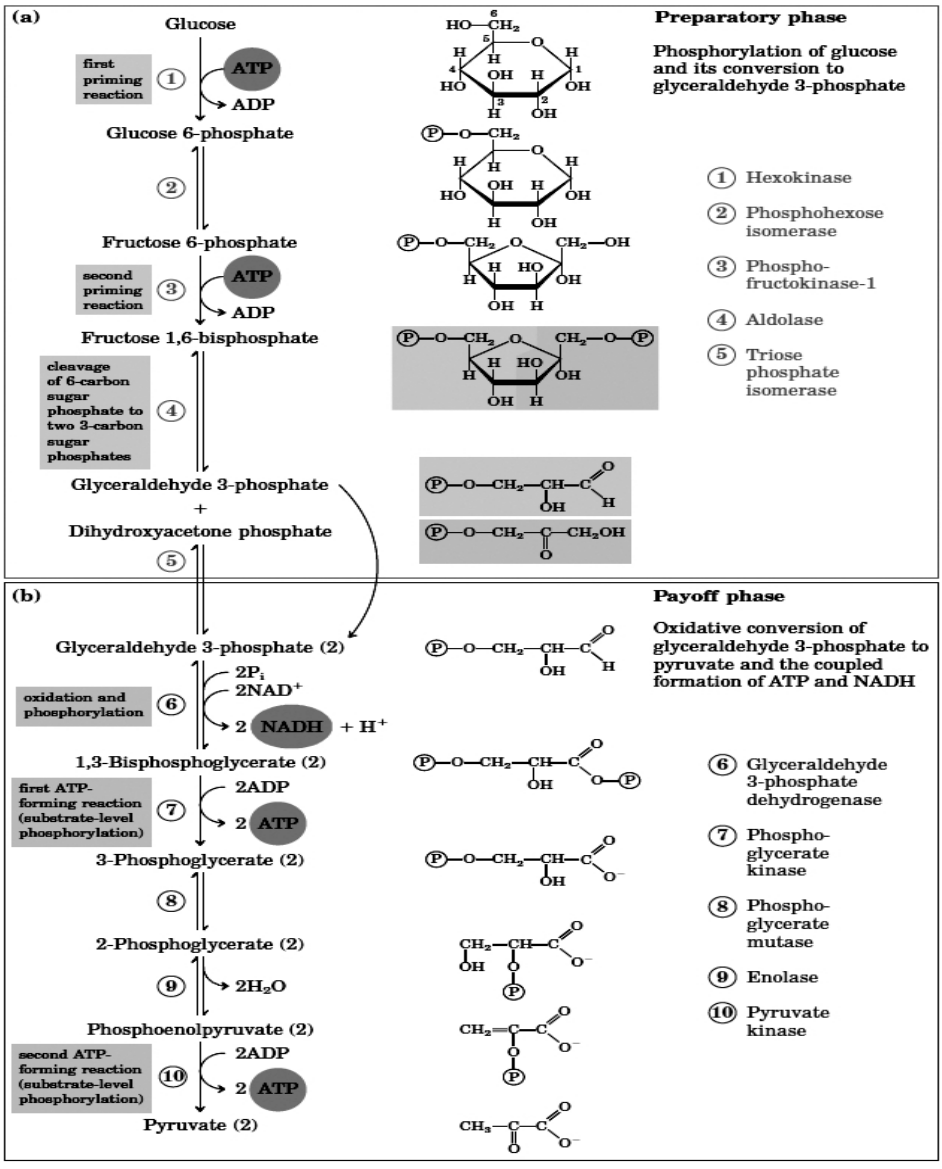


Fig. 4.2: Process of phosphorylation

Step 3

The kinase **phosphofructokinase** uses another ATP molecule to transfer a phosphate group to F6P in order to form fructose 1,6-bisphosphate or FBP. Two ATP molecules have been used so far.

Step 4

The enzyme **aldolase** splits fructose 1,6-bisphosphate into a ketone and an aldehyde molecule. These sugars, dihydroxyacetone phosphate (DHAP) and glyceraldehyde 3-phosphate (GAP), are isomers of each other.

Step 5

The enzyme **triose-phosphate isomerase** rapidly converts DHAP into GAP (these isomers can inter-convert). GAP is the substrate needed for the next step of glycolysis.

Step 6

The enzyme **glyceraldehyde 3-phosphate dehydrogenase** (GAPDH) serves two functions in this reaction. First, it dehydrogenates GAP by transferring one of its hydrogen (H^+) molecules to the oxidizing agent nicotinamide adenine dinucleotide (NAD^+) to form $NADH + H^+$.

Next, GAPDH adds a phosphate from the cytosol to the oxidized GAP to form 1,3-bisphosphoglycerate (BPG). Both molecules of GAP produced in the previous step undergo this process of dehydrogenation and phosphorylation.

Step 7

The enzyme **phosphoglycerokinase** transfers a phosphate from BPG to a molecule of ADP to form ATP. This happens to each molecule of BPG. This reaction yields two 3-phosphoglycerate (3 PGA) molecules and two ATP molecules.

Step 8

The enzyme **phosphoglyceromutase** relocates the P of the two 3 PGA molecules from the third to the second carbon to form two 2-phosphoglycerate (2 PGA) molecules.

Step 9

The enzyme **enolase** removes a molecule of water from 2-phosphoglycerate to form phosphoenolpyruvate (PEP). This happens for each molecule of 2 PGA from step eight.

Step 10

The enzyme **pyruvate kinase** transfers a P from PEP to ADP to form pyruvate and ATP. This happens for each molecule of PEP. This reaction yields two molecules of pyruvate and two ATP molecules.

4.2.2. Fates of Pyruvate

Pyruvate is a versatile molecule which feeds into numerous pathways. After glycolysis, it can be converted to **acetyl CoA** for complete oxidation by entering the citric acid cycle and oxidative phosphorylation. It can also be converted to lactate which enters the Cori cycle in absence of mitochondria or oxygen or used in ketogenesis where acetyl CoA can instead be used to make ketones when glucose levels are low. Finally, acetyl CoA can be used to make fatty acids in states of high energy.

4.3. Fermentation

Fermentation is a chemical process by which molecules such as glucose are broken down anaerobically. More broadly, fermentation is the foaming that occurs during the manufacture of wine and beer, a process at least 10,000 years old. The frothing results from the evolution of carbon dioxide gas, though this was not recognized until the 17th century. French chemist and microbiologist Louis Pasteur in the 19th century used the term ***fermentation*** in a narrow sense to be described the changes brought about by yeasts and other microorganisms growing in the absence of air (anaerobically); he also recognized that ethyl alcohol and carbon dioxide are not the only products of fermentation.

4.3.1. Examples of Products Formed by Fermentation

Most people are aware of food and beverages that are fermentation products, but may not realize many important industrial products resulting from fermentation.

- Beer
- Wine
- Yogurt
- Cheese
- Certain sour foods containing lactic acid, including sauerkraut, kimchi, and pepperoni
- Bread leavening by yeast
- Sewage treatment
- Some industrial alcohol production, such as biofuels
- Hydrogen gas

4.3.2. Anaerobic Breakdown of Molecules

In 1920s it was discovered that, in the absence of air, extracts of muscle catalyze the formation of lactate from glucose and that the same intermediate compounds formed in the fermentation of grain are produced

by muscle. An important generalization thus emerged those fermentation reactions are not peculiar to the action of yeast but also occur in many other instances of glucose utilization.

Glycolysis, the breakdown of sugar, was originally defined during 1930 as the metabolism of sugar into lactate. It can be further defined as that form of fermentation, characteristic of cells in general, in which the six-carbon sugar glucose is broken down into two molecules of the three-carbon organic acid, pyruvic acid (the nonionized form of pyruvate), coupled with the transfer of chemical energy to the synthesis of adenosine triphosphate (ATP). The pyruvate may then be oxidized in the presence of oxygen, through the tricarboxylic acid cycle, or in the absence of oxygen, be reduced to lactic acid, alcohol, or other products (Fig. 4.3). The sequence from glucose to pyruvate is often called the Embden–Meyerhof pathway, named after two German biochemists who in the late 1920s and ’30s postulated and analyzed experimentally the critical steps in that series of reactions.

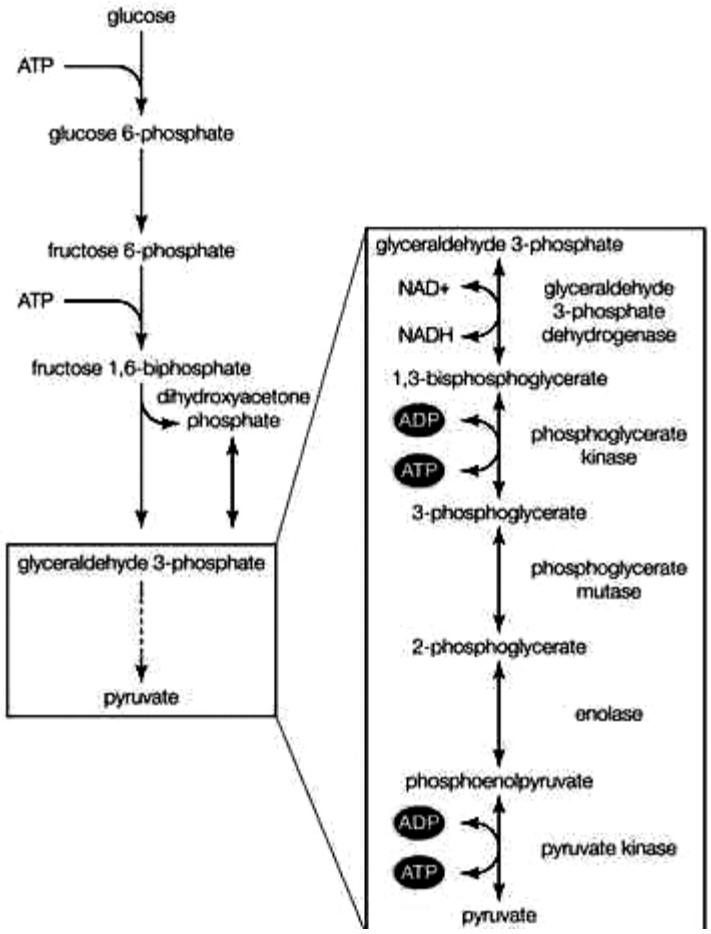


Fig. 4.3: Glycolysis

The term ***fermentation*** now denotes the enzyme-catalyzed, energy-yielding pathway in cells involving the anaerobic breakdown of molecules such as glucose. In most cells the enzymes occur in the soluble portion of the cytoplasm. The reactions leading to the formation of ATP and

pyruvate thus are common to sugar transformation in muscle, yeasts, some bacteria and plants.

4.3.3. Lactic acid fermentation

In lactic acid fermentation, NADH transfers its electrons directly to pyruvate, generating lactate as a byproduct. Lactate, which is just the deprotonated form of lactic acid, gives the process its name. The bacteria that make yogurt carry out lactic acid fermentation.

Muscle cells also carry out lactic acid fermentation, though only when they have too little oxygen for aerobic respiration to continue for instance, when a person has been exercising very hard. It was once thought that the accumulation of lactate in muscles was responsible for soreness caused by exercise, but recent researches suggest that this is probably not the case.

Lactic acid produced in muscle cells is transported through the bloodstream to the liver, where converted back to pyruvate and processed normally in the remaining reactions of cellular respiration.

4.3.4. Alcohol fermentation

Another familiar fermentation process is **alcohol fermentation**, in which NADH donates its electrons to a derivative of pyruvate, producing ethanol.

Ethanol Fermentation

Yeast and certain bacteria perform ethanol fermentation where pyruvate (from glucose metabolism) is broken into ethanol and carbon dioxide. The net chemical equation for the production of ethanol from glucose is:

$C_6H_{12}O_6$ (glucose) $\rightarrow$ 2 C_2H_5OH (ethanol) + 2 CO_2 (carbon dioxide)

Ethanol fermentation has used the production of beer, wine, and bread. It is worth noting that fermentation in the presence of high level of pectin results in the production of small amounts of methanol which is toxic when consumed.

Alcohol fermentation by yeast produces the ethanol found in alcoholic drinks like beer and wine. However, alcohol is toxic to yeast in large quantities (just as it is to humans), which puts an upper limit on the percentage alcohol in these drinks. Ethanol tolerance of yeast ranges from about 555 percent to 212121 percent, depending on the yeast strain and environmental conditions.

Hydrogen and Methane Gas Production

The process of fermentation may yield hydrogen gas and methane gas. Methanogenic archaea undergo a disproportionation reaction in which one electron is transferred from a carbonyl of a carboxylic acid group to a methyl group of acetic acid to yield methane and carbon dioxide.

Many types of fermentation yield hydrogen. The product may be used by the organism to regenerate NAD^+ from NADH. Hydrogen gas may be used as a substrate by sulfate reducers and methanogens. Humans experience hydrogen gas production from intestinal bacteria, producing flatus.

Industrial Fermentation

Industrial fermentation processes begin with suitable microorganisms and specified conditions, such as careful adjustment of nutrient concentration. The products are of many types: alcohol, glycerol, and carbon dioxide from yeast fermentation of various sugars; butyl alcohol, acetone, lactic acid, monosodium glutamate, and acetic acid from various bacteria; and citric acid, gluconic acid, and small amounts of antibiotics, vitamin B_{12}, and riboflavin (vitamin B_2) from mold fermentation. Ethyl alcohol produced via the fermentation of starch or sugar is an important source of liquid biofuel.

4.4. The TCA Cycle

The TCA cycle is a central pathway into which many metabolites are involved. It consists of a number of reactions which generate NADH and FADH2 which can in turn be used by the oxidative phosphorylation pathway to generate ATP. The TCA cycle occurs in the **matrix** of the mitochondria.

5.4.1. Steps of the citric acid cycle

A preview of the molecules produced during the citric acid cycle already has been given. But how, exactly, are those molecules made. The cycle show the following steps for production of NADH, 2FADH2, ATP, GTP release of carbon dioxide molecules (Fig. 4.4).

- **Step 1.** In the first step of the citric acid cycle, acetyl CoA joins with a four-carbon molecule, oxaloacetate, releasing the CoA group and forming a six-carbon molecule called citrate.
- **Step 2.** In the second step, citrate is converted into its isomer, isocitrate. This is actually a two-step process, involving first the removal and then the addition of a water molecule, that is why the

citric acid cycle is sometimes described as having nine steps—rather than the eight listed here^33cubed.

- **Step 3.** In the third step, isocitrate is oxidized releasing a molecule of carbon dioxide, leaving behind a five-carbon molecule α-ketoglutarate. During this step, NAD^+, is reduced to NADH. The enzyme catalyzing this steps **isocitrate dehydrogenase** which is important in regulating the speed of the citric acid cycle.

- **Step 4.** The fourth step is similar to the third. In this case, it is α-ketoglutarate that is oxidized, reducing NAD+, NADH and releasing a molecule of carbon dioxide in the process. The remaining four-carbon molecules pick up Coenzyme A, forming the unstable compound succinyl-CoA. The enzyme catalyzing these steps **is α-ketoglutarate dehydrogenase** which is important in the regulation of the citric acid cycle.

- **Step5.** In step five, the CoA of succinyl CoA is replaced by a phosphate group, which is then transferred to ADP to make ATP. In some cells, GDP guanosine diphosphate is used instead of ADP, forming GTP (guanosine triphosphate) as a product. The four-carbon molecule produced in this step is called succinate.

- **Step 6.** In step six, succinate is oxidized, forming another four-carbon molecule called fumarate. In this reaction, two hydrogen atoms with their electrons are transferred to FAD, producing $FADH_2$. The enzyme that carries out this step is embedded in the inner membrane of the mitochondrion, so $FADH_2$ can transfer its electrons directly into the electron transport chain.

- **Step 7.** In step seven, water is added to the four-carbon molecule fumarate, converting it into another four-carbon molecule called malate.

- **Step 8.** In the last step of the citric acid cycle, oxaloacetate the starting four-carbon compound is regenerated by oxidation of malate. Another molecule of NAD^+ is reduced to NADH in the process.

- It is important to be aware that whilst the primary role of the TCA cycle is production of NADH and $FADH_2$. It also produces molecules that supply various biosynthetic processes, which can enter or exit the cycle at various points depending on the demand on different reactions for example, alpha-ketoglutarate can leave the cycle to be converted into amino acids or **succinate** can be converted to them.

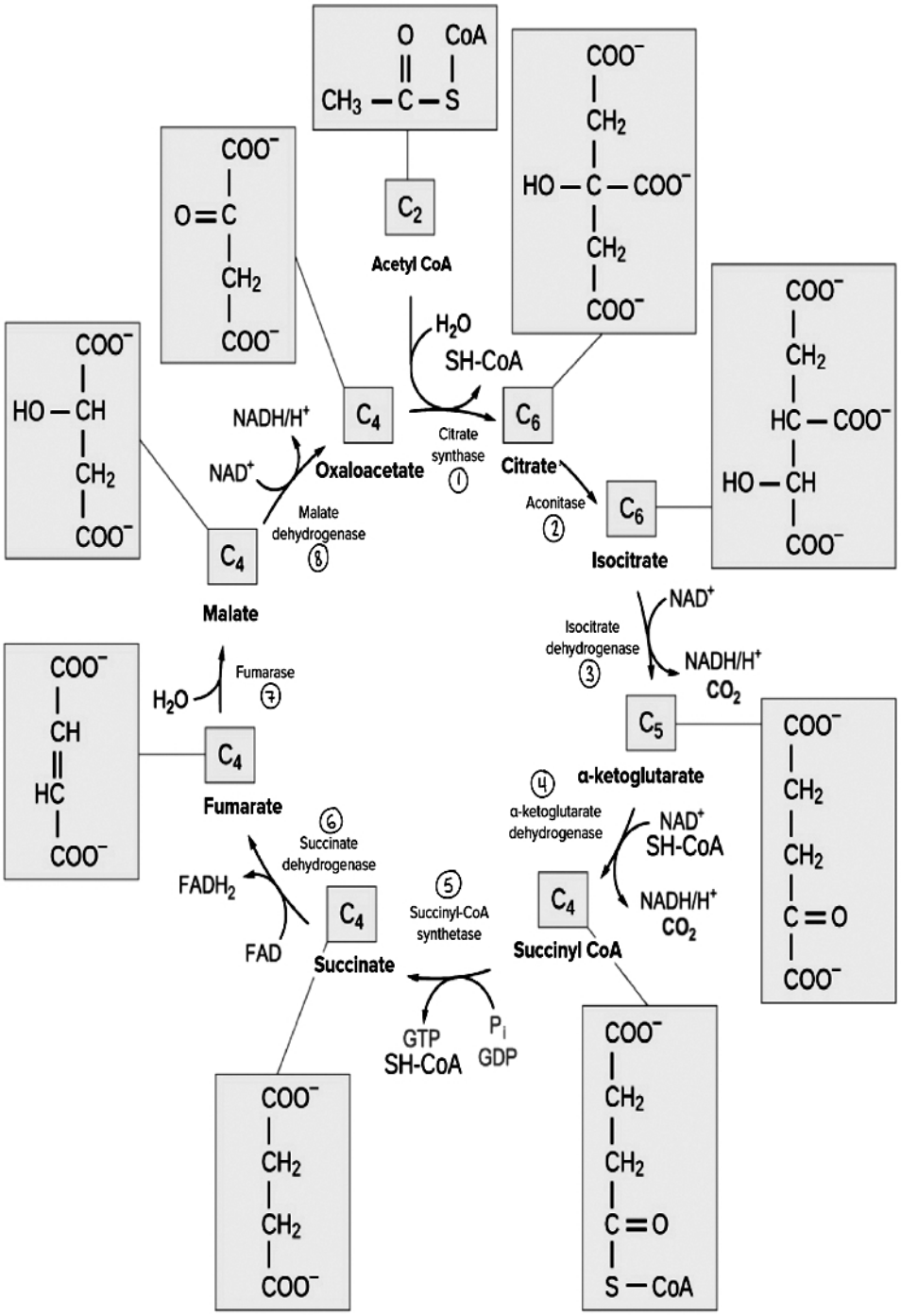


Fig. 4.4: Steps of the citric acid cycle

Each cycle produces two molecules of carbon dioxide, three molecules of NADH, three hydrogen ions, one molecule of $FADH_2$ and one molecule of GTP. As such each molecule of glucose produces double of this (2 carbon dioxide, 6 NADH, 6 hydrogen ions, 2 $FADH_2$ and 2 GTP).

4.4.2. Regulation of the TCA Cycle

The TCA Cycle is regulated in a variety of ways. As already mentioned isocitrate dehydrogenase regulates step 3 of the TCA cycle,

making it the rate limiting step of the cycle. In addition to this energy availability also regulates the cycle so low energy signals, such as ADP activate the cycle and high levels of NADH (a product of the cycle) inhibit it.

4.5. Electron Transport Chain

Earlier have been explained two pathways in cellular respiration glycolysis and the citric acid cycle that generate ATP. However, most of the ATP generated during the aerobic catabolism of glucose is not generated directly from these pathways. Rather, it is derived from a process that begins with moving electrons through a series of electron transporters that undergo redox reactions: **the electron transport chain (**Fig. 4.5**)**. This causes hydrogen ions to accumulate within the matrix space. Therefore, a concentration gradient is formed in which hydrogen ions diffuse out of the matrix space by passing through ATP synthase. The current of hydrogen ions power the catalytic action of ATP synthase, which phosphorylates ADP, producing ATP.

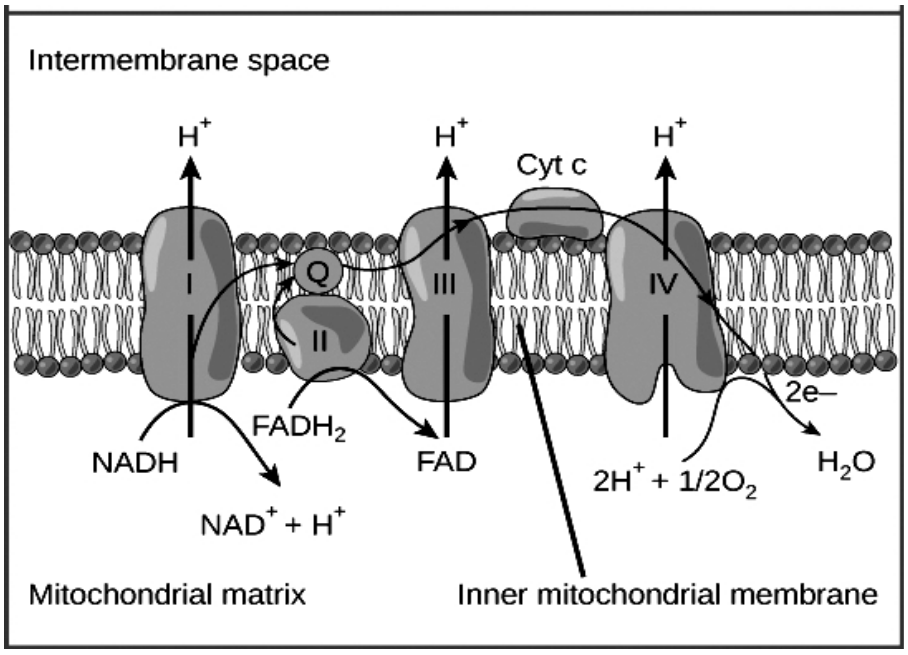


Fig.4.5: Electron transport chain

Fig. 4.5. The electron transport chain is a series of electron transporters embedded in the inner mitochondrial membrane that shuttles electrons from NADH and $FADH_2$ to molecular oxygen. In the process, protons are pumped from the mitochondrial matrix to the intermembrane space and oxygen is reduced to form water.

The electron transport chain (Fig. 4.5) is the last component of aerobic respiration and is the only part of glucose metabolism that uses atmospheric oxygen. Oxygen continuously diffuses into plants but; in animals, it enters the body through the respiratory system. Electron transport is a series of redox reactions that resemble a relay race or bucket

brigade in that electrons are passed rapidly from one component to the next, to the endpoint of the chain where the electrons reduce molecular oxygen, producing water. There are four complexes composed of proteins, labeled I through IV in Fig. 4.5 and the aggregation of these four complexes, together with associated mobile, accessory electron carriers, is called the electron transport chain. The electron transport chain is present in multiple copies in the inner mitochondrial membrane of eukaryotes and the plasma membrane of prokaryotes. Note, however, that the electron transport chain of prokaryotes may not require oxygen as some live in anaerobic conditions. The common feature of all electron transport chains is the presence of a proton pump to create a proton gradient across a membrane.

4.5.1. Complex I

To start, two electrons are carried to the first complex aboard NADH. This complex, labeled I, is composed of flavin mononucleotide (FMN) and an iron-sulfur (Fe-S)-containing protein. FMN, which is derived from vitamin B_2, also called riboflavin, is one of several prosthetic groups or co-factors in the electron transport chain. A **prosthetic group** is a non-protein molecule required for the activity of a protein. Prosthetic groups are organic or inorganic, non-peptide molecules bound to a protein that facilitates its function; prosthetic groups include co-enzymes, which are the prosthetic groups of enzymes. The enzyme in complex I is NADH dehydrogenase and is a very large protein, containing 45 amino acid chains. Complex I can pump four hydrogen ions across the membrane from the matrix into the intermembrane space, and it is in this way that the hydrogen ion gradient is established and maintained between the two compartments separated by the inner mitochondrial membrane.

4.5.2. Q and Complex II

Complex II directly receives $FADH_2$, which does not pass through complex I. The compound connecting the first and second complexes to the third is **ubiquinone** (Q). The Q molecule is lipid soluble and freely moves through the hydrophobic core of the membrane. Once it is reduced, (QH_2), ubiquinone delivers its electrons to the next complex in the electron transport chain. Q receives the electrons derived from NADH from complex I and the electrons derived from $FADH_2$ from complex II, including succinate dehydrogenase. This enzyme and $FADH_2$ form a small complex that delivers electrons directly to the electron transport chain, bypassing the first complex. Since these electrons bypass and thus do not energize the proton pump in the first complex, fewer ATP molecules are made from the $FADH_2$ electrons. The number of ATP molecules ultimately obtained is directly proportional to the number of protons pumped across the inner mitochondrial membrane.

4.5.3. Complex III

The third complex is composed of cytochrome b, another Fe-S protein, Rieske center (2Fe-2S center), and cytochrome c proteins; this

complex is also called cytochrome oxidoreductase. Cytochrome proteins have a prosthetic group of heme. The heme molecule is similar to the heme in hemoglobin, but it carries electrons, not oxygen. As a result, the iron ion at its core is reduced and oxidized as it passé the electrons, fluctuating between different oxidation states: Fe^{++} (reduced) and Fe^{+++} (oxidized). The heme molecules in the cytochromes have slightly different characteristics due to the effects of the different proteins binding them, giving slightly different characteristics to each complex. Complex III pumps protons through the membrane and passes its electrons to cytochrome c for transport to the fourth complex of proteins and enzymes (cytochrome c is the acceptor of electrons from Q; however, whereas Q carries pairs of electrons, cytochrome c can accept only one at a time).

4.5.4. Complex IV

The fourth complex is composed of cytochrome proteins c, a and a_3. This complex contains two heme groups (one in each of the two cytochromes, a, and a_3) and three copper ions (a pair of Cu_A and one Cu_B in cytochrome a_3). The cytochromes hold an oxygen molecule very tightly between the iron and copper ions until the oxygen is completely reduced. The reduced oxygen then picks up two hydrogen ions from the surrounding medium to make water (H_2O). The removal of the hydrogen ions from the system contributes to the ion gradient used in the process of chemiosmosis.

4.5.5. Chemiosmosis

In chemiosmosis, the free energy from the series of redox reactions just described is used to pump hydrogen ions (protons) across the membrane. The uneven distribution of H^+ ions across the membrane establishes both concentration and electrical gradients (thus, an electrochemical gradient), owing to the hydrogen ions' positive charge and their aggregation on one side of the membrane.

If the membrane were open to diffusion by the hydrogen ions, the ions would tend to diffuse back across into the matrix, driven by their electrochemical gradient. Recall that many ions cannot diffuse through the nonpolar regions of phospholipid membranes without the aid of ion channels. Similarly, hydrogen ions in the matrix space can only pass through the inner mitochondrial membrane through an integral membrane protein called ATP synthase. This complex protein acts as a tiny generator, turned by the force of the hydrogen ions diffusing through it, down their electrochemical gradient. The turning of parts of this molecular machine facilitates the addition of a phosphate to ADP, forming ATP, using the potential energy of the hydrogen ion gradient.

4.5.6. ATP Yield

The number of ATP molecules generated from the catabolism of glucose varies for example, the number of hydrogen ions that the electron transport chain complexes can pump through the membrane varies between species. Another source of variance stems from the shuttle of

electrons across the membranes of the mitochondria. (The NADH generated from glycolysis cannot easily enter mitochondria.) Thus, electrons are picked up on the inside of mitochondria by either NAD^+ or FAD^+. As has been explain earlier, these FAD^+ molecules can transport fewer ions; consequently, fewer ATP molecules are generated when FAD^+ acts as a carrier. NAD^+ is used as the electron transporter in the liver and FAD^+ acts in the brain.

Another factor that affects the yield of ATP molecules generated from glucose is the fact that intermediate compounds in these pathways are used for other purposes. Glucose catabolism connects with the pathways that build or break down all other biochemical compounds in cell, and the result is somewhat messier than the ideal situations already described. For example, sugars other than glucose are fed into the glycolytic pathway for energy extraction. Moreover, the five-carbon sugars that form nucleic acids are made from intermediates in glycolysis. Certain nonessential amino acids can be made from intermediates of both glycolysis and the citric acid cycle. Lipids, such as cholesterol and triglycerides, are also made from intermediates in these pathways and both amino acids and triglycerides are broken down for energy through these pathways. Overall, in living systems, these pathways of glucose catabolism extract about 34 percent of the energy contained in glucose.

4.6. Oxidative Phosphorylation

The electron transport chain forms a proton gradient across the inner mitochondrial membrane, which drives the synthesis of ATP via chemiosmosis. The NADH and $FADH_2$ formed in glycolysis, fatty acid oxidation and the citric acid cycle are energy-rich molecules because each contains a pair of electrons having a high transfer potential. When these electrons are used to reduce molecular oxygen to water, a large amount of free energy is liberated, which can be used to generate ATP. *Oxidative phosphorylation is the process in which ATP is formed as a result of the transfer of electrons from NADH or* $FADH_2$ *to* O_2 *by a series of electron carriers.* This process, which takes place in mitochondria, is the major source of ATP in aerobic organisms for example, oxidative phosphorylation generates 26 of the 30 molecules of ATP that are formed when glucose is completely oxidized to CO_2 and H_2O.

The flow of electrons from NADH or $FADH_2$ to O_2 through protein complexes located in the mitochondrial inner membrane leads to the pumping of protons out of the mitochondrial matrix. The resulting uneven distribution of protons generates a pH gradient and a transmembrane electrical potential that creates a *proton-motive force*. ATP is synthesized when protons flow back to the mitochondrial matrix through an enzyme complex. Thus, *the oxidation of fuels and the phosphorylation of ADP are coupled by a proton gradient across the inner mitochondrial membrane.*

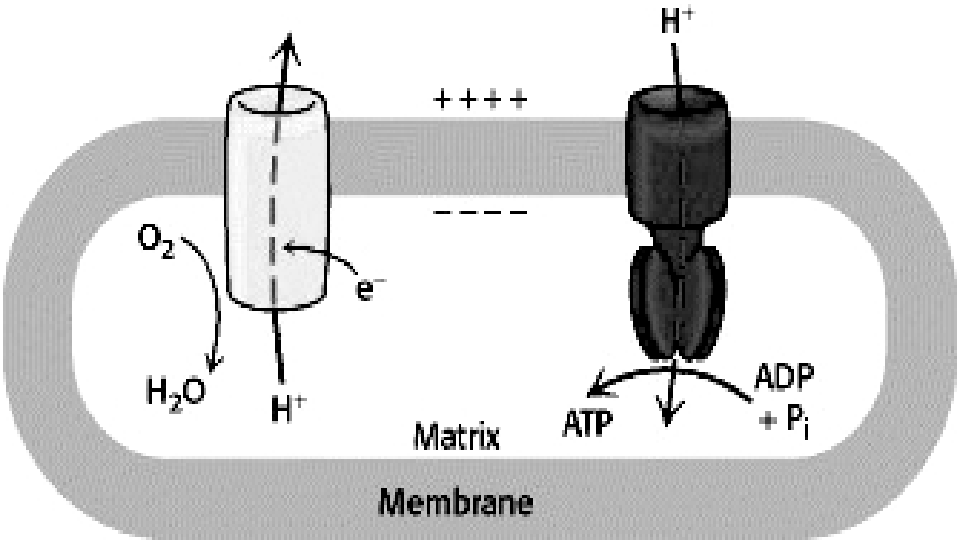


Fig. 4.6: Oxidative Phosphorylation

4.6.1. ATP Synthase and ATP Generation

The inner mitochondrial membrane is impermeable to ions and has one dedicated channel for the flow of protons back into the matrix. This is the membrane-bound ATP synthase enzyme. It consists of two parts the F_0 and F_1 regions. F_0 forms the proton pore and is embedded within the membrane. When protons flow back into the matrix from the inter-membrane space, the catalytic activity of the enzyme uses the potential energy released due to chemiosmosis to synthesis ATP from ADP and P_i.

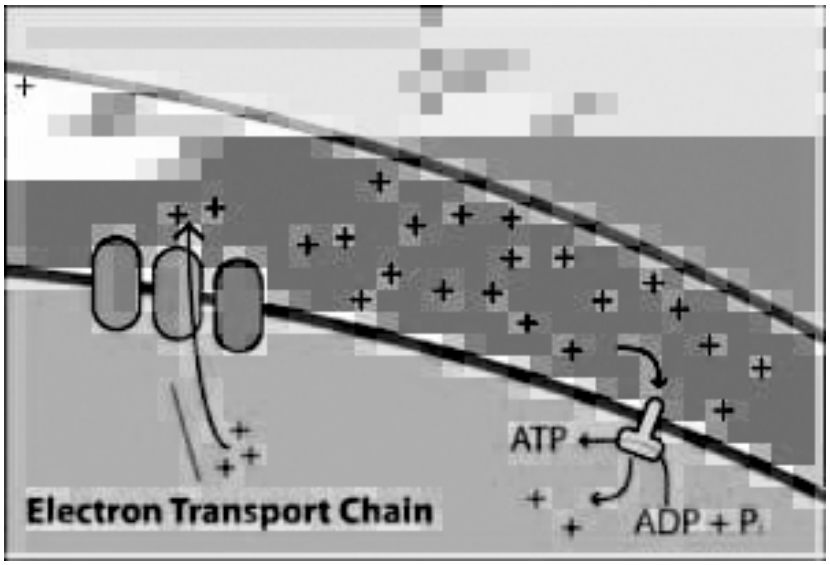


Fig. 4.7: ETC

4.7. Gluconeogenesis

Gluconeogenesis quite literally translates as 'the production of new glucose'. It is a metabolic pathway that results in the generation of glucose

from **non-carbohydrate** carbon substrates such as lactate, glycerol and glycogenic amino acids.

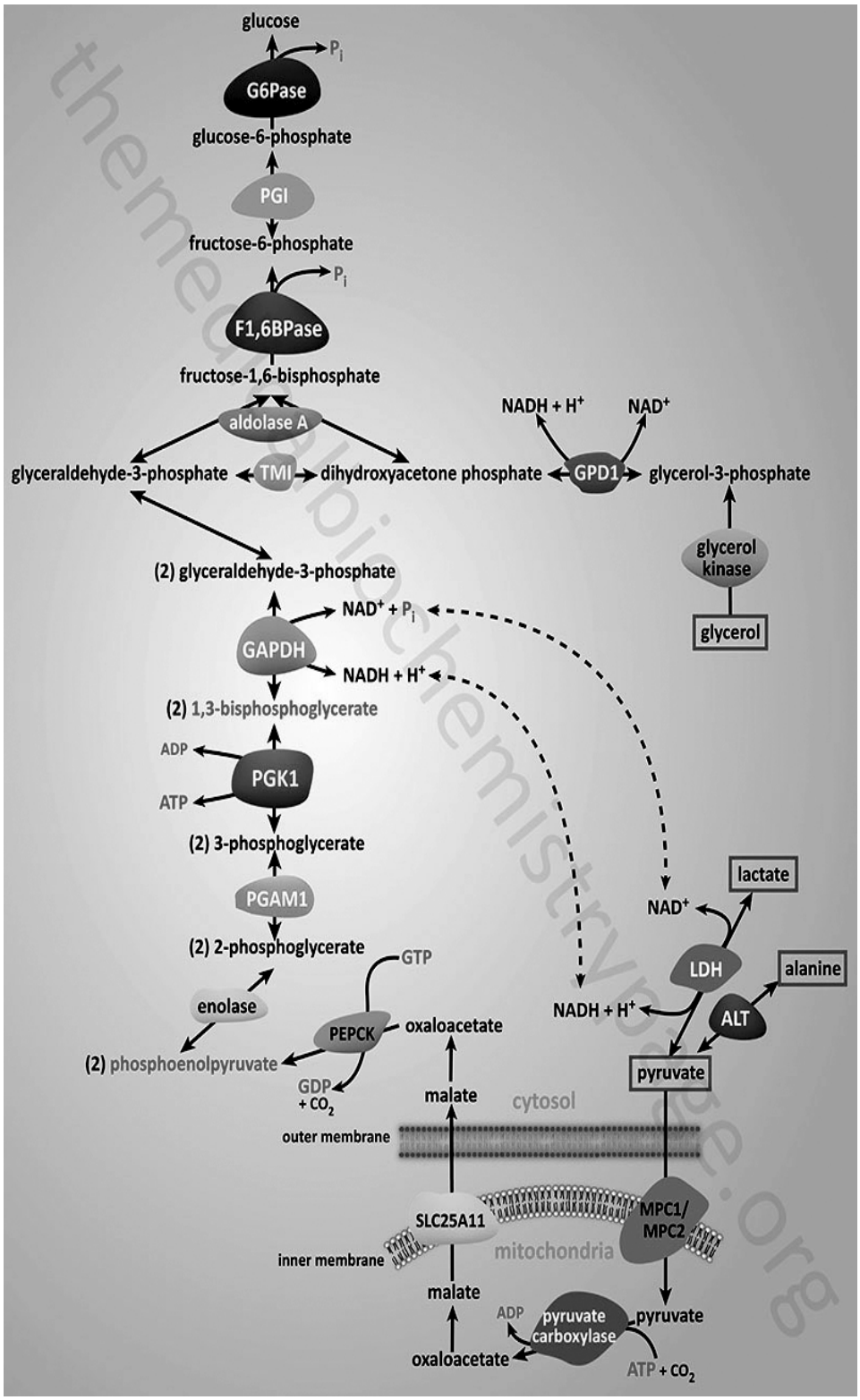


Fig. 4.8: Gluconeogenesis pathway

Gluconeogenesis occurs beyond around 8 hours of fasting when liver glycogen stores start to deplete and an alternative source of glucose is

required (Fig. 4.8). It occurs mainly in the liver and the kidney. There are three main precursors: Lactate from anaerobic glycolysis in exercising muscle and red blood cells via the Cori Cycle; **Glycerol** which is released from adipose tissue breakdown of triglycerides and amino acids (mainly alanine).

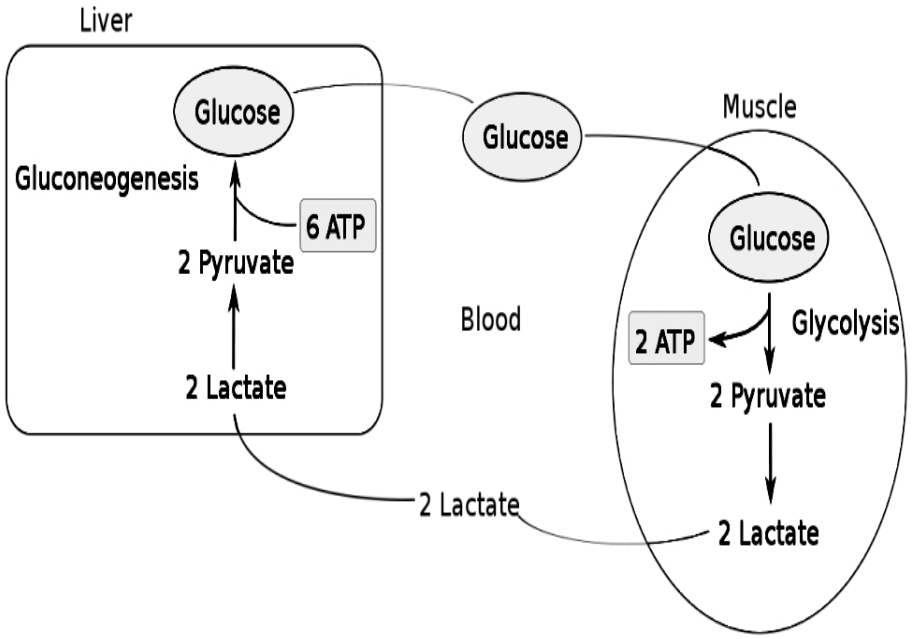


Fig.4.9

Whilst glycolysis is the breaking of glucose, gluconeogensis is the creation of glucose. However, gluconeogenesis is not as simple as reversing glyolysis, as there are irreversible steps in glycolysis.

Therefore, to circumvent this, some more enzymes are important in gluconeogensis, such as Phosphoenolpyruvate carboxykinase (PEPCK) which converts oxaloacetate to phosphoenolpyruvate. Fructose 1,6-bisphosphatase is also important, converting fructose 1,6-bisphosphatase to fructose fructose 6-phosphate. Finally, glucose-6-phosphatase turns glucose 6-phosphate to glucose.

The three reactions of glycolysis that proceed with a large negative free energy change are bypassed during gluconeogenesis by using different enzymes. These three are the pyruvate kinase, phosphofructokinase-1 (PFK-1) and hexokinase/glucokinase catalyzed reactions.

7.1. Regulation of Gluconeogenesis

Obviously the regulation of gluconeogenesis will be in direct contrast to the regulation of glycolysis (Fig.4.10). In general, negative effectors of glycolysis are positive effectors of gluconeogenesis. Regulation of the activity of PFK-1 and F1, 6BPase is the most

significant site for controlling the flux toward glucose oxidation or glucose synthesis. As described in control of glycolysis, this is predominantly controlled by fructose-2,6-bisphosphate, F2,6BP which is a powerful negative allosteric effector of F1,6Bpase activity.

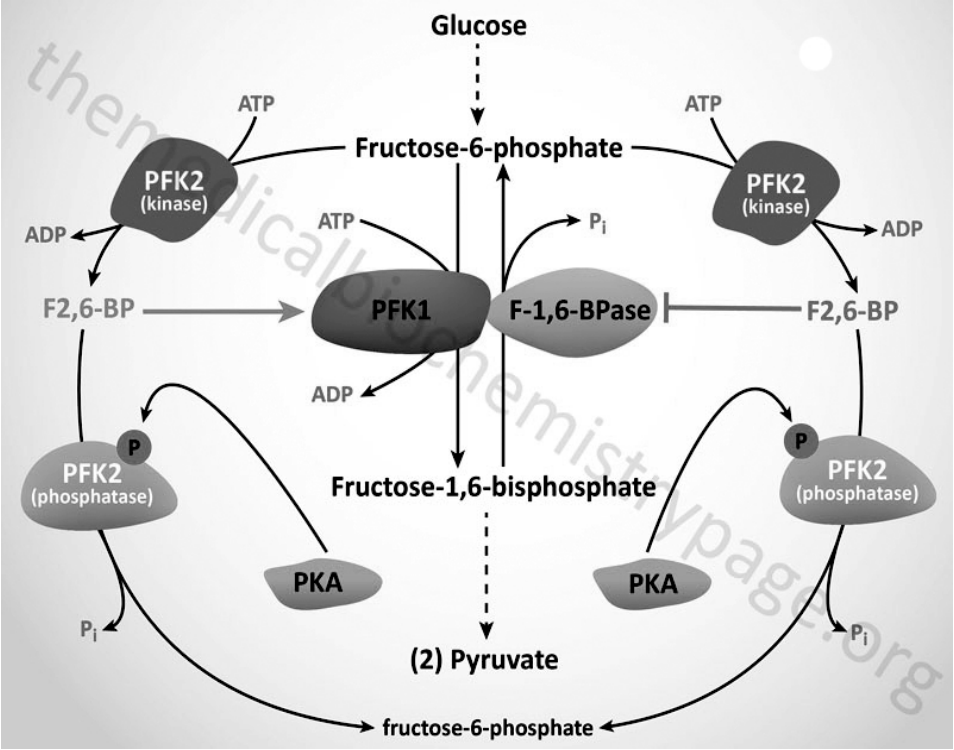


Fig. 4.10: Regulation of gluconeogenesis

Regulation of glycolysis and gluconeogenesis by fructose 2,6-bisphosphate (F2,6BP). The major sites for regulation of glycolysis and gluconeogenesis are the phosphofructokinase-1 (PFK-1) and fructose-1,6-bisphosphatase (F-1,6-BPase) catalyzed reactions. PFK-2 is the kinase activity and F-2,6-BPase is the phosphatase activity of the bi-functional regulatory enzyme, phosphofructokinase-2/fructose-2,6-bisphosphatase. PKA is cAMP-dependent protein kinase which phosphorylates PFK-2/F-2,6-BPase turning on the phosphatase activity. Green arrow indicates positive actions. Red T-lines represent inhibitory actions.

4.8. Glycogenesis

Glycogenesis is the biological process of forming glycogen from ***glucose***, the simplest cellular sugar. The body creates glycogen through the process of glycogenesis to store these molecules for later use, when the body does not have readily available glucose. Glycogen is not the same as ***fat***, which is stored for long term energy. Glycogen stores are often resorted to between meals, when the blood glucose concentration has dropped. In this case, the cells of the body resort to their stores of glycogen, undergoing the reverse process from glycogenesis. This process is called glycogenolysis.

4.8.1 Glycogenesis Process

To start the process, the cell must have an excess of glucose. Glucose is the starting molecule, and is modified through the process of glycogenesis. Through the modifications, it gains the ability to be stored in long chains. The process starts when the cell receives a signal from the body to enter glycogenesis. These signals could come from a number of different routes and are discussed in a later section. When glucose enters the glycogenesis process, it must be acted on by a number of enzymes as seen.

First, the glucose molecule interacts with the enzyme ***glucokinase***, which adds a phosphate group to the glucose. In the next step of glycogenesis, the phosphate group is transferred to the other side of the molecule, using the enzyme ***phosphoglucomutase***. A third enzyme, ***UDP-glucose pyrophosphorylase***, *takes* this molecule and creates ***uracil-diphosphate glucose***. This form of glucose has two phosphate groups, as well as the nucleic acid ***uracil***. These additions aid in the next step, creating a chain of molecules.

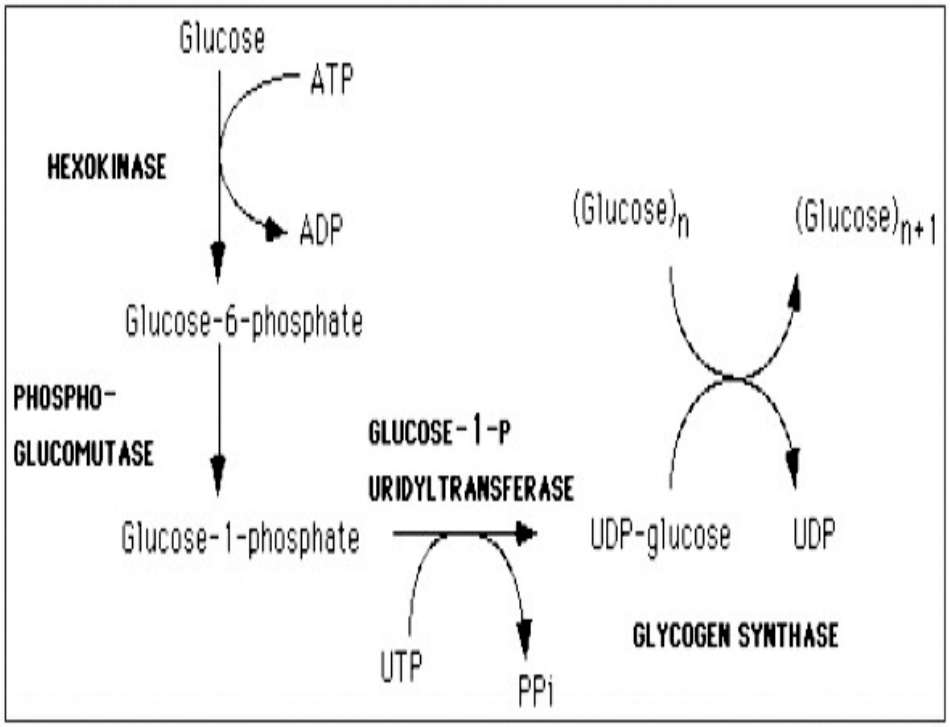


Fig. 4.12: Glycogenesis pathway

A special enzyme, ***glycogenin***, takes the lead in this part of glycogenesis. The UDP-diphosphate glucose can form short chains by binding to this molecule. After around 8 of these molecules chain together, more enzymes come in to finish the process. ***Glycogen synthase*** adds to the chain (**Fig. 4.12**), while ***glycogen branching enzyme*** helps create branches in the chains. This leads to a more compact macromolecule, and thus more efficient storage of energy.

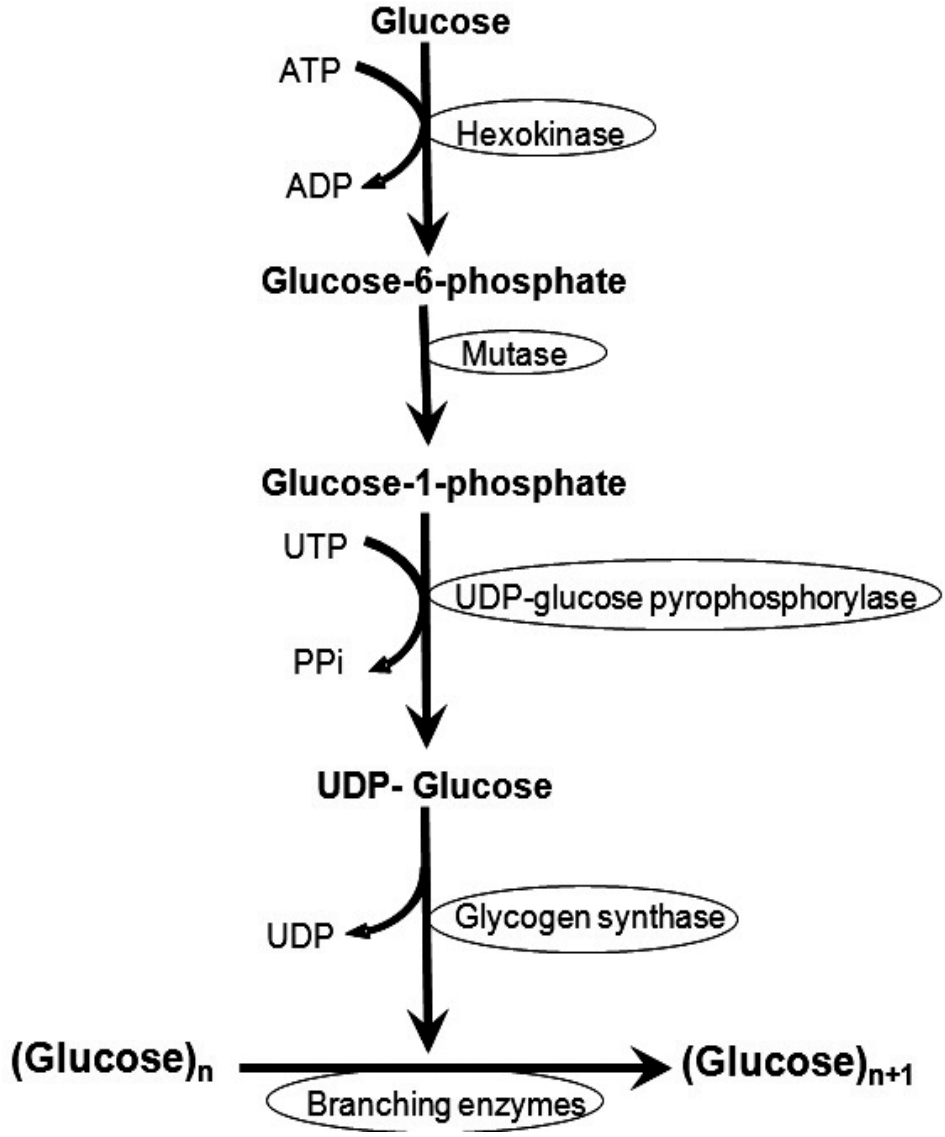


Fig. 4.13

4.6.2 Regulation of Glycogenesis

Epinephrine

Epinephrine, also called ***adrenaline***, is the hormone released in the ***fight-or-flight response***. In other words, it is the chemical released when the body is frightened or stressed. Epinephrine negatively regulates glycogenesis, by disrupting the proteins necessary for the process. During a flight-or-fight response, the body wants all the available energy ready for disposal by the cells. Thus, it stops glycogenesis, starts glycogenolysis, and starts converting the glucose into energy. The cells will need much ATP to overcome the threat presented.

Insulin

When the body receives a large meal and the blood glucose levels are up, the ***pancreas*** releases ***insulin***, a hormone which tells the cells to uptake and store glucose. This stimulates the process of glycogenesis,

causing the cells to store glycogen and reduce the sugar present in the blood. Lots of glycogen is stored in the liver, which can then release it as glucose back into the blood stream between meals to keep the blood glucose at tolerable levels

4.9. Energetic and regulation of metabolic cycles

Scientists use the term bioenergetics to describe the concept of energy flow through living systems, such as cells (**Fig.4.14). Cellular processes** such as the building and breaking down of complex molecules **occur through stepwise chemical reactions**. Some of these chemical reactions are spontaneous and release energy, whereas others require energy to proceed. Just as living things must continually consume food to replenish their energy supplies, cells must continually produce more energy to replenish that used by the many energy-requiring chemical reactions that constantly take place. Together, **all of the chemical reactions** that take place inside cells, including those that consume or generate energy, are referred to as the **cell's metabolism.**

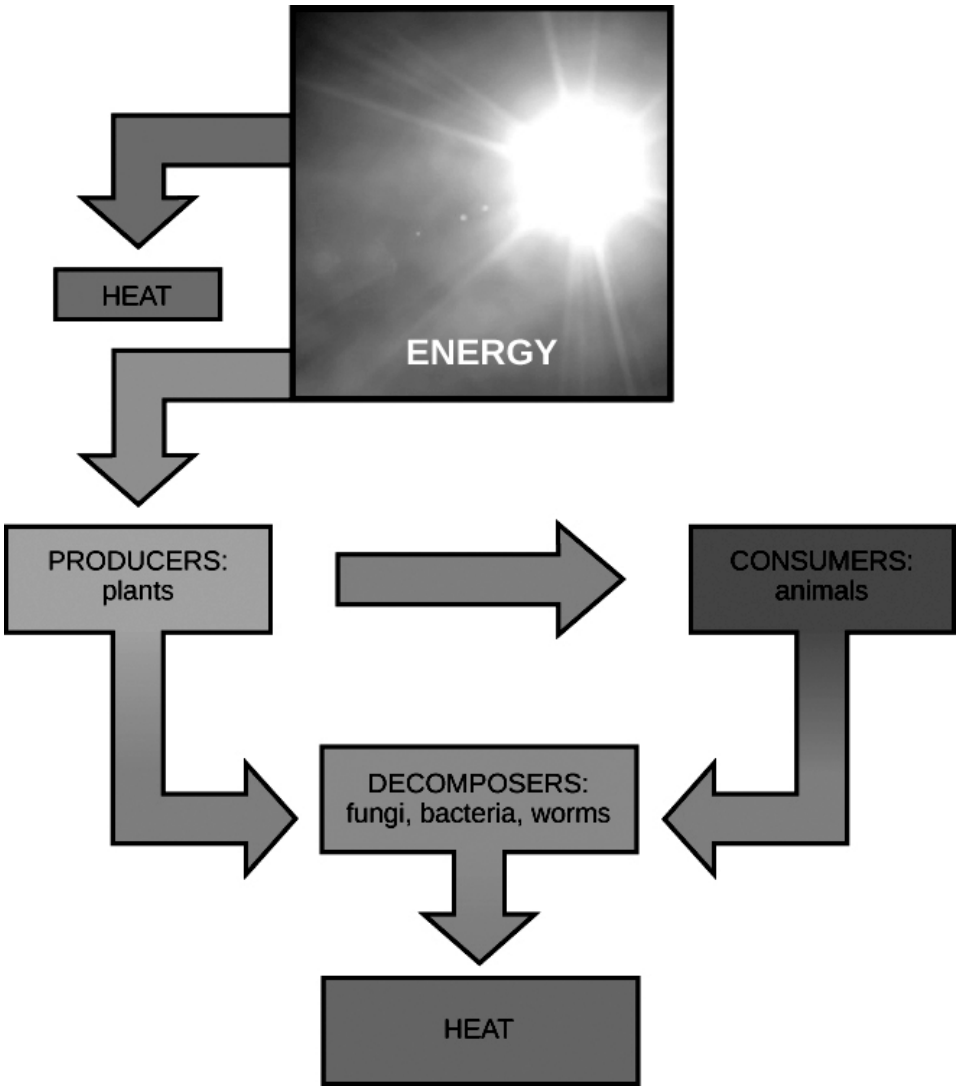
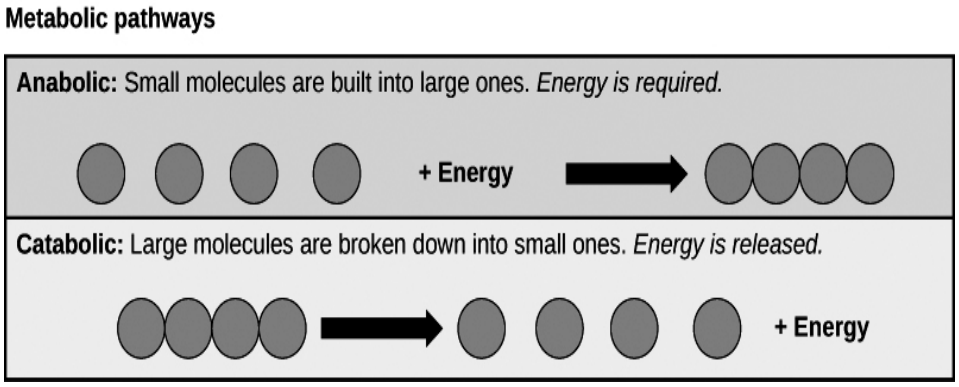


Fig. 4.14: Energy transfer

4.9.1. Metabolic Pathways

It is important to know that the chemical reactions of metabolic pathways do not take place on their own. Each reaction step is facilitated, or catalyzed, by a protein called an enzyme. Enzymes are important for catalyzing all types of biological reactions those that require energy as well as those that release energy.



Catabolic pathways are those that generate energy by breaking down larger molecules. Anabolic pathways are those that require energy to synthesize larger molecules. Both types of pathways are required for maintaining the cell's energy balance.
An important concept in physical systems is that of order and disorder. The more energy that is lost by a system to its surroundings, the less ordered and more random the system is. Scientists refer to the measure of randomness or disorder within a system as entropy. High entropy means high disorder and low energy. Molecules and chemical reactions have varying entropy as well for example, entropy increases as molecules at a high concentration in one place diffuse and spread out. The second law of thermodynamics says that energy will always be lost as heat in energy transfers or transformations.

4.9.2. Feedback Inhibition in Metabolic Pathways

Molecules can regulate enzyme function in many ways such as allosteric modulation, competitive and non-competitive inhibition. Perhaps the most relevant sources of regulatory molecules, with respect to enzymatic cellular metabolism are the products of the cellular metabolic reactions themselves. In a most efficient and elegant way, cells have evolved to use the products of their own reactions for feedback inhibition of enzyme activity. The cell responds to an abundance of the products by slowing down production during anabolic or catabolic reactions.

The production of both amino acids and nucleotides is controlled through feedback inhibition. Additionally, ATP is an allosteric regulator of some of the enzymes involved in the catabolic breakdown of sugar, the process that creates ATP. In this way when ATP is in abundant supply, the cell can prevent the production of ATP. On the other hand, ADP serves as a positive allosteric regulator (an allosteric activator) for some of the same enzymes that are inhibited by ATP. Thus, when relative levels of ADP are

high as compared to ATP, the cell is triggered to produce more ATP through sugar catabolism.

4.9.3. Hormone control

Hormone control is mainly affected through the action of two hormones synthesized by the pancreas: insulin and glucagon. Insulin is released by the pancreas when blood glucose level is high, i.e., after a meal. Insulin stimulates glucose uptake by the muscle, glycogen synthesis, and triacylglyceride synthesis by the adipose tissue. It inhibits gluconeogenesis and glycogen degradation. Glucagon is released by pancreas when blood glucose level drops too much. Its effects are opposite to those of insulin: in liver, glucagon stimulates glycogen degradation and the absorption of gluconeogenic aminoacids. It inhibits glycogen synthesis and promotes the release of fatty acids by adipose tissue.

4.10. Metabolism of disaccharides

Glucose is the most common monosaccharide consumed by humans. Two other monosaccharides that occur in significant amounts in the diet are fructose and galactose. The major source of fructose is the disaccharide sucrose. Entry of fructose is not dependent on insulin. Galactose is an important component of cell structural carbohydrates.

Diets containing large amounts of sucrose (a disaccharide of glucose and fructose) can utilize the fructose as a major source of energy. Fructose is found in foods containing sucrose (fruits), high-fructose corn syrups and honey. The pathway to utilization of fructose differs in muscle and liver.

There are two functionally different classes of disaccharides:

- Reducing disaccharides, in which one monosaccharide, the reducing sugar of the pair, still has a free hemiacetal unit that can perform as a reducing aldehyde group; lactose, maltose and cellobiose are examples of reducing disaccharides, each with one hemiacetal unit, the other occupied by the glycosidic bond, which prevents it from acting as a reducing agent. They can easily be detected by the Woehlk test or Fearon's test on methylamine.

- Non-reducing disaccharides, in which the component monosaccharides bond through an acetal linkage between their anomeric centers. This results in neither monosaccharide being left with a hemiacetal unit that is free to act as a reducing agent. Sucrose and trehalose are examples of non-reducing disaccharides because their glycosidic bond is between their respective hemiacetal carbon atoms. The reduced chemical reactivity of the non-reducing sugars in comparison to reducing sugars may be an advantage where stability in storage is important.

Table 4.1: Name of common disaccharides

Common disaccharides	**Unit 1**	**Unit 2**	**Bond**
Sucrose (*table sugar*, *cane sugar*, *beet sugar*, or *saccharose*)	Glucose	Fructose	α(1→2) β
Lactulose	Galactose	Fructose	β(1→4)
Lactose (*milk sugar*)	Galactose	Glucose	β(1→4)
Maltose (*malt sugar*)	Glucose	Glucose	α(1→4)
Trehalose	Glucose	Glucose	α(1→1) α
Cellobiose	Glucose	Glucose	β(1→4)
Chitobiose	Glucosamine	Glucosamine	β(1→4)

Maltose, cellobiose, and chitobiose are hydrolysis products of the polysaccharides starch, cellulose and chitin, respectively.

4.10.1.Sucrose

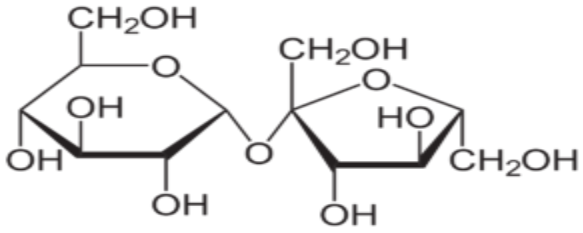


Fig. 4.15

This is the most important disaccharide. It is popularly known as ***table sugar.*** Sucrose is found in all photosynthetic plants. It is commercially obtained from sugarcane and sugar beets via an industrial process. Some chemical properties of sucrose are as under:

The molecular formula of sucrose is $\mathbf{C_{12}H_{22}O_{11}}$.

- If sucrose goes through acid catalysed hydrolysis it will give one mole of D-Glucose and one mole of D-Fructose.

- The chemical structure of sucrose comprises of α form of glucose and β form of fructose.
- The glycosidic linkage is α linkage because the molecule formation is in α orientation.
- Sucrose is a non-reducing sugar. It is combined (linked) at the hemiacetal oxygen and does not have a free hemiacetal hydroxide.
- Since it has no free hemiacetal hydroxide it does not show mutarotation (α to β conversion). Sucrose also does not form osazones for the same reason.
- One can prove the structural formula of sucrose by hydrolysing it with α-glycosidase enzymes which only hydrolyses α glucose. This test is positive for sucrose.

4.10.2. Lactose

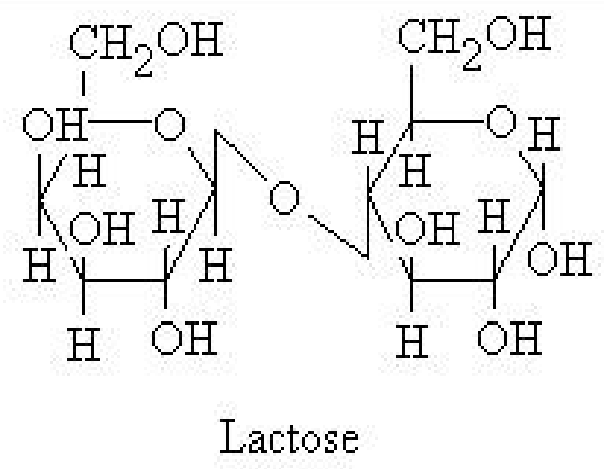


Fig. 4.16

This is a disaccharide show in **Fig. 4.16.** Lactose is the primary ingredient found in the milk familiar to all mammals. Unlike the majority of saccharides, lactose is not sweet to taste. Lactose consists of one galactose carbohydrate and one glucose carbohydrate. These are bound together by a 1-4 glycosidic bond in a beta orientation.

The structure of lactose shows that there is one significant difference between galactose and glucose. Galactose's fourth carbon has a different orientation in galactose than in sucrose. If it was not so the resulting molecule would have just been sucrose (glucose+glucose) instead of lactose.

Also from the structure, one can notice that lactose is a reacting sugar since it has one free hemiacetal hydroxide. So when we reacts Lactose with bromine water it will give monocarboxylic acid.

4.10.3. Maltose

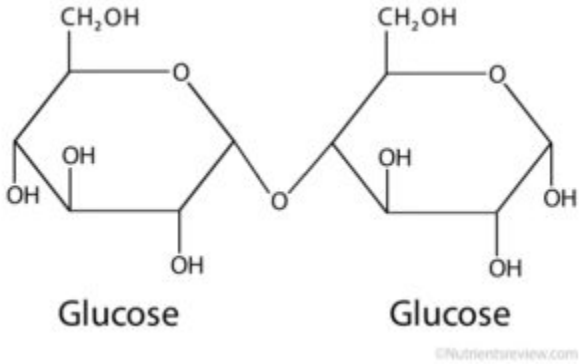


Fig. 4.17

Maltose is another disaccharide commonly found show in Fig. 4.17. It has two monosaccharide glucose molecules bound together; The link is between the first carbon atom of glucose and the fourth carbon of another glucose molecule. This, as you know, is the one-four glycosidic linkage. A few of its properties are as under:

- On acid catalysed hydrolysis one mole of maltose gives two moles of D-glucose.
- Maltose has a free hemiacetal hydroxide, hence it undergoes mutarotation. It exists as both α-Maltose and also β-Maltose
- For the same reasons it also gives a positive test with Benedicts and Tollens reagent.

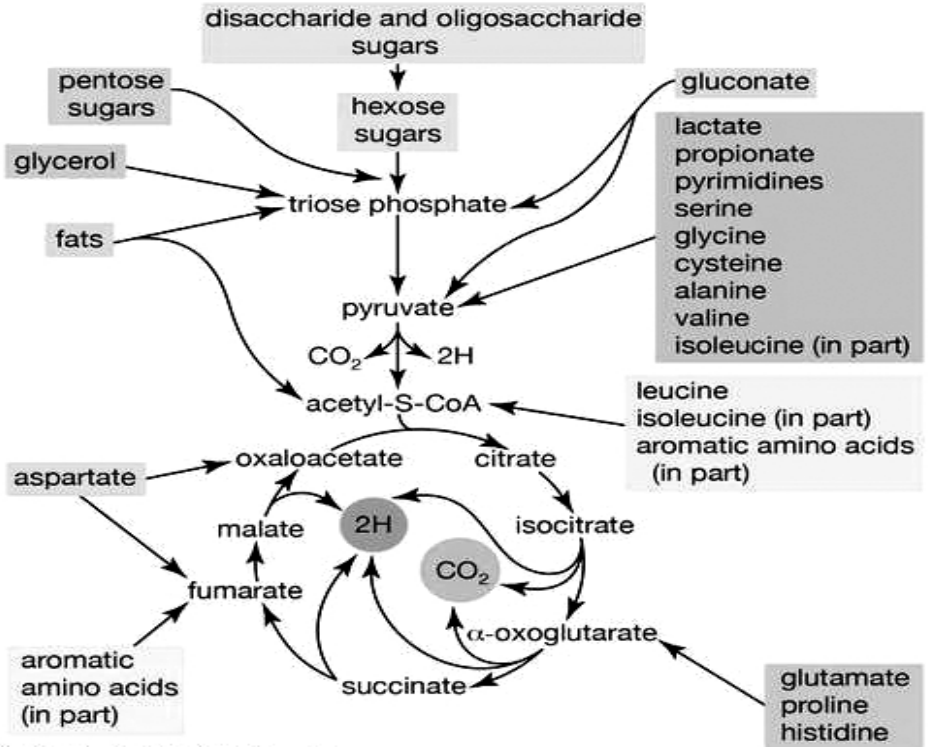


Fig. 4.18

4.11.Hormone Control of Carbohydrate Metabolism

The metabolism of carbohydrates is regulated by a variety of hormones and other molecules. Some of these have already been mentioned in previous sections. The proper functions of the body are dependent on precise control of the glucose concentration in the blood. The normal fasting level of glucose in the blood is 70-90 mg/100 ml.

If the concentration of glucose in blood is too high (above 120 mg/100 mL) a condition known as hyperglycemia results. Hyperglycemia may temporarily exist as a result of eating a meal rich in carbohydrates.

If the concentration of glucose is too low (below 70 mg/100 ml) a condition of hypoglycemia exists. Hypoglycemia is characterized by general weakness, trembling, drowsiness, headache, profuse perspiration, rapid heartbeat, and possible loss of consciousness. In addition, the hormone regulators are as under:

4.11.1 Insulin

Insulin, a polypeptide, is secreted from the pancreas in response to a hyperglycemia condition which usually results shortly after ingesting a meal.

The major effect of insulin is to promote the transport of sugar across the cell membrane of fat and muscle cells. In addition, insulin promotes anabolic processes such as increasing the rate of synthesis for glycogen (glycogenesis), fatty acids, and proteins. Insulin inhibits the catabolic processes such as the breakdown of glycogen and fat.

A deficiency of insulin (hypoinsulinism) results in a permanent hyperglycemic condition known as diabetes mellitus. If little or no insulin is present, glucose cannot be utilized properly by the cells and accumulates in the blood. Fatty acid metabolism is also upset. For this reason, a detailed study of diabetes mellitus must wait until the next chapter.

Hyperinsulinism (too much insulin) leads to the hypoglycemic condition. Excessive amount of glucose is removed from the blood. Severe hypoglycemia may result when a diabetic injects too much insulin. A severe insulin shock may result in a coma since glucose does not reach the brain. A diabetic usually carries a glucose rich food, such as candy, to provide a quick supply of glucose to replenish depleted glucose levels caused by too much insulin.

A functional type of hypoglycemia results in some individuals from an over stimulation of insulin. The causes of hypoglycemia are not completely understood, but it occurs in some people after eating heavily sugared food such as heavily sugared cereal and/or coffee and sweet rolls. The initial high glucose levels over stimulates the pancreas to produce too much insulin. The excess insulin causes blood sugar levels to drop below normal after 2-3 hours which may cause the person to feel sleepy, irritable,

and generally tired. The condition is only exacerbated by a "quick fix" of more sweetened coffee, pastry, or candy since more insulin is produced again. A protein rich breakfast would correct the condition by allowing glucose to enter the blood stream more slowly.

4.11.2. Glucagon

If one hormone, insulin, controls the excess of glucose in the blood by stimulating synthesis of glycogen, then other hormones must respond to low levels of glucose. The liver is more responsive to glucagon, a peptide also secreted by the pancreas.

Glucagon increases glucose levels in the blood by stimulating the breakdown of glycogen (glycogenolysis) in the liver into glucose which leaves the liver cells and enters the blood stream. The method of hormone stimulation is a complex cascade effect. The exact sequence has been worked out in the most details for epinephrine (adrenalin) although glucagon works in a similar fashion.

4.12. Summary

Most cells are capable of regulating their carbohydrate metabolism based on their current energy necessities. This is attained by feedback inhibition or stimulation of regulatory enzymes by various metabolites. The important metabolites involved in regulation of carbohydrate metabolism include ATP, NADH, glucose-6- phosphate, citrate, and fructose-2,6- bisphosphate. Many cell types respond to hormonal and neuronal signals that allow the coordination of metabolism at the level of the entire organism. For carbohydrate metabolism, the liver and skeletal muscles have the most important roles. The liver either takes up glucose for storage in the form of glycogen, or releases glucose for use by other tissues. The muscle takes up glucose for storage or for conversion to mechanical energy; it can release free amino acids derived from protein breakdown to act as substrates for liver gluconeogenesis. Both synthesis and degradation are controlled through a complex mechanism involving insulin, glucagon and epinephrine, as well as allosteric regulators. Glucagon is released from the pancreas when blood glucose level drop in the hours after a meal. It binds to receptors on hepatocytes and initiates a signal transduction process that elevates intracellular cAMP levels. CAMP increases the original glucagon signal and initiates a phosphorylation cascade that leads to the activation of glycogen phosphorylase along with a number of other proteins. Within seconds, glycogenolysis leads to the release of glucose into the bloodstream. When occupied, the insulin receptor becomes an active tyrosine kinase enzyme that causes a phosphorylation cascade that ultimately has the opposite effect of the glucagon/CAMP system: the enzymes of glycogenolysis are inhibited and the enzymes of glycogenesis are activated. Insulin also increases the rate of glucose uptake into several types of target cells, but not liver or brain cells. Emotional or physical stress releases the hormone epinephrine from the adrenal medulla. Epinephrine stimulates glycogenolysis and inhibits

glycogenesis. In emergency conditions, when epinephrine is released in relatively large quantities, enormous production of glucose provides the energy required to manage the situation.

4.13. Terminal Questions

Q.1. Glycolysis is the most important evolutionary evidence of link between prokaryotes and eukaryotes" Explain above statement.

Answer:----------

Q.2. Describe all 10 sequential reaction of glycolysis and make balance sheet of energy dynamics.

Answer:----------

Q.3. What are the conditions required for glucose fermentation?

Answer:----------

Q.4. Why TCA cycle is found only in aerobic organism? Explain with stepwise reactions of TCA cycle.

Answer:----------

Q.5. What is membrane potential and how this potential difference used for storage in bond energy? Show proper energy balance in electron transport chain.

Answer:----------

Q.6. Discuss energy extraction efficiency from substrate of glycolysis, TCA and fermentation.

Answer:----------

4.14. Further Readings

1. A.L. Lehninger, Principles of Biochemistry, 4th edition, W.H Freeman and Company, 2004.

2. Fundamentals of Biochemistry: Dr J L Jain, S. Chand and Company

3. Textbook of Biochemistry and Human Biology: Talwar and Srivastava. Eastern Economy Edition, Prentice Hall, India.

4. V. Voet and J.G.Voet, Biochemistry, 3rd edition, John Wiley, New York, 2004.

5. L. Stryer, Biochemistry, 5th edition, W.H. Freeman and Company, 2002.

Uttar Pradesh Rajarshi Tandon
Open University

PGBCH-103

Bioenergetics and Metabolism

BLOCK

3

Metabolism of Amino Acids And Nitrogen Compounds

Curriculum Design Committee

Dr. (Prof.) Ashutosh Gupta, **Chairman**
School of Science, UPRTOU, Prayagraj

Prof. Prof. Umesh Nath Tripathi **Member**
Department of chemistry
Deen Dayal Upadhyaya Gorakhpur University, Gorakhpur

Prof. S.I. Rizvi **Member**
Department of Biochemistry
University of Allahabad, Prayagraj

Prof. Dinesh Yadav **Member**
Department of Biotechnology
Deen Dayal Upadhyaya Gorakhpur University, Gorakhpur

Prof. Sharad Kumar Mishra **Member**
Department of Biotechnology
Deen Dayal Upadhyaya Gorakhpur University, Gorakhpur

Dr. Ravindra Pratap Singh **Member**
Academic Consultant (Biochemistry)
School of Science, UPRTOU, Prayagraj

Dr. Dharmveer Singh **Course Coordinator**
Academic Consultant (Biochemistry)
School of Science, UPRTOU, Prayagraj

Course Preparation Committee

Dr. Sanjeev Kumar Maurya Associate Professor Department of biotechnology, Invertis University, Bareilly	**Author**	**Block-1&2 (Unit: 1- 4)**
Dr. Shashank Upadhaya Asst. Professor Department of biotechnology, Invertis University, Bareilly	**Author**	**Block-3 (Unit: 5, 6)**
Dr. V.C. Srivastava Former Associate Professor, Department of Zoology, University of Allahabad, Prayagraj	**Editor**	**(All blocks and units)**
Dr. Dharmveer Singh School of Sciences, UPRTOU, Prayagraj		**(Course Coordinator)**

ISBN :

Introduction

This is the third block on Bioenergetics. It consists of following two units:

Unit-5 This unit covers the general reactions of amino acid metabolism, transamination, and decarboxylation. The process of oxidative and non-oxidative deamination of amino acids is also cover in this unit. The special metabolism of methionine, histidine, phenylalanine, tyrosine, tryptophan, lysine, valine, leucine, isoleucine and polyamines are briefly discussed.

Unit-6 The molecular nitrogen that makes up 80% of the earth's atmosphere is unavailable to living organisms until it is reduced. The metabolism and regulation of nitrogen is cover in this unit. Fixation of atmospheric N_2 takes place in certain free-living soil bacteria and in symbiotic bacteria in the root nodules of leguminous plants, by the action of the complex nitrogenase system. The process of decarboxylation, transamination, metabolism and regulation of urea is briefly discussed in this unit.

UNIT-5

AMINO ACIDS

Structure

5.1. Introduction

The 20 amino acids commonly found as hydrolysis products of proteins contain an α-carboxyl group, an α-amino group, and a distinctive

R group substituted on the α-carbon atom. The α-carbon atom of the amino acids (except glycine) is asymmetric, and thus amino acids can exist in at least two stereoisomeric forms. Only the L stereoisomers, which are related to the absolute configuration of L-glyceraldehyde, are found in proteins. The amino acids are classified on the basis of the polarity of their R groups. The nonpolar, aliphatic class includes alanine, glycine, isoleucine, leucine, proline, and valine. Phenylalanine, tryptophan, and tyrosine have aromatic side chains and are also relatively hydrophobic. The polar, uncharged class includes asparagine, cysteine, glutamine, methionine, serine, and threonine. The negatively charged (acidic) amino acids are aspartate and glutamate; the positively charge (basic) ones are arginine, histidine, and lysine. There are also a large number of nonstandard amino acids that occur in some proteins (as a result of the modification of standard amino acids) or as free metabolites in cells.

Objectives

- List and name the 20 amino acids that commonly occur in proteins and classify them according to chirality, polarity, size, and charge.
- Describe the bonds and forces (peptide, disulfide, and hydrogen bonds; hydrophobic, dipoledipole, van der Waals and electrostatic interactions) that contribute to the conformation of proteins and the interaction of proteins with other biomolecules.
- Describe the dynamics of the free amino acid pool, including (A) inputs from diet, body protein breakdown, and de novo synthesis (B) outputs to protein synthesis, urea production, synthesis of specialized products and other metabolic processes.

5.2. Some basic facts of amino acids

Monoamino monocarboxylic amino acids are diprotic acids (+H3NCH(R)COOH) at low pH. As the pH is raised to about 6, near the isoelectric point, the proton is lost from the carboxyl group to form the dipolar or zwitterionic species +H3NCH(R)COO-, which is electrically neutral. Further increase in pH causes loss of the second proton, to yield the ionic species H2NCH(R)COO-. Amino acids with ionizable R groups may exist in additional ionic species, depending on the pH and the pKa of the R group. Thus amino acids vary in their acid-base properties. Amino acids form colored derivatives with ninhydrin. Other colored or fluorescent derivatives are formed in reactions of the α-amino group of amino acids with fluorescamine, dansyl chloride, dabsyl chloride, and 1-fluoro-2,4-dinitrobenzene. Complex mixtures of amino acids can be separated and identified by ionexchange chromatography or HPLC.

Amino acids can be joined covalently through peptide bonds to form peptides, which can also be formed by incomplete hydrolysis of polypeptides. The acid-base behavior and chemical reactions of a peptide are functions of its amino-terminal amino group, its carboxyl-terminal carboxyl group, and its R groups. Peptides can be hydrolyzed to yield free

amino acids. Some peptides occur free in cells and tissues and have specific biological functions. These include some hormones and antibiotics, as well as other peptides with powerful biological activity.

Proteins can be reduced to their constituent amino acids by a variety of methods, and the earliest studies of proteins naturally focused on the free amino acids derived from them. The first amino acid to be discovered in proteins was asparagine, in 1806. The last of the 20 to be found, threonine, was not identified until 1938. All the amino acids have trivial or common names, in some cases derived from the source from which they were first isolated. Asparagine was first found in asparagus, as one might guess; glutamate was found in wheat gluten; tyrosine was first isolated from cheese (thus its name is derived from the Greek *tyros*, "cheese") and glycine (Greek *glykos*, "sweet") was so named because of its sweet taste.

5.2.1. Amino acids have common structural features

All of the 20 amino acids found in proteins have a carboxyl group and an amino group bonded to the same carbon atom (the α carbon) (Fig. 5.1). They differ from each other in their side chains, or R groups, which vary in structure, size, and electric charge, and influence the solubility of amino acids in water. When the R group contains additional carbons in a chain, they are designated β, γ, δ, ε, etc., proceeding out from the α carbon. The 20 amino acids of proteins are often referred to as the standard, primary, or normal amino acids, to distinguish them from amino acids within proteins that are modified after the proteins are synthesized, and from many other kinds of amino acids present in living organisms but not in proteins. The standard amino acids have been assigned three-letter abbreviations and one-letter symbols (Table 5.1), which are used as shorthand to indicate the composition and sequence of amino acids in proteins.

They note in Fig. 5.1 that for all the standard amino acids except one (glycine) the α carbon is asymmetric, bonded to four different substituent groups: a carboxyl group, an amino group, an R group, and a hydrogen atom. The α-carbon atom is thus a **chiral center**. Because of the tetrahedral arrangement of the bonding orbitals around the α-carbon atom of amino acids, the four different substituent groups can occupy two different arrangements in space, which are nonsuperimposable mirror images of each other (Fig. 5.2). These two forms are called **enantiomers** or **stereoisomers**. All molecules with a chiral center are also **optically active**-i.e., they can rotate plane-polarized light, with the direction of the rotation dif fering for different stereoisomers.

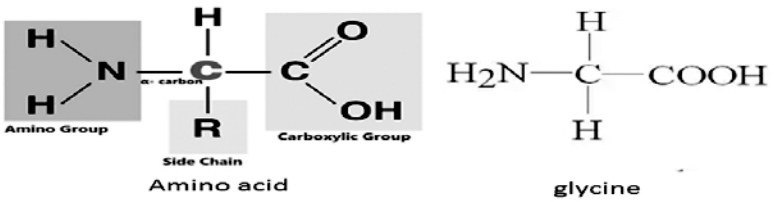


Fig. 5.1: General structure of the amino acids found in proteins.

With the exception of the nature of the R group, this structure is common to all the α-amino acids. (Proline, because it is an imino acid, is an exceptional component of proteins.) The α carbon is shown in blue. R (in red) represents the R group or side chain, which is different in each amino acid. In all amino acids except glycine (shown for comparison) the α-carbon atom has four difl'erent substituent groups.

Table 5.1: Illustration of different amino acids and their respective information.

Amino acid	*Mr*	*pK$_1$ (—COOH)*	*pK$_2$ (—NH$_3^+$)*	*pKR (R group)*	*pI*	*Hydropathy index**	*Occurrence in proteins (%)†*
Nonpolar R groups							
Alanine	89	2.34	9.69		6.01	1.8	9.0
Valine	117	2.32	9.62		5.97	4.2	6.9
Leucine	131	2.36	9.60		5.98	3.8	7.5
Isoleucine	131	2.36	9.68		6.02	4.5	4.6
Proline	115	1.99	10.96		6.48	– 1.6	4.6
Phenylalanine	165	1.83	9.13		5.48	2.8	3.5
Tryptophan	204	2.38	9.39		5.89	– 0.9	1.1
Methionine	149	2.28	9.21		5.74	1.9	1.7
Polar, uncharged R groups							
Glycine	75	2.34	9.60		5.97	– 0.4	7.5
Serine	105	2.21	9.15	13.60	5.68	–0.8	7.1
Threonine	119	2.11	9.62	13.60	5.87	– 0.7	6.0
Tyrosine	181	2.20	9.11	10.07	5.66	– 1.3	3.5
Cysteine	121	1.96	8.18	10.28	5.07	2.5	2.8
Asparagine	132	2.02	8.80		5.41	– 3.5	4.4
Glutamine	146	2.17	9.13		5.65	– 3.5	3.9
Negatively-charged R groups							
Aspartate	133	1.88	9.60	3.65	2.77	– 3.5	5.5
Glutamate	147	2.19	9.67	4.25	3.22	– 3.5	6.2
Positively-charged R groups							
Lysine	146	2.18	8.95	10.53	9.74	– 3.9	7.0
Arginine	174	2.17	9.04	12.48	10.76	– 4.5	4.7
Histidine	154	1.82	9.17	6.00	7.59	– 3.2	2.1

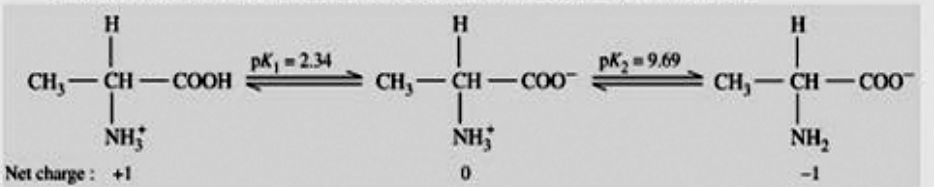
* A scale combining hydrophobicity and hydrophilicity ; can be used to predict which amino acid will be found in an aqueous environment (-values) and which will be found in a hydrophobic environment (+ values).

† Average occurrence in over 200 proteins

Note that in nature, the commonest amino acid is alanine and the rarest amino acid is tryptophan.

(Adapted from Klapper MH, 1977)

As an instance, ionization of the amino acid, **alanine** (Fig. 9–4) is discussed.



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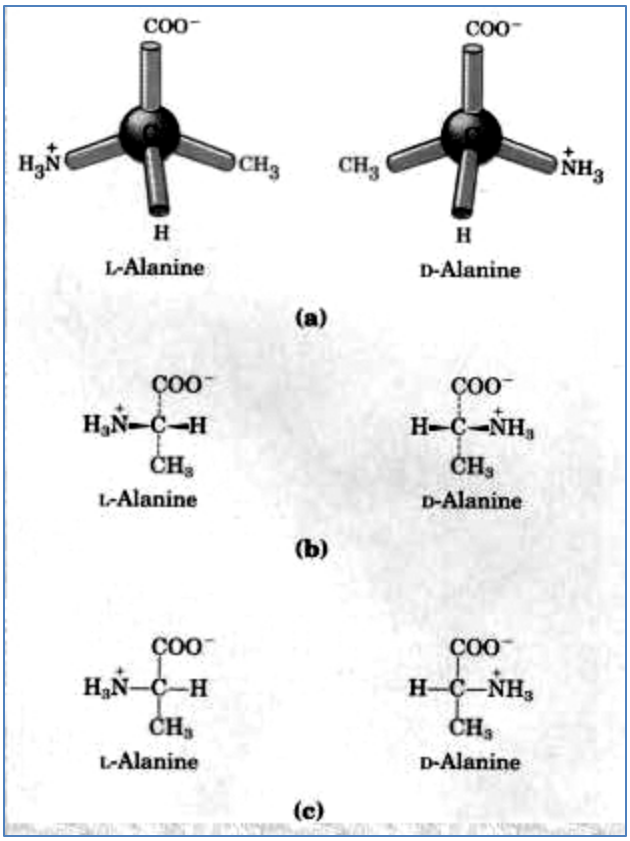


Fig. 5.2: (a) The two stereoisomers of alanine. L- and D-alanine are nonsuperimposable mirror images of each other. (b, c) 'Two different conventions for showing the configurations in space of stereoisomers. In perspective formulas (b) the wedge-shaped bonds project out of the plane of the paper, the dashed bonds behind it. In projection formulas (c) the horizontal bonds are assumed to project out of the plane of the paper, the vertical bonds behind. However, projection formulas are often used casually without reference to stereochemical configuration.

5.2.2. Amino acids can be classified by R group

An understanding of the chemical properties of the standard amino acids is central to an understanding of much of biochemistry. The topic can be simplified by grouping the amino acids into classes based on the properties of their R groups (Table 5-1), in particular, their **polarity** or tendency to interact with water at biological pH (near pH 7.0). The polarity of the R groups varies widely, from totally nonpolar or hydrophobic (water-insoluble) to highly polar or hydrophilic (water-soluble). The structures of the 20 standard amino acids are shown in Fig. 5.3, and many of their properties are listed in Table 5.1. There are five main classes of amino acids, those whose R groups are: nonpolar and aliphatic; aromatic (generally nonpolar); polar but uncharged; negatively charged; and positively charged. Within each class there are gradations of polarity, size, and shape of the R groups. Let us begin with a mental exercise in recording the three-dimensional data of a biopolymer. Consider how one might record, on paper, all the details and dimensions of a three-

dimensional ball-and-stick model of a protein like myoglobin . One way to begin is with the sequence, which can be obtained by tracing out the backbone of the three-dimensional model. Beginning from the NH_2-terminus, one could identify each amino acid side chain by comparing the atomic structure of each residue with the chemical structure of the 20 common amino acids.

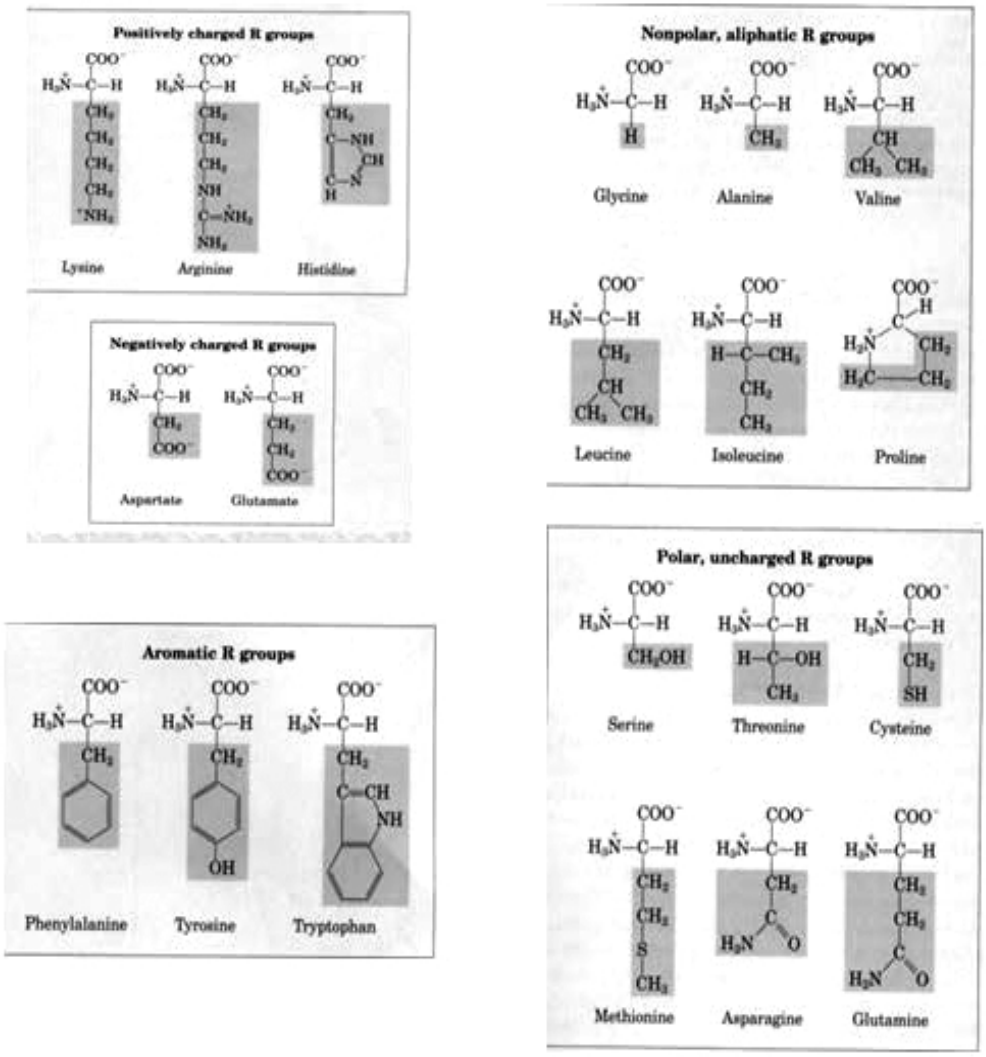


Fig. 5.3: The 20 standard amino acids of proteins. They are shown with their amino and carboxyl groups ionized, as they would occur at pH 7.0. The portions in black are those common to all the amino acids; the portions shaded in red are the R groups.

Nonpolar, Alzphatic R Groups: The hydrocarbon R groups in this class of amino acids are nonpolar and hydrophobic (Fig. 5.3). The bulky side chains of alanine, valine, leucine, and isoleucine, with their distinctive shapes, are important in promoting hydrophobic interactions within protein structures. Glycine has the simplest amino acid structure. Where it is present in a protein, the minimal steric hindrance of the glycine side chain allows much more structural flexibility than the other amino acids. Proline represents the opposite structural extreme. The secondary amino

(imino) group is held in a rigid conformation that reduces the structural flexibility of the protein at that point.

Aromatic R Groups: Phenylalanine, tyrosine, and tryptophan, with their aromatic side chains (Fig. 5.3), are relatively nonpolar (hydrophobic). All can participate in hydrophobic interactions, which are particularly strong when the aromatic groups are stacked on one another. The hydroxyl group of tyrosine can form hydrogen bonds, and it acts as an important functional group in the activity of some enzymes. Tyrosine and tryptophan are significantly more polar than phenylalanine because of the tyrosine hydroxyl group and the nitrogen of the tryptophan indole ring.

Tryptophan and tyrosine, and to a lesser extent phenylalanine, absorb ultraviolet light. This accounts for the characteristic strong absorbance of light by proteins at a wavelength of 280 nm, and is a property exploited by researchers in the characterization of proteins.

Polar, Uncharged R Groups: The R groups of these amino acids (Fig. 5.3) are more soluble in water, or hydrophilic, than those of the nonpolar amino acids, because they contain functional groups that form hydrogen bonds with water. This class of amino acids includes serine, threonine, cysteine, methionine, asparagine, and glutamine. The polarity of serine and threonine is contributed by their hydroxyl groups; that of cysteine and methionine by their sulfur atom and that of asparagine and glutamine by their amide groups. Asparagine and glutamine are the amides of two other amino acids also found in proteins, aspartate and glutamate, respectively, to which asparagine and glutamine are easily hydrolyzed by acid or base. Cysteine has an R group (a thiol group) that is approximately as acidic as the hydroxyl group of tyrosine. Cysteine requires special mention for another reason. It is readily oxidized to form a covalently linked dimeric amino acid called cystine, in which two cysteine molecules are joined by a disulfide bridge. Disulfide bridges of this kind occur in many proteins, stabilizing their structures.

Negatiuely Charged (Acidic) R Groups: The two amino acids having R groups with a net negative charge at pH 7.0 are aspartate and glutamate, each with a second carboxyl group (Fig. 5.3). These amino acids are the parent compounds of asparagine and glutamine, respectively.

Positiuely Charged (Basic) R Groups: The amino acids in which the R groups have a net positive charge at pH 7.0 are lysine, which has a second amino group at the e position on its aliphatic chain; arginine, which has a positively charged guanidino group; and histidine, containing an imidazole group (Fig. 5.3). Histidine is the only standard amino acid having a side chain with a pKa near neutrality.

5.3. Amino acid oxidation and the production of urea

Amino acids, derived largely from protein in the diet or from degradation of intracellular proteins, are the final class of biomolecules

whose oxidation makes a significant contribution to the generation of metabolic energy. The fraction of metabolic energy derived from amino acids varies greatly with the type of organism and with the metabolic situation in which an organism finds itself. Carnivores, immediately following a meal, may obtain up to 90% of their energy requirements from amino acid oxidation. Herbivores may obtain only a small fraction of their energy needs from this source. Most microorganisms can scavenge amino acids from their environment if they are available; these can be oxidized as fuel when required by metabolic conditions. Photosynthetic plants, on the other hand, rarely, if ever, oxidize amino acids to provide energy. Instead, they convert CO_2 and H_2O into the carbohydrate that is used almost exclusively as an energy source. The amount of amino acids in plant tissues in carefully regulated to just meet the requirements for biosynthesis of proteins, nucleic acids and a few other molecules needed to support growth. Amino acid catabolism does occur in plants, but it is generally concerned with the production of metabolites for other biosynthetic pathways.

In animals, amino acids can undergo oxidative degradation in three different metabolic circumstances. (1) During the normal synthesis and degradation of cellular proteins some of the amino acids released during protein breakdown will undergo oxidative degradation if they are not needed for new protein synthesis. (2) When a diet is rich in protein and amino acids are ingested in excess of the body needs for protein synthesis, the surplus may be catabolized; amino acids cannot be stored. (3) During starvation or in diabetes mellitus, when carbohydrates are either unavailable or not properly utilized, body proteins are called upon as fuel. Under these different circumstances, amino acids loose their amino groups, and the a-keto acids so formed may undergo oxidation to CO_2 and H_2O. In addition, and often equally important, the carbon skeletons of the amino acids provide three- and four-carbon units that can be converted to glucose, which in turn can fuel the functions of the brain, muscle, and other tissues.

Amino acid degradative pathways are quite similar in most organisms. The focus of this chapter is on vertebrates, because amino acid catabolism has received the most attention in these organisms. As is the case for sugar and fatty acid catabolic pathways, the processes of amino acid degradation converge on the central catabolic pathways for carbon metabolism. The carbon skeletons of the amino acids generally find their way to the citric acid cycle, and from there they are either oxidized to produce chemical energy or funneled into gluconeogenesis. In some cases the reaction pathways closely parallel steps in the catabolism of fatty acids.

Some organisms can synthesize all of the 20 standard amino acids, others cannot. Nonessential amino acids are those that can be synthesized, essential amino acids have to be taken in the diet. The 20 standard amino acids can be grouped into six biosynthetic families depending on the metabolic intermediate from which their carbon skeleton is derived.

Amino acids are degraded by the removal of the _-amino group and the conversion of the resulting carbon skeleton into one or more metabolic intermediates. Amino acids are termed glucogenic if their carbon skeletons can give rise to the net synthesis of glucose, and ketogenic if they can give rise to ketone bodies. Some amino acids give rise to more than one intermediate and these lead to the synthesis of glucose as well as ketone bodies. Thus these amino acids are both glucogenic and ketogenic.

5.3.1 Biosynthesis of amino acids

All amino acids are derived from intermediates in glycolysis, the citric acid cycle, or the pentose phosphate pathway (Fig. 5.4). Nitrogen enters these pathways by way of glutamate and glutamine. Some pathways are simple, others are not. Ten of the amino acids are produced when only one or a few enzymatic steps are removed from their precursors. The pathways for others, such as the aromatic amino acids, are more complex. Different organisms vary greatly in their ability to synthesize the 20 amino acids, whereas most bacteria and plants can synthesize all 20, mammals can synthesize only about half of them.

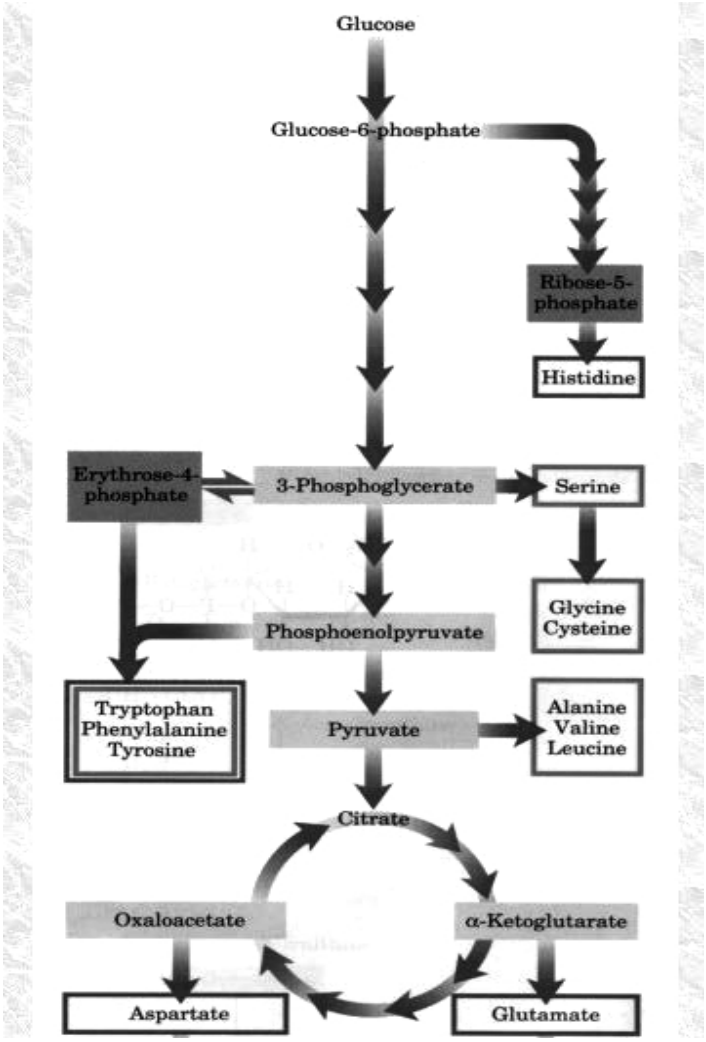


Fig. 5.4: Overview of amino acid biosynthesis. Precursors from glycolysis (red), the citric acid cycle (blue), and the pentose phosphate

pathway (purple) are shaded, and the amino acids derived from them are boxed in the corresponding colors. The same device-color-matching precursors with pathway end products-will be used in illustrations of the individual pathways

Those that are synthesized in mammals are generally those with simple pathways. These are called the nonessential amino acids to denote the fact that they are not needed in the diet. The remainder, the essential amino acids, must be obtained from food. Unless otherwise indicated, the pathways presented below are those operative in bacteria.

5.3.2. α-Ketoglutarate gives rise to glutamate, glutamine, proline, and arginine

The biosynthesis of glutamate and glutamine was described earlier in this chapter. The formation of proline, a cyclized derivative of glutamate, is shown in Fig. 5.5. In the first reaction, ATP reacts with the γ-carboxyl group of glutamate to form an acyl phosphate, which is reduced by NADPH to form glutamate γ-semialdehyde. This intermediate is then cyclized and reduced further to yield proline.

Arginine is synthesized from glutamate via ornithine and the urea cycle. Ornithine could also be synthesized from glutamate γ-semialdehyde by transamination, but the cyclization of the semialdehyde that occurs in the proline pathway is a rapid spontaneous reaction that precludes a sufficient supply of this intermediate for ornithine synthesis. The biosynthetic pathway for ornithine therefore parallels some steps of the proline pathway, but includes two additional steps to chemically block the amino group of glutamate γ-semialdehyde and prevent cyclization (Fig. 5.5). At the outset the α-amino group of glutamate is blocked by acetylation in a reaction involving acetyl-CoA, and after the transamination step the acetyl group is removed to yield ornithine. Most of the arginine formed in mammals is cleaved to form urea, a process that depletes the available arginine and makes it an essential amino acid in young animals that require higher amounts of amino acids for growth.

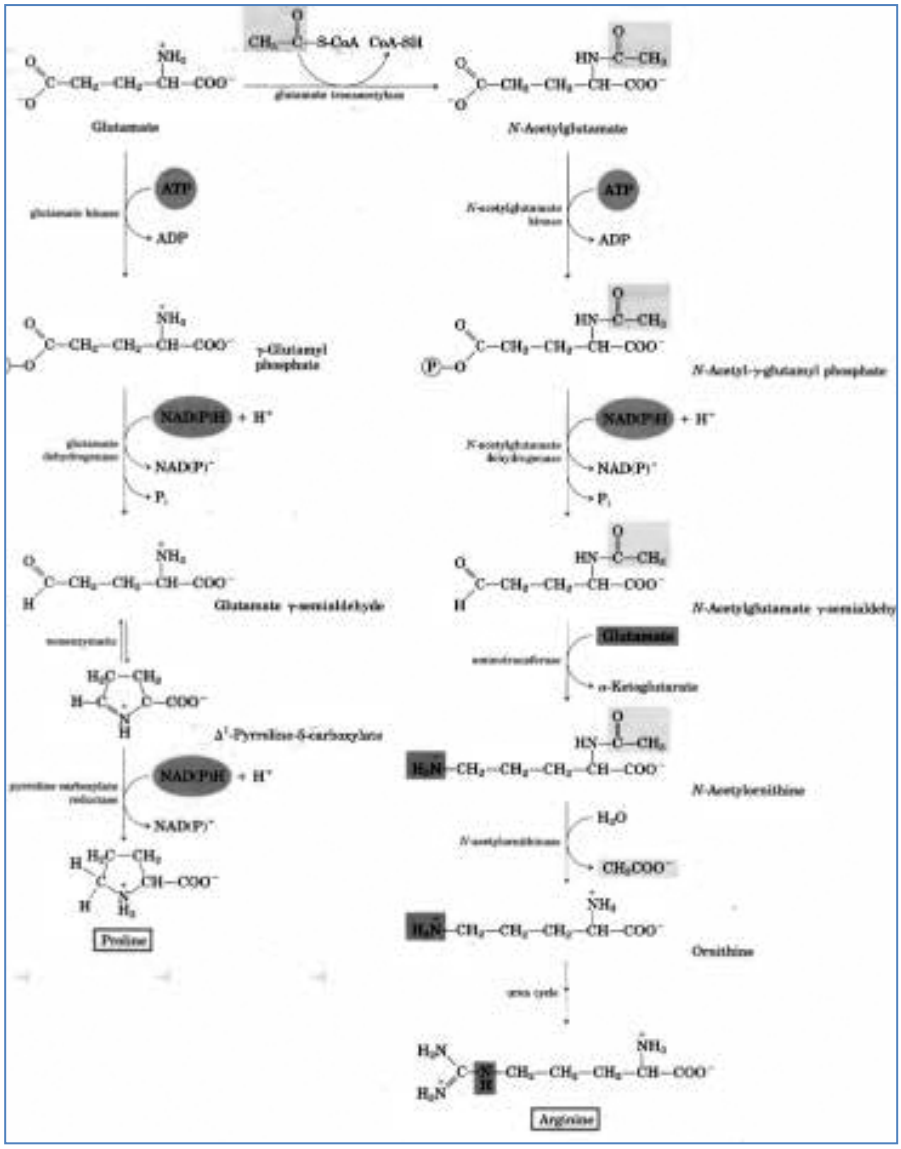


Fig. 5.5: Showing the production of proline and arginine from α-Ketoglutarate.

5.3.3. Serine, glycine, and cysteine are derived from 3-phosphoglycerate

The major pathway for the formation of serine is shown in Fig. 5.6. In the first step the hydroxyl group of 3-phosphoglycerate is oxidized by NAD+ to yield 3-phosphohydroxypyruvate. Transamination from glutamate yields 3-phosphoserine, which undergoes hydrolysis by phosphoserine phosphatase to yield free serine.

The three-carbon amino acid serine is the precursor of the twocarbon glycine through removal of one carbon atom by serine hydroxymethyl transferase (Fig. 5.6). Tetrahydrofolate is the acceptor of the β-carbon atom of serine during its cleavage to yield glycine. This carbon atom forms a methylene bridge between N-5 and N-10 of tetrahydrofolate to yield N5, N10 methylenetetrahydrofolate. The overall

reaction, which is reversible, also requires pyridoxal phosphate. In the liver of vertebrates, glycine can be made by another route catalyzed by the enzyme glycine synthase:

$CO_2 + NH_4^+ + NADH + H^+ + N_5,N_{10}$-methylenetetrahydrofolate $\longrightarrow$ glycine + NAD+ + tetrahydrofolate

In mammals, cysteine is made from two other amino acids: methionine furnishes the sulfur atom and serine furnishes the carbon skeleton. In a series of reactions the -OH group of serine is replaced by an -SH group derived from methionine to form cysteine. In the first reaction methionine is converted into S-adenosylmethionine. After the enzymatic transfer of the methyl group to any of a number of different acceptors, S-adenosylhomocysteine, the demethylated product, is hydrolyzed to free homocysteine. Homocysteine next reacts with serine in a reaction catalyzed by cystathionine β-synthase to yield cystathionine (Fig. 5.6 A). In the last step cystathionine-γ-lyase, a PLP-requiring enzyme, catalyzes the removal of ammonia and cleavage of cystathionine to yield free cysteine.

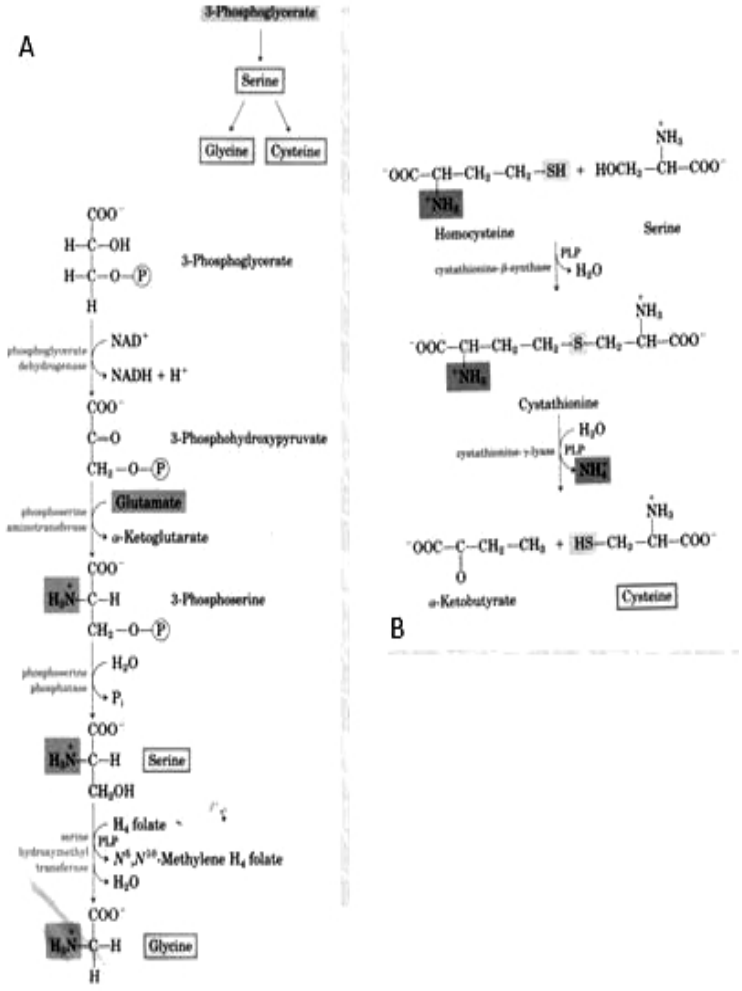


Fig. 5.6: A) Biosynthesis of serine from 3-phosphoglycerate and the subsequent conversion of serine into glycine. Glycine is also made from CO_2 and NH_4^+ by the action of glycine synthase, which uses N_5,N_{10}-

methylenetetrahydrofolate as methyl group donor. B) Biosynthesis of cysteine from homocysteine and serine.

5.3.4. Three nonessential and six essential amino acids are synthesized from oxaloacetate and pyruvate

Alanine and aspartate are synthesized from pyruvate and oxaloacetate, respectively, by transamination from glutamate. Asparagine is then synthesized by amidation of aspartate, with glutamine donating the NH_4^+ . These amino acids are nonessential and their simple biosynthetic pathways are found in all organisms.

The amino acids methionine, threonine, lysine, isoleucine, valine, and leucine are essential amino acids. The biosynthetic pathways for these amino acids are complex and interconnected. In some cases there are significant differences in the pathways present in bacteria, fungi, and plants. Aspartate gives rise to methionine, threonine, and lysine. Branch points occur at aspartate-β-semialdehyde, an intermediate in all three pathways, and at homoserine, a precursor of threonine and methionine. Threonine, in turn, is one of the precursors of isoleucine. The valine and isoleucine pathways share four enzymes. Pyruvate gives rise to valine and isoleucine in pathways that begin with the condensation of two carbons of pyruvate (in the form of hydroxyethyl thiamine pyrophosphate; see Fig. 5.7) with another molecule of pyruvate or with α-ketobutyrate (isoleucine path). The α-ketobutyrate is derived from threonine in a reaction that requires pyridoxal phosphate. An intermediate in the valine pathway, a-ketoisovalerate, is the starting point for a four-step branch pathway leading to leucine.

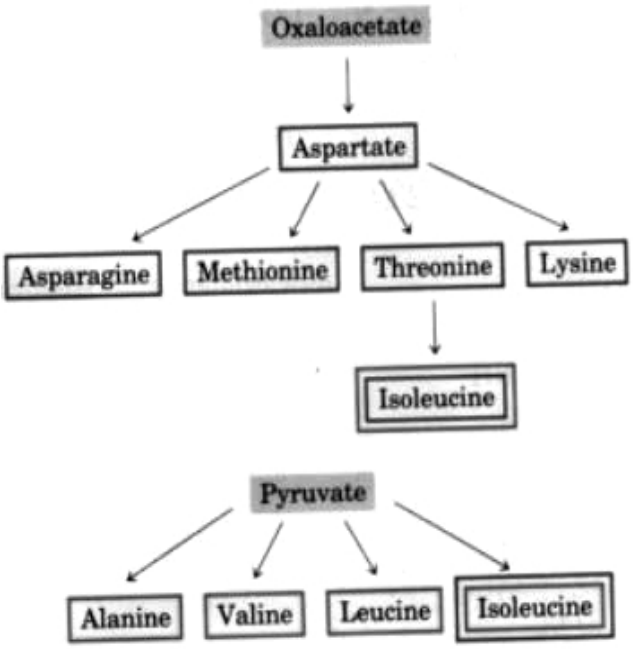


Fig. 5.7: Showing the production of different amino acids from Pyruvate and Oxoloacetate.

5.4. Chorismate is a key intermediate in the synthesis of tryptophan, phenylalanine, and tyrosine

Tryptophan, phenylalanine, and tyrosine are synthesized in bacteria by well-understood pathways that share a number of early steps. The first four steps result in the production of shikimate, in which the seven carbons are derived from erythrose-4-phosphate and phosphoenolpyruvate (Fig. 5.8). Shikimate is converted to chorismate in three more steps that include the addition of three more carbons from another molecule of phosphoenolpyruvate. Chorismate is the first branch point, with one branch leading to tryptophan and the other to phenylalanine and tyrosine.

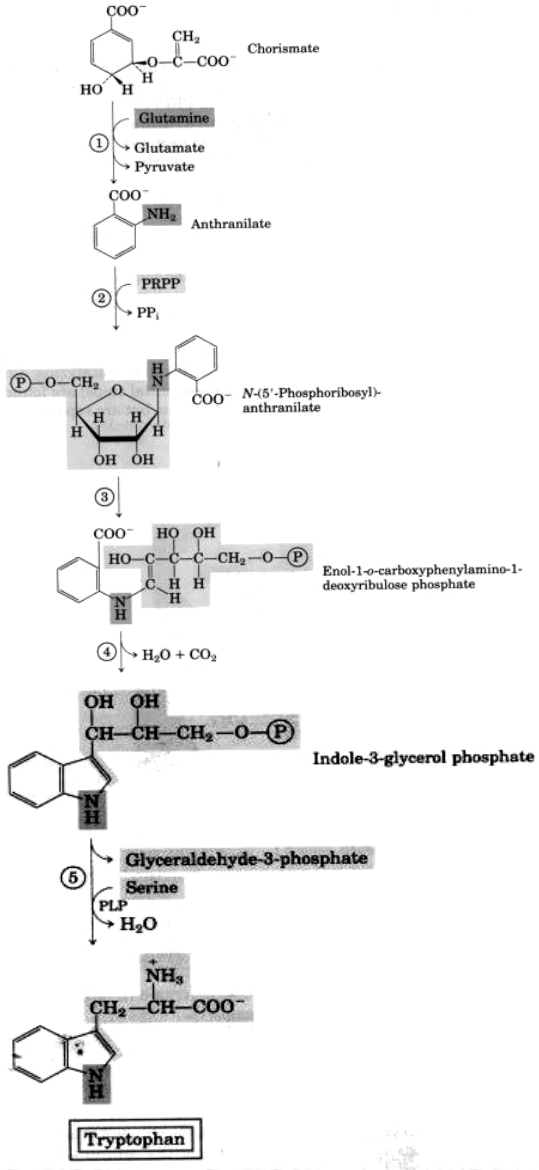


Fig. 5.8: Synthesis of chorismate, a key intermediate in the synthesis of the aromatic amino acids. All carbons are derived from either

erythrose-4-phosphate (purple) or phosphoenolpyruvaten (red). The pathway enzymes are: (l) 2-keto-3-deoxy- D-arabinoheptulosonate-7-phosphate synthase, (2) dehydroquinate synthase, (3) 5-dehydroquinate dehydratase, (4) shikimate dehydrogenase, (5) shikimate kinase, 3-enoylpyruvylshikimmate-5phosphate synthase, and (7) chorismate synthase. Note that step (2) requires NAD+ as a cofactor, and NAD+ is released unchanged. It may be transiently reduced to NADH during the reaction, to produce an oxidized reaction intermediate.

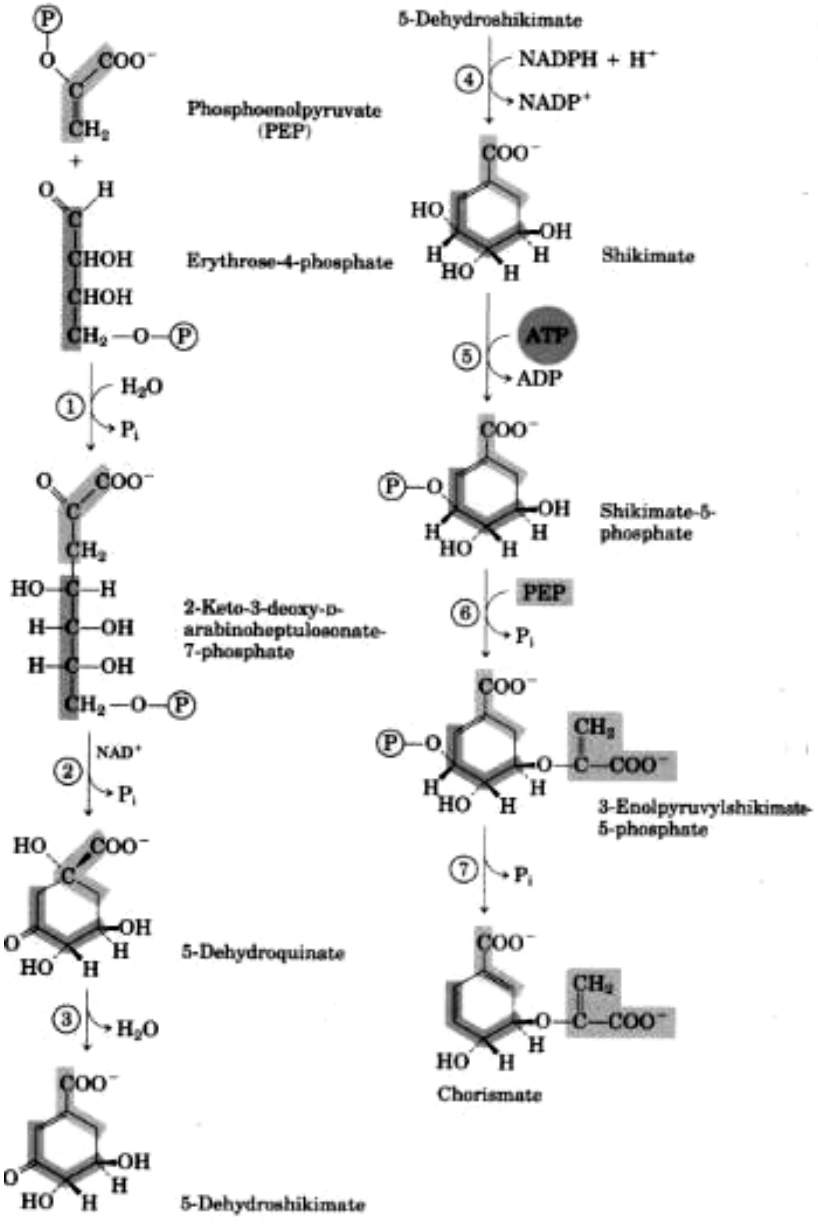
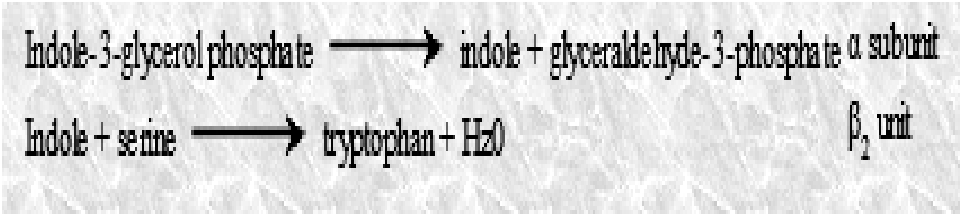


Fig. 5.9: Synthesis of chorismate, a key intermediate in the synthesis of the aromatic amino acids. All carbons are derived from either erythrose-4-phosphate (purple) or phosphoenolpyruvate (red). The pathway enzymes are: (l) 2-keto-3-deoxy- D-arabinoheptulosonate-7-

phosphate synthase, (2) dehydroquinate synthase, (3) 5-dehydroquinate dehydratase, (4) shikimate dehydrogenase, (5) shikimate kinase, 3-enoylpyruvylshikimmate-5phosphate synthase, and (7) chorismate synthase. Note that step (2) requires NAD+ as a cofactor, and NAD+ is released unchanged. It may be transiently reduced to NADH during the reaction, to produce an oxidized reaction intermediate.

On the tryptophan branch (Fig. 5.9), chorismate is converted first to anthranilate. In this reaction, glutamine donates a nitrogen that ultimately becomes part of the completed indole ring.

Anthranilate then condenses with PRPP. The indole ring of tyrptophan is derived from the ring carbons and amino group of anthranilate plus two carbons derived from PRPP. The final reaction in the sequence is catalyzed by tryptophan synthase, an enzyme with an α2β2 subunit structure. The enzyme can be dissociated into two a subunits and a β2 unit that catalyze different parts of the overall reaction:



The second part of the reaction requires a pyridoxal phosphate cofactor. Indole is rapidly channeled from the α-subunit active site to the β-subunit active site, where it is condensed with a Schiff base intermediate derived from serine and PLP. This kind of channeling of intermediates may be a feature of the entire pathway from chorismate to tryptophan. Enzyme active sites catalyzing different steps of the pathway (sometimes not sequential steps) are found on single polypeptides in some fungi and bacteria, but are separate proteins in others. In addition, the activity of some of these enzymes requires a noncovalent association with other enzymes of the pathway. These observations suggest that all are parts of a large multienzyme complex in both prokaryotes and eukaryotes. Although such complexes are generally not preserved intact when the enzymes are isolated using traditional biochemical methods, evidence for the existence of multienzyme complexes in cells is accumulating for a number of metabolic pathways.

Phenylalanine and tyrosine are synthesized from chorismate in plants and microorganisms via simpler pathways using the common intermediate prephenate (Fig. 5.10). The paths branch at prephenate and the final step in both cases is transamination with glutamate as amino group donor.

Tyrosine can also be made by animals directly from phenylalanine via hydroxylation at C-4 of the phenyl group by phenylalanine hydroxylase, which also participates in the degradation of phenylalanine. Tyrosine is considered a nonessential amino acid only because it can be synthesized from the essential amino acid phenylalanine.

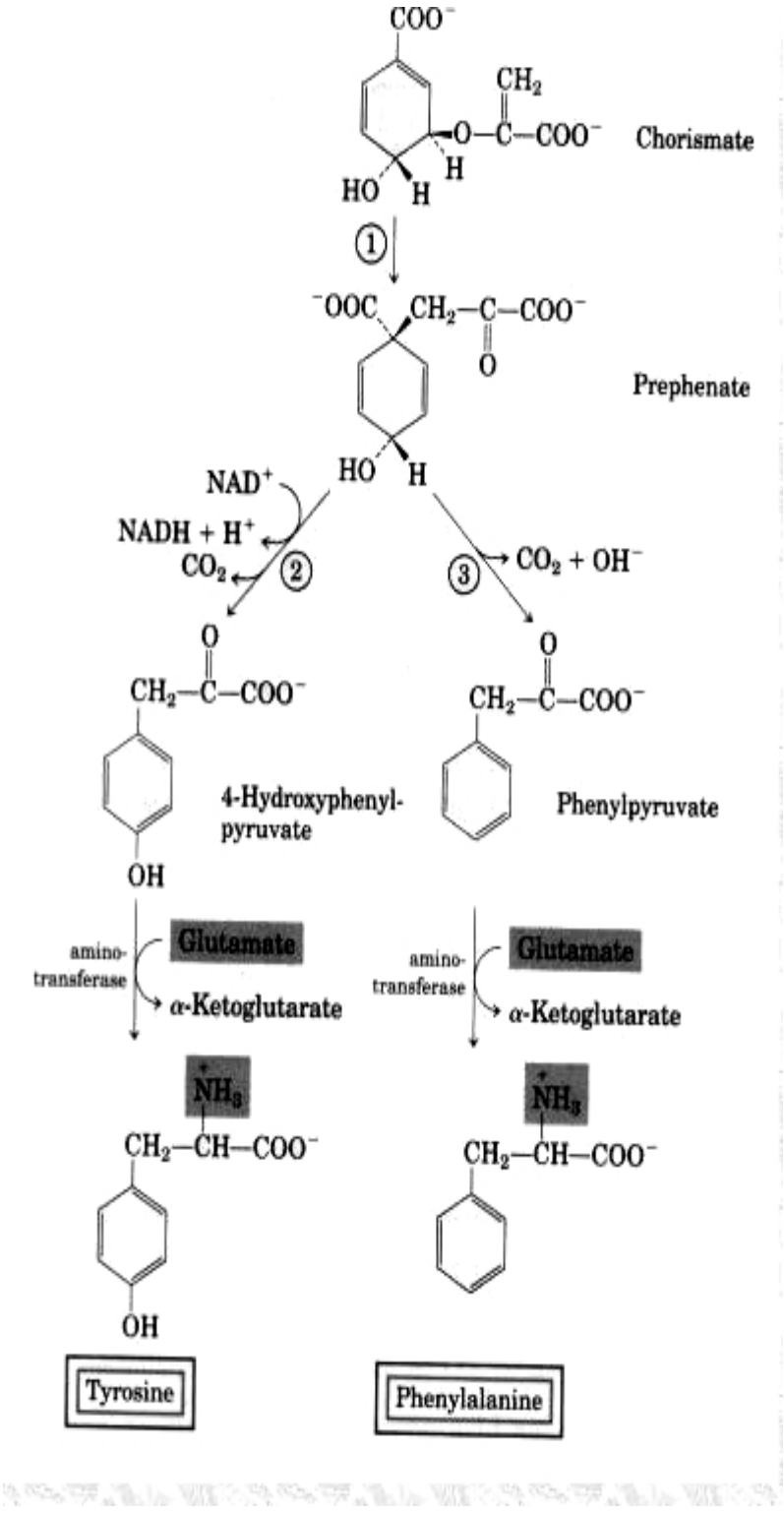


Fig. 5.10: Biosynthesis of phenylalanine and tyrosine from chorismate. The enzymes are: (l) chorismate mutase, (2) prephenate dehydrogenase, and (3) prephenate dehydratase.

5.4.1. Histidine biosynthesis uses precursors of purine biosynthesis

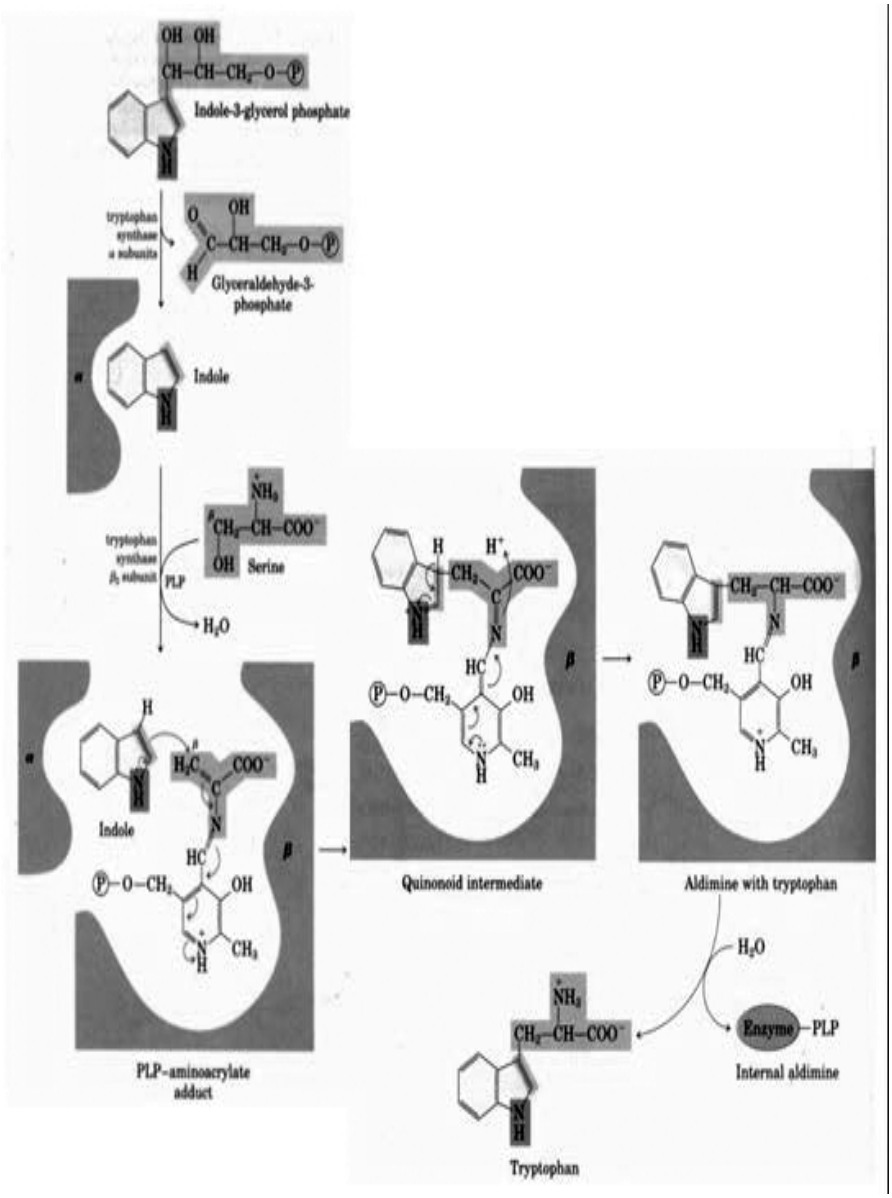


Fig. 5.11: Biosynthesis of histidine.

Two of the histidine nitrogens are derived from glutamine and glutamate (green). The pathway enzymes are: (1) ATP phosphoribosyl transferase, (2) pyrophosphoh drolase (3) phosphoribosyl-AMP cyclohydrolase, (4) phosphoribosylformimino-5-aminoimidazole-4-carboxamide ribonucleotide isomerase, (5) glutamine amidotransferase, (6) imidazole glycerol-3-phosphate dehydratase, (7) L-histidinol phosphate aminotransferase, (8) histidinol phosphate phosphatase, and (9) histidinol dehydrogenase. Note that the derivative of ATP remaining after step (5) is an intermediate in purine biosynthesis, so that ATP is rapidly regenerated.

The histidine biosynthetic pathway in all plants and bacteria is novel in several respects. Histidine is derived from three precursors (Fig. 5.11): PRPP contributes five carbons, the purine ring of ATP contributes a nitrogen and a carbon, and the second ring nitrogen comes from glutamine. The key steps are the condensation of ATP and PRPP (N-1 of the purine ring becomes linked to the activated C-1 of the ribose in PRPP) (step l in Fig. 5.11), purine ring opening that ultimately leaves N-1 and C-2 linked to the ribose (step 3), and formation of the imidazole ring in a reaction during which glutamine donates a nitrogen (step 5). The use of ATP as a metabolite rather than a highenergy cofactor is unusual, but not wasteful because it dovetails with the purine biosynthetic pathway. The remnant of ATP that is released after the transfer of N-1 and C-2 is 5-aminoimidazole-4-carboxamide ribonucleotide, an intermediate in the biosynthesis of purines that can rapidly be recycled to ATP.

5.5. Amino acid biosynthesis is under allosteric regulation

The most responsive manner in which amino acid synthesis is controlled is through feedback inhibition of the first reaction in the biosynthetic sequence by its fmal end product. The first reaction of such a sequence, which is usually irreversible, is catalyzed by an allosteric enzyme. As an example, Fig. 5.12 shows the allosteric regulatior of the synthesis of isoleucine from threonine. The end product, isoleucine, is a negative modulator of the firs~ reaction in the sequence. Such allosteric or noncovalent modulation o amino acid synthesis is responsive on a minute-to-minute basis in bacteria.

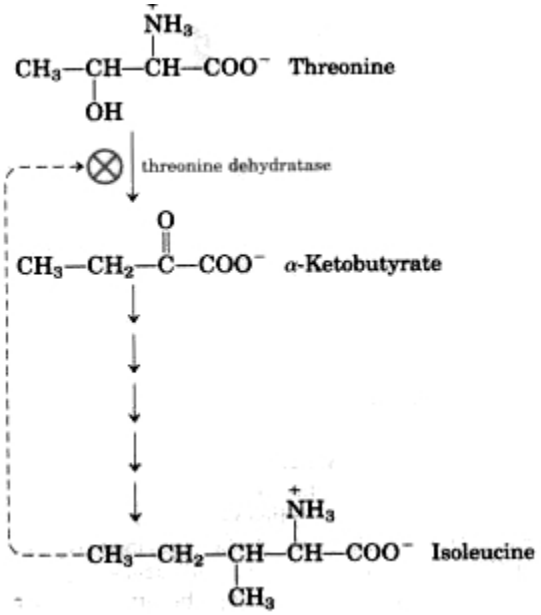


Fig. 5.12: The first reaction in the pathway leading from threonine to isoleucine is inhibited by the end product, isoleucine. This was one of

the first examples of allosteric feedback inhibition to be discovered. The steps from α-ketobutyrate to isoleucine.

Allosteric regulation can be considerably more complex. For An example the remarkable set of allosteric controls exerted on the activity of glutamine synthetase of *E. coli*. Six products of glutamim metabolism in E. coli are now known to serve as negative feedback modulators of the activity of glutamine synthetase, and the overall effects of these and other modulators are more than additive. This kind of regulation is called concerted inhibition.

Because the 20 amino acids must be made in the correct proportions for protein synthesis, cells have developed ways not only of controlling the rate of synthesis of individual amino acids but also co-ordinating their formation. Such coordination is especially well developed in fast-growing bacterial cells. Cells coordinate the synthesis of lysine, methionine, threonine, and isoleucine, all made from aspartate. Several important types of inhibition patterns are evident. The step from aspartate to aspartyl-β-phosphate is catalyzed by three isozyme forms, each of which can be independently controlled by different modulators. This enzyme multiplicity prevents one biosynthetic end product from shutting down key steps in a pathway when other products of the same pathway are required. The steps from aspartate-β-semialdehyde to homoserine and from threonine to α-ketobutyrate (Fig. 5.6) are also catalyzed by dual, independently controlled isozymes. One of the isozymes for the conversion of aspartate to aspartyl-β-phosphate can be allosterically inhibited by two different modulators, lysine and isoleu~ine, whose action is more than additive. This is another example of concerted inhibition. The sequence from aspartate to isoleucine shows multiple, overlapping negative feedback inhibition; for example, isoLeucine inhibits the conversion of threonine to α-ketobutyrate (as described above), and threonine inhibits its own formation at three points: from homoserine, from aspartate-β-semialdehyde, and from aspartate. This overall action is called sequential feedback inhibition.

5.6. Transamination, deamination and decarboxylation

A amino groups are removed from amino acids by a process called transamination. The acceptor for this reaction is usually the α keto acid called α ketoglutarate which results in the formation of glutamate and the corresponding α keto acid. The coenzyme of all transaminases is pyridoxal phosphate which is derived from vitamin B6 and which is transiently converted during transamination into pyridoxamine phosphate. The glutamate produced by transamination is oxidatively deaminated by glutamate dehydrogenase to produce ammonia. This enzyme is unusual in being able to use either NAD+ or NADP+, and is subject to allosteric regulation. GTP and ATP are allosteric inhibitors, whereas GDP and ADP

are allosteric activators. Small amounts of amino acids are degraded by L- and D-amino acid oxidases that utilize flavin mononucleotide (FMN) or flavin adenine dinucleotide (FAD) as coenzyme, respectively.

Some of the key reaction and important factors are asunder:

- ❖ Protein metabolism is a key physiological process in all forms of life.
- ❖ Proteins are converted to amino acids and then catabolised.
- ❖ The complete hydrolysis of a polypeptide requires mixture of peptidases because individual peptidases do not cleave all peptide bonds.
- ❖ Both exopeptidases and endopeptidases are required for complete conversion of protein to amino acids.
- ❖ The amino acids not only function as energy metabolites but also used as precursors of many physiologically important compounds such as heme, bioactive amines, small peptides, nucleotides and nucleotide coenzymes.
- ❖ In normal human beings about 90% of the energy requirement is met by oxidation of carbohydrates and fats. The remaining 10% comes from oxidation of the carbon skeleton of amino acids.
- ❖ Since the 20 common protein amino acids are distinctive in terms of their carbon skeletons, amino acids require unique degradative pathway.
- ❖ The degradation of the carbon skeletons of 20 amino acids converges to just seven metabolic intermediates namely.

  i. Pyruvate, ii. Acetyl CoA,

  ii. Acetoacetyl CoA,

  iii. α-Ketoglutarate,

  iv. Succinyl CoA,

  v. Fumarate,

  vi. Oxaloacetate**.**

The important reaction commonly employed in the breakdown of an amino acid is always the removal of its □-amino group. The product ammonia is excreted after conversion to urea or other products and the carbon skeleton is degraded to CO_2 releasing energy. The important reactions involved in the deamination of amino acids are:

i. Transamination

ii. Oxidative deamination

iii. Non oxidative deamination

Most amino acids are deaminated by transamination reaction catalysed by aminotransferases or transaminases. The amino group present in an amino acid is transferred to keto acid to yield a new amino acid and the α-keto acid of the original amino acid. The predominant amino group acceptor is α-keto glutarate. Glutamate's amino group is then transferred to oxaloacetate in a second transamination reaction yielding aspartate.

Glutamate + oxaloacetate ⟶ ketoglutarate + aspartate + pyridoxal phosphate

Pyridoxal phosphate, the coenzyme of pyridoxine (vitamin B6) plays an important role in these reactions. Amino transferase reactions occur in two stages. Pyridoxal phosphate is covalently attached to the amino transferases via a Schiff's base linkage formed between the aldehyde group of pyridoxal phosphate and the epsilon amino group of lysine residue of the enzyme. Pyridoxal phosphate is converted to pyridoxamine phosphate. In the second stage, the amino group attached to pyridoxamine phosphate is transferred to a different keto acid to yield a new amino acid and releases pyridoxal phosphate

Oxidative deamination

Transamination does not result in net deamination, since one amino acid is replaced by another amino acid. The function of transamination is to funnel the amino nitrogen into one or a few amino acids. For glutamate to play a role in the net conversion of amino groups to ammonia, a mechanism for glutamate deamination is needed so that □-ketoglutarate can be regenerated for further transamination. The generation is accomplished by the oxidative deamination of glutamate by glutamate dehydrogenase. Glutamate is oxidatively deaminated in the mitochondrion by glutamate dehydrogenase. NAD+ or NADP+ functions as the coenzyme. Oxidation is thought to occur with the transfer of a hydride ion from glutamate's carbon to NAD(P)+ to form □-iminoglutarate, which is then hydrolysed to α-ketoglutarate and ammonia. The ammonia produced is then converted to urea in mammals.

Two non-specific amino acid oxidases namely, L-amino acid and D-amino acid oxidases catalyse the oxidation of L and D-amino acids utilizing FAD as their coenzymes.

Amino acid + FAD + H_2O ⟶ α-Keto acid + NH_3 + $FADH_2$

Non-oxidative deamination

Amino acids such as serine and histidine are deaminated non-oxidatively. The other reactions involved in the catabolism of amino acids are decarboxylation, transulfuration, desulfuration and dehydration etc.

Decarboxylation

The decarboxylation process is important since the products of decarboxylation reactions give rise to physiologically active amines. The

enzymes, amino acid decarboxylases are pyridoxal phosphatedpendent enzymes. Pyridoxal phosphate forms a Schiff's base with the amino acid so as to stabilise the carbanion formed by the cleavage of bond between carboxyl and α carbon atom. The physiologically active amines epinephrine, nor-epinephrine, dopamine, serotonin, α-amino butyrate and histamine are formed through decarboxylation of the corresponding precursor amino acids.

5.7. Amino acid oxidation and production of urea

The major site of amino acid degradation in mammals is the liver. The α-ketoacids that result from the deamination of amino acids are metabolized so that the carbon skeletons can enter the metabolic mainstream as precursors to glucose or citric acid cycle intermediates. Oxidative deamination is a form of deamination that generates α-keto acids and other oxidized products from amine-containing compounds, and occurs only in the liver. This is a common pathway during amino acid catabolism.

Oxidative deamination is a form of deamination that generates α-keto acids and other oxidized products from amine-containing compounds, and occurs only in the liver. Oxidative deamination is an important step in the catabolism of amino acids, generating a more metabolizable form of the amino acid, and also generating ammonia as a toxic byproduct. The ammonia generated in this process can then be neutralized into urea via the urea cycle.

Much of the oxidative deamination occurring in cells involves the amino acid glutamate, which can be oxidatively deaminated by the enzyme glutamate dehydrogenase (GDH), using NAD or NADP as a coenzyme. This reaction generates α-ketoglutarate (α-KG) and ammonia. Glutamate can then be regenerated from α-KG via the action of transaminases or aminotransferase, which catalyze the transfer of an amino group from an amino acid to an α-keto acid. In this manner, an amino acid can transfer its amine group to glutamate, after which GDH can then liberate ammonia via oxidative deamination. This is a common pathway during amino acid catabolism.

Another enzyme responsible for oxidative deamination is monoamine oxidase, which catalyzes the deamination of monoamines via addition of oxygen. This generates the corresponding ketone- or aldehyde-containing form of the molecule, and generates ammonia. Monoamine oxidases MAO-A and MAO-B play vital roles in the degradation and inactivation of monoamine neurotransmitters such as serotonin and epinephrine. Monoamine oxidases are important drug targets, targeted by MAO inhibitors (MAOIs) such as selegiline.

Amino acids, derived largely from protein in the diet or from degradation of intracellular proteins, are the final class of biomolecules whose oxidation makes a significant contribution to the generation of

metabolic energy. The fraction of metabolic energy derived from amino acids varies greatly with the type of organism and with the metabolic situation in which an organism finds itself. Carnivores, immediately following a meal, may obtain up to 90% of their energy requirements from amino acid oxidation. Herbivores may obtain only a small fraction of their energy needs from this source. Most microorganisms can scavenge amino acids from their environment if they are available; these can be oxidized as fuel when required by metabolic conditions. Photosynthetic plants, on the other hand, rarely, if ever, oxidize amino acids to provide energy. Instead, they convert CO_2 and H_2O into the carbohydrate that is used almost exclusively as an energy source. The amounts of amino acids in plant tissues are carefully regulated to just meet the requirements for biosynthesis of proteins, nucleic acids, and a few other molecules needed to support growth. Amino acid catabolism does occur in plants, but it is generally concerned with the production of metabolites for other biosynthetic pathways.

In animals, amino acids can undergo oxidative degradation in three different metabolic circumstances:

1. During the normal synthesis and degradation of cellular proteins some of the amino acids released during protein breakdown will undergo oxidative degradation if they are not needed for new protein synthesis.

2. When a diet is rich in protein, and amino acids are ingested in excess of the body's needs for protein synthesis, the surplus may be catabolised as amino acids cannot be stored.

3. During starvation or in diabetes mellitus, when carbohydrates are either unavailable or not properly utilized, body proteins are called upon as fuel. Under these different circumstances, amino acids loose their amino groups, and the α-keto acids so formed may undergo oxidation to CO_2 and H_2O. In addition, and often equally important, the carbon skeletons of the amino acids provide three- and four-carbon units that can be converted to glucose, which in turn can fuel the functions of the brain, muscle, and other tissues.

Amino acid degradative pathways are quite similar in most organisms. The focus of this chapter is on vertebrates, because amino acid catabolism has received the most attention in these organisms. As is the case for sugar and fatty acid catabolic pathways, the processes of amino acid degradation converge on the central catabolic pathways for carbon metabolism. The carbon skeletons of the amino acids generally find their way to the citric acid cycle, and from there they are either oxidized to produce chemical energy or funneled into gluconeogenesis. In some cases the reaction pathways closely parallel steps in the catabolism of fatty acids.

However, one major factor distinguishes amino acid degradation from the catabolic processes described to this point: every amino acid contains an amino group. Every degradative pathway therefore passes

through a key step in which the a-amino group is separated from the carbon skeleton and shunted into the specialized pathways for amino group metabolism in previous sections. This biochemical fork in the road is the point around which this chapter is organized.

5.7.1. Metabolic fates of amino groups

Nitrogen ranks fourth, behind carbon, hydrogen, and oxygen, in its contribution to the mass of living cells. Atmospheric nitrogen, N_2, is abundant but is too inert for use in most biochemical processes. Because only a few microorganisms can convert N_2 to biologically useful forms such as NH_3, amino groups are used with great economy in biological systems.

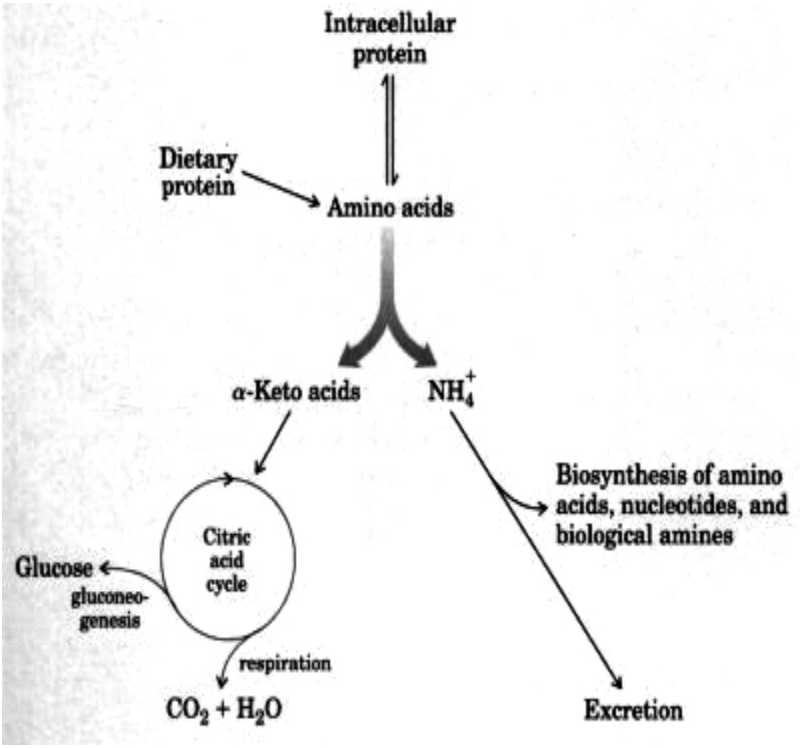


Fig. 5.12: Overview of the catabolism of amino acids. The separate paths taken by the carbon skeleton and the amino groups are emphasized.

An overview of the catabolism of ammonia and amino groups in vertebrates is provided in Fig. 5.12. Amino acids derived from dietary proteins are the source of most amino groups. Most of the amino acids are metabolized in the liver. Some of the ammonia that is generated is recycled and used in a variety of biosynthetic processes; the excess is either excreted directly or converted to uric acid or urea for excretion, depending on the organism. Excess ammonia generated in other (extrahepatic) tissues is transported to the liver (in the form of amino groups, as described below) for conversion to the appropriate excreted form. With these reactions one encounters the coenzyme pyridoxal phosphate, the functional form of vitamin B6 and a coenzyme of major importance in nitrogen metabolism.

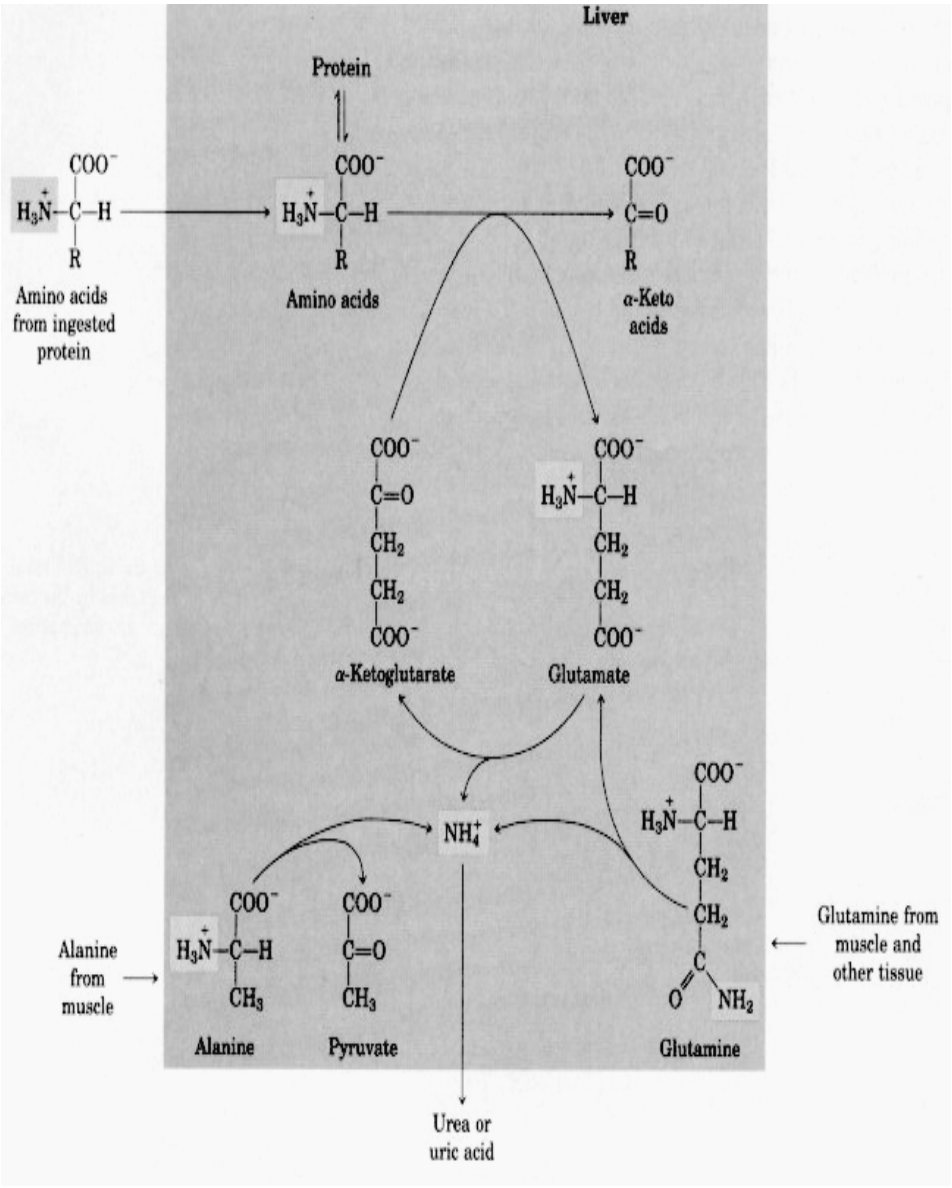


Fig. 5.13: Overview of amino group catabolism in the vertebrate liver (shaded). Excess NH4+ is excreted as urea or uric acid.

The amino acids glutamate and glutamine play especially critical roles in these pathways (Fig. 5.13). Amino groups from amino acids are generally first transferred to α-ketoglutarate in the cytosol of liver cells (hepatocytes) to form glutamate. Glutamate is then transported into the mitochondria; only here is the amino group removed to form NH_4^+. Excess ammonia generated in most other tissues is converted to the amide nitrogen of glutamine, and then transported to liver mitochondria. In most tissues, one or both of these amino acids are found in elevated concentrations relative to other amino acids.

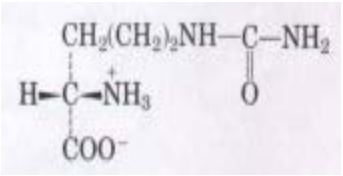
In muscles, excess amino groups are generally transferred to pyruvate to form alanine. Alanine is another important molecule in the transport of amino groups, conveying them from muscle to the liver.

5.8. Summary

A small fraction of oxidative energy in humans comes from the catabolism of amino acids. Amino acids are derived from the normal breakdown (recycling) of cellular proteins, degradation of ingested proteins, or breakdown of body proteins in lieu of other fuel sources during starvation or in untreated diabetes mellitus. Ingested proteins are degraded in the stomach and small intestine by proteases. Most proteases are initially synthesized as inactive zymogens, which are activated in the stomach or intestine by proteolytic removal of parts of their polypeptide chains. An early step in the catabolism of amino acids is the separation of the amino group from the carbon skeleton. In most cases, the amino group is transferred to α-ketoglutarate to form glutamate. This type of reaction is called a transamination and requires the coenzyme pyridoxal phosphate. Glutamate is transported to liver mitochondria, where an amino group is liberated as ammonia ($NH4^+$) by the enzyme glutamate dehydrogenase. Ammonia formed in other tissues is transported to liver mitochondria as the amide nitrogen of glutamine or as the amino group of alanine. Most of the alanine is generated in muscle and transported in the blood to the liver. After deamination the resulting pyruvate is converted to glucose, which is transported back to muscle as part of the glucose-alanine cycle.

5.9. Terminal questions

Q.1. Absolute Configuration of Citrulline is citrulline isolated from watermelons (shown below) a D- or L-amino acid? Explain?



Answer: ----------

Q.2. Preparation of a Glycine Buffer Glycine is commonly used as a buffer. Preparation of a 0.1 M glycine buffer starts with 0.1 M solutions of glycine hydrochloride ($HOOC\text{-}CH_2\text{-}NH_3{+}Cl^-$) and glycine ($^-OOC\text{-}CH_2\text{-}NH_3$), two commercially available forms of glycine What volumes of these two solutions must be mixed to prepare 1 L of 0.1 M glycine buffer having a pH of 3.2?

Answer: ----------

Q.3. Naming the Stereoisomers of Isoleucine. In the structure of the amino acid isoleucine is:

Answer: ----------

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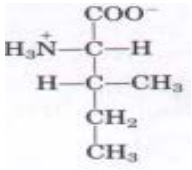
a. How many chiral centers does it have?

b. How many optical isomers are present.

c. Draw perspective formulas for all the optical isomers of isoleucine.



Answer: ----------

Q.4. Nucleotide Biosynthesis in Amino Acid Auxotrophic Bacteria although normal *E. coli* cells can synthesize all the amino acids, some mutants, called amino acid auxotrophs, are unable to synthesize specific amino acids and require the addition of that amino acid to the culture medium for optimal growth. In addition to their role in protein synthesis, specific amino acids are also required in the biosynthesis of other nitrogenous cell products. Consider the three amino acid auxotrophs that are unable to synthesize glycine, glutamine, and aspartate, respectively. For each mutant what nitrogenous cell products other than proteins would fail to be synthesized?

Answer: ----------

Q.5. In the treatment of Gout Allopurinol, an inhibitor of xanthine oxidase, is used to treat chronic gout. Explain the biochemical basis for this treatment. Patients treated with allopurinol sometimes develop xanthine stones in the kidneys, although the incidence of kidney damage is much lower than in untreated gout. Explain this observation in light of the following solubilities in urine: uric acid, 0.15 g/L; xanthine, 0.05 g/L; and hypoxanthine, 1.4 g/L.

Answer: ----------

Q.6. Nucleotides Are Poor Sources of Energy In most organisms, nucleotides are not employed as energy-yielding fuels. What observations support this conclusion? Why are nucleotides relatively poor sources of energy in mammals?

Answer: ----------

5.10. Further readings

1. A.L. Lehninger, Principles of Biochemistry, 4th edition, W.H Freeman and Company, 2004.
2. L. Stryer, Biochemistry, 5th edition, W.H. Freeman and Company, 2002.
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UNIT-6

METABOLISM OF NITROGEN COMPOUNDS

Structure

6.1. Introduction

The molecular nitrogen that makes up 80% of the earth's atmosphere is unavailable to living organisms until it is reduced. Fixation of atmospheric N2 takes place in certain free-living soil bacteria and in symbiotic bacteria in the root nodules of leguminous plants, by the action of the complex nitrogenase system. Formation of ammonia by bacterial fixation of N2, nitrification of ammonia to form nitrate by soil organisms, conversion of nitrate to ammonia by higher plants, synthesis of amino acids from ammonia by plants and animals, and conversion of nitrate to N_2 by some soil bacteria in the process of Denitrification. The fixation of N_2 as NH3 is carried out by a protein complex called the nitrogenase complex, in a reaction that requires ATP. The nitrogenase complex is very labile in the presence of O_2. In living systems, reduced nitrogen is incorporated first into amino acids and then into a variety of other biomolecules including nucleotides. The key entry point is the amino acid glutamate. Glutamate and glutamine are the nitrogen donors in a wide variety of biosynthetic reactions. Glutamine synthetase, which catalyzes the formation of glutamine from glutamate, is a key regulatory enzyme of nitrogen metabolism.

Objectives

- Describe the modes of nitrogen fixation (both biological and abiological
- Explain the steps involved in nitrogen fixation by free living organisms
- Explain the mode of symbiotic nitrogen fixation in leguminous plants
- Describe the assimilation of nitrate and ammonia by plants
- Describe amino acid synthesis in plants.

6.2. Introduction for nitrogen fixation

The amino acid and nucleotide biosynthetic pathways make repeated use of the biological cofactors pyridoxal phosphate, tetrahydrofolate, and S-adenosylmethionine. Pyridoxal phosphate is required for transamination reactions involving glutamate and for a number of other amino acid transformations. One-carbon transfers are carried out using S-adenosylmethionine (at the -CH3 oxidation level) and tetrahydrofolate (usually at the -CHO and -CH2OH oxidation levels). Enzymes called glutamine amidotransferases are used in reactions that incorporate nitrogen derived from glutamine.

Mammals (e.g., humans and the albino rat) can synthesize 10 of the 20 amino acids of proteins. The remainder, which are required in the diet (essential amino acids), can be synthesized by plants and bacteria. Among the nonessential amino acids, glutamate is formed by reductive amination of α-ketoglutarate and is the precursor of glutamine, proline, and arginine. Alanine and aspartate (and thus asparagine) are formed from pyruvate and oxaloacetate, respectively, by transamination. The carbon chain of serine is derived from 3-phosphoglycerate. Serine is a precursor of glycine; the β-carbon atom of serine is transferred to tetrahydrofolate. Cysteine is formed from methionine and serine by a series of reactions in which S-adenosylmethionine and cystathionine are intermediates. The aromatic amino acids (phenylalanine, tyrosine, and tryptophan) are formed via a pathway in which the intermediate chorismate occupies a key branch point. Phosphoribosyl pyrophosphate is a precursor of tryptophan and histidine, both essential amino acids. The biosynthetic pathway to histidine is interconnected with the purine synthetic pathway. Tyrosine can also be formed by hydroxylation of phenylalanine, an essential amino acid. The pathways for biosynthesis of the other essential amino acids in bacteria and plants are complex. The amino acid biosynthetic pathways are subject to allosteric end-product inhibition; the regulatory enzyme is usually the first in the sequence. The regulation of these synthetic pathways is coordinated.

Many other important biomolecules are derived from amino acids. Glycine is a precursor of porphyrins; porphyrins, in turn, are degraded to form bile pigments. Glycine and arginine give rise to creatine and phosphocreatine. Glutathione, a tripeptide, is an important cellular reducing agent. D-amino acids are synthesized from L-amino acids in bacteria in racemization reactions requiring pyridoxal phosphate. The PLP-dependent decarboxylation of certain amino acids yields some important biological amines, including neurotransmitters. The aromatic amino acids are precursors of a number of plant substances.

The purine ring system is built up in a step-bystep fashion on 5-phosphoribosylamine. The amino acids glutamine, glycine, and aspartate furnish all the nitrogen atoms of purines. Two ring-closure steps ensue to form the purine nucleus. Pyrimidines are synthesized from carbamoyl phosphate and aspartate. Ribose-5-phosphate is then attached to yield the

pyrimidine ribonucleotides. Purine and pyrimidine biosynthetic pathways are regulated by feedback inhibition. Nucleoside monophosphates are converted to their triphosphates by enzymatic phosphorylation reactions. Ribonucleotides are converted to deoxyribonucleotides by the action of ribonucleotide reductase, an enzyme with novel mechanistic and regulatory characteristics. The thymine nucleotides are derived from the deoxyribonucleotides dCDP and dUMP. Uric acid and urea are the end products of purine and pyrimidine degradation. Free purines can be salvaged and rebuilt into nucleotides by a separate pathway. Genetic deficiencies in certain salvage enzymes cause serious genetic diseases such as Lesch-Nyhan syndrome and severe immunodeficiency disease. Another genetic deficiency results in the accumulation of uric acid crystals in the joints, causing gout. The enzymes of the nucleotide biosynthetic pathways are targets for an array of chemotherapeutic agents used to treat cancer and other diseases..

6.2.1. Overview of nitrogen metabolism

The biosynthetic pathways to the amino acids and nucleotides share a requirement for nitrogen, but soluble, biologically useful nitrogen compounds are generally scarce in natural environments. For this reason ammonia, amino acids, and nucleotides are used economically by most organisms. Indeed, it will be seen that free amino acids, purines, and pyrimidines, formed during metabolic turnover, are often salvaged and reused. The pathways by which nitrogen from the environment is introduced into biological systems are hereby mentioned.

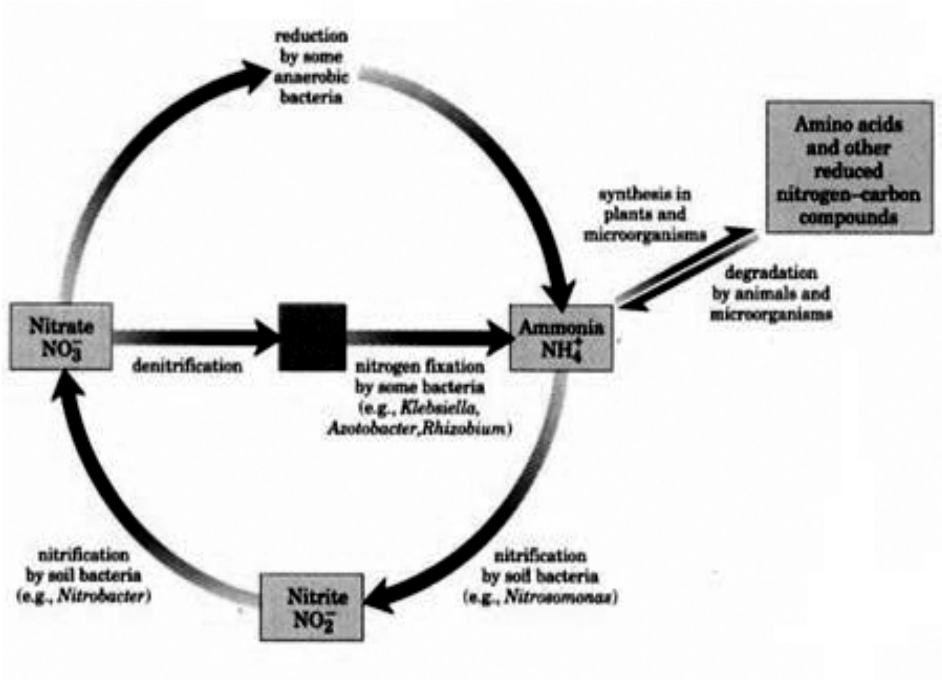

Fig. 6.1: The nitrogen cycle. The total amount of nitrogen fixed annually in the biosphere exceeds 10^{11} kg.

6.2.2. The nitrogen cycle maintains a pool of biologically available nitrogen

Only a relatively few species of microorganisms, all of them prokaryotes, can six atmospheric nitrogen. The cyanobacteria, which inhabit soils and fresh and salt waters, as well as other kinds of free-living soil bacteria, such as Azotobacter species, are capable of fixing atmospheric nitrogen. Other nitrogen-fixing bacteria live as symbionts in the root nodules of leguminous plants. The first important product of nitrogen fixation in all of these organisms is ammonia, which can be used by other organisms, either directly or after its conversion into other soluble compounds, such as nitrites, nitrates, or amino acids.

The reduction of nitrogen to ammonia is an exergonic reaction:

$$N_2 + 3H_2 \quad 2NH_3 \longrightarrow \Delta G^{\circ\prime} = -33.5 \text{ kJ/mol}$$

The N≡N triple bond, however, is very stable, with a bond energy of 942 kJ/mol. Nitrogen fixation therefore has an extremely high activation energy, and atmospheric nitrogen is almost chemically inert under normal conditions. Ammonia is produced industrially by the Haber process (named for Fritz Haber, who invented it in 1910), which uses temperatures of 400 to 500 °C and pressures of tens of thousands of kilopascals (several hundred atmospheres) of N_2 and H_2 to provide the necessary activation energy. Biological nitrogen fixation must occur at 0.8 atm of nitrogen, and the high activation barrier is overcome, at least in part, by the binding and hydrolysis of ATP (described below). The overall reaction can be written as under:

$$N_2 + lOH^+ + 8e^- + l6ATP \longrightarrow 2NH_4+ + l6ADP + 16Pi + H_2$$

Biological nitrogen fixation is carried out by a highly conserved complex of proteins called the nitrogenase complex (Fig. 6.1). The two key components of this complex are dinitrogenase reductase and dinitrogenase. Dinitrogenase reductase (Mr 60,000) is a dimer of two identical subunits. It contains a single Fe_4-S_4 redox center and can be oxidized and reduced by one electron. It also has two binding sites for ATP. Dinitrogenase is a tetramer with two copies of two different subunits (combined Mr 240,000). Dinitrogenase contains both iron and molybdenum, and its redox centers have a total of 2 Mo, 32 Fe, and 30 S per tetramer. About half of the Fe and S is present as four Fe_4-S_4 centers. The remainder is present as part of a novel iron-molybdenum cofactor of unknown structure. A form of nitrogenase that contains vanadium rather than molybdenum has been detected and both types of nitrogenase systems can be produced by some bacterial species. The vanadium enzyme may be the primary nitrogen fixation system under some environmental conditions but it has not been well characterized (Fig. 6.2).

6.2.3. Ammonia is incorporated into biomolecules through glutamate and glutamine

Reduced nitrogen in the form of NH_4^+ can be assimilated, first into amino acids and then into other nitrogen-containing biomolecules. Two amino acids, glutamate and glutamine provide the critical entry point. Recall that the same two amino acids play central roles in amino acid catabolism (Unit 5). The amino groups of most other amino acids are derived from glutamate via transamination reactions. The amide nitrogen of glutamine is the source of amino groups in a wide range of biosynthetic processes. In most types of cells and intercellular fluids in higher organisms, one or both of these amino acids are present at elevated concentrations, sometimes of an order of magnitude or more higher than those of other amino acids. In *E. coli* so much glutamate is required that it is one of the primary solutes in the cell. Its concentration is regulated and varied, not only in response to nitrogen requirements, but also to keep the interior of the cell in osmotic balance with the external medium.

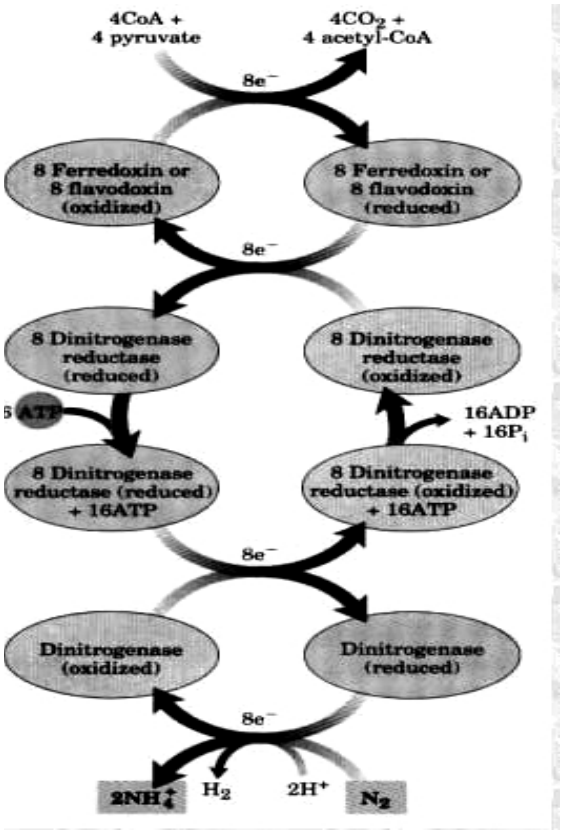

Fig. 6.2: Nitrogen fixation by the nitrogenase complex. Electrons are transferred from pyruvate to dinitrogenase via ferredoxin (or flavodoxin) and dinitrogenase reductase. Dinitrogenase is reduced one electron at a time by dinitrogenase reductase and must be reduced by at least six electrons to fix one molecule of N_2. An additional two electrons (thus a total of eight) are used to reduce two H' to H_2 in a process that obligatorily accompanies nitrogen fixation in anaerobes. The subunit

structures and metal cofactors of the dinitrogenase reductase and dinitrogenase proteins are described in the text.

The biosynthetic pathways to glutamate and glutamine are simple and appear to be similar in all forms of life. The most important pathway for the assimilation of NH_4^+ into glutamate requires two reactions. First, glutamate and NH_4 react to yield glutamine by the action of **glutamine synthetase**, which has a high affinity for NH_4^+ and is found in all organisms:

$$\text{Glutamate} + NH_4^+ + \text{ATP} \longrightarrow \text{glutamine} + \text{ADP} + \text{Pi} + H^+$$

Recall that this reaction takes place in two steps, with enzyme-bound γ-glutamyl phosphate as an intermediate:

$$\text{(1) Glutamate} + \text{ATP} \longrightarrow \gamma\text{-glutamyl phosphate} + \text{ADP}$$

$$\text{(2) } \gamma\text{-Glutamyl phosphate} + NH_4^+ \longrightarrow \text{glutamine} + \text{Pi} + H^+$$

$$\text{Sum: Glutamate} + NH_4^+ + \text{ATP} \longrightarrow \text{glutamine} + \text{ADP} + \text{Pi} + H^+$$

In addition to its importance for NH_4^+ assimilation in bacteria, this is a central reaction in amino acid metabolism in mammals. It is the main pathway for converting toxic free ammonia into the nontoxic glutamine for transport in the blood.

In bacteria, glutamate is then produced by the action of the enzyme glutamate synthase. This enzyme catalyzes the reductive amination of a-ketoglutarate, an intermediate of the citric acid cycle, using glutamine as nitrogen donor.

$$\alpha\text{-Ketoglutarate} + \text{glutamine} + \text{NADPH} + H^+ \longrightarrow \text{glutamate} + NADP^+$$

The net reaction of these two enzymes (glutamate synthase and glutamine synthetase) in bacteria is

$$\alpha\text{-Ketoglutarate} + NH_4^+ + \text{NADPH} + \text{ATP} \longrightarrow \text{L-glutamate} + NADP^+ + \text{ADP} + \text{Pi}$$

Thus there is a net synthesis of one molecule of glutamate.

In animals, glutamate synthase is not known to occur; glutamate is maintained at high levels by processes such as the transamination of α-ketoglutarate during amino acid catabolism.

Glutamate can also be formed from α-ketoglutarate and NH_4^+ by the action of L-glutamate dehydrogenase, present in all organisms.

The required reducing power is furnished by NADPH:

$$\alpha\text{-Ketoglutarate} + NH_4^+ + \text{NADPH} \longrightarrow \text{L-glutamate} + NADP^+ + H_2O$$

This reaction is encountered in the catabolism of amino acids. In eukaryotic cells, L-glutamate dehydrogenase is located in the mitochondrial matrix. The equilibrium for the reaction favors reactants and the Km for NH4+ (~1 mM) is so high that this reaction probably makes only a modest contribution to NH_4^+ assimilation. (Recall that the glutamate dehydrogenase reaction, in reverse, is a primary source of NH_4^+ destined for the urea cycle.) Soil bacteria and plants rarely encounter sufficiently high NH_4^+ concentrations for this reaction to make a significant contribution to glutamate levels and generally rely on the two-enzyme pathway outlined above

6.3. Glutamine synthetase is a primary regulatory point in nitrogen metabolism

Glutamine synthetase in bacteria is one of the most complex regulatory enzymes known-not surprising in light of its central role as the entry point for reduced nitrogen in metabolism. It is subject to both allosteric regulation and control by covalent modification. The enzyme has 12 identical subunits. At least six end products of glutamine metabolism plus alanine and glycine are allosteric inhibitors of the enzyme (Fig. 6.3), and each subunit (Mr 50,000) has binding sites for all eight inhibitors as well as an active site for catalysis. Each inhibitor alone gives only partial inhibition. The effects of the different inhibitors, however, are more than additive, and all eight together virtually shut down the enzyme. This control mechanism provides a minute-by-minute adjustment of the supply of glutamine to the metabolic processes that require it.

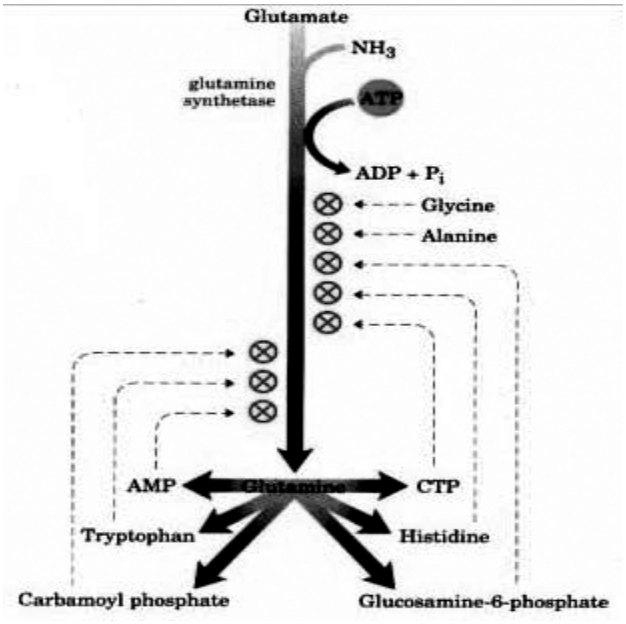

Fig. 6.3: Cumulative allosteric regulation of glutamine synthetase by six end products of glutamine metabolism. Alanine and glycine probably serve as indicators of the general status of cellular amino acid metabolism

Superimposed on the allosteric regulation is inhibition by adenylylation of (addition of AMP to) Tyr^{397} (Fig. 6.4 a), which is located near the enzyme's active site. This covalent modification increases the enzyme's sensitivity to the allosteric inhibitors, and the enzyme's activity decreases as more of the 12 subunits are adenylylated. Both adenylylation and deadenylylation are promoted by the enzyme adenylyl transferase, part of a complex enzymatic cascade that responds to levels of glutamine, α-ketoglutarate, ATP, and Pi (Fig. 6.4 b). The activity of adenylyl transferase is modulated by binding to a regulatory protein called PII. The effect of PII, in turn, is regulated by covalent modification (uridylylation), again at a Tyr residue. The adenylyl transferase complex with PII-UMP stimulates deadenylylation, whereas the same complex with deuridylylated PII stimulates adenylylation of glutamine synthetase. The uridylylation and deuridylylation of PII is brought about by a single enzyme, uridylyl transferase, with both uridylylation and deuridylylation activities. Uridylylation is stimulated by α-ketoglutarate and ATP but inhibited by glutamine and Pi. The deuridylylation activity is not regulated.

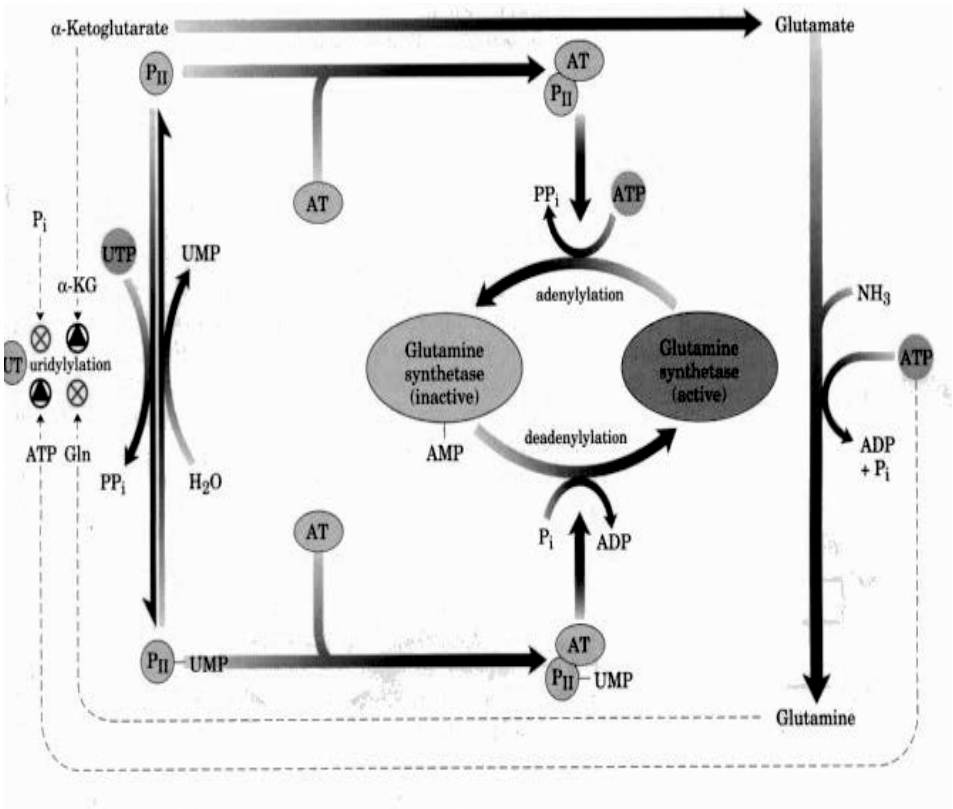

Fig. 6.4: Second level of regulation of glutamine synthetase: covalent modifications. **(a)** Structure of an adenylylated Tyr residue. **(b)** Cascade leading to adenylylation (inactivation) of glutamine synthetase. AT represents adenylyl transferase; UT, uridylyl transferase. The details of this cascade are discussed in the text.

The net result of this complex mechanism is a decrease in glutamine synthetase activity when glutamine levels are high and an

increase in activity when glutamine levels are low and the α-ketoglutarate and ATP substrates are available.

6.3.1. Several classes of reactions play special roles in the biosynthesis of amino acids and nucleotides

The pathways described in this chapter offer examples of a variety of interesting chemical rearrangements. Several of these recur and deserve special note before the pathways are discussed. These are (1) the transamination reactions and other rearrangements promoted by enzymes containing pyridoxal phosphate, (2) the transfer of one-carbon groups using either tetrahydrofolate or S-adenosylmethionine as a cofactor, and (3) the transfer of amino groups derived from the amide nitrogen of glutamine.

There are over a dozen known biosynthetic reactions in which glutamine is the major physiological source of ammonia and most of these appear in the pathways outlined in this chapter. As a class, the enzymes catalyzing these reactions are called glutamine amidotransferases, and all have two structural domains. One domain binds glutamine and the other binds the second substrate, which serves as amino group acceptor. In the reaction, a conserved Cys residue in the glutamine-binding domain is believed to act as a nucleophile, cleaving the amide bond of glutamine and forming a covalent glutamyl-enzyme intermediate. The NH_3 produced in this reaction remains at the active site and reacts with the second substrate to form the aminated product. The covalent intermediate is hydrolyzed to form the free enzyme and glutamate. If the second substrate must be activated, ATP is generally used to generate an acyl phosphate intermediate. The enzyme glutaminase is similar but has no second substrate, and this reaction simply yields NH_4^+ and glutamate.

6.4 Molecules derived from amino acids

In addition to their role as the building blocks of proteins, amino acids are precursors of many specialized biomolecules, including hormones, coenzymes, nucleotides, alkaloids, cell-wall polymers, porphyrins, antibiotics, pigments and neurotransmitters all of which serve essential biological roles. A number of pathways in which amino acids serve as precursors for other biomolecules will be described here.

6.4.1. Glycine is a precursor of porphyrins

The biosynthesis of porphyrins, for which glycine is a major precursor, is the first example because of the central importance of the porphyrin nucleus in heme proteins such as hemoglobin and the cytochromes, and the Mg^{2+}-containng porphyrin derivative chlorophyll. The porphyrins are constructed from four molecules of the nonopyrrole derivative porphobilinogen (Fig. 6.5). In the first reaction, glycine reacts with succinyl-CoA to yield α-amino-β-ketoadipate, which is then decarboxylated to give δ-aminolevulinate. Two molecules of δ-

aminolevulinate condense to form porphobilinogen, and four molecules of porphobilinogen come together to form protophorphyrin through a series of complex enzymatic reactions. The iron atom is incorporated after the protoporphyrin has been assembled. Porphyrin biosynthesis is regulated by the concentration of the heme irotein product, such as haemoglobin, which can serve as a feedback inhibitor of early steps in porphyrin synthesis.

In humans, genetic defects of certain enzymes in the biosynthetic athway from glycine to porphyrins lead to the accumulation of speific porphyrin precursors in erythrocytes, in body fluids, and in the licer. These genetic discases are known as porphyrias, In one of the porphyrias, which affects mainly erythroeytes, there is an accumulaion ofIt stains the urine red and causes the teeth to fluoresce.

Strongly in ultraviolet light the skin shows abnormal sensitivity to sunlight. Because insufficient heme is synthesized, patients with this disease are anemic, shy away from sunlight, and have a propensity to drink blood. This condition may have given rise to the vampix myths in medieval folk legend. Another type of porphyria causes accumulation of porphobilinogen in the liver, as well as intermittent neurological and behavioural aberrations.

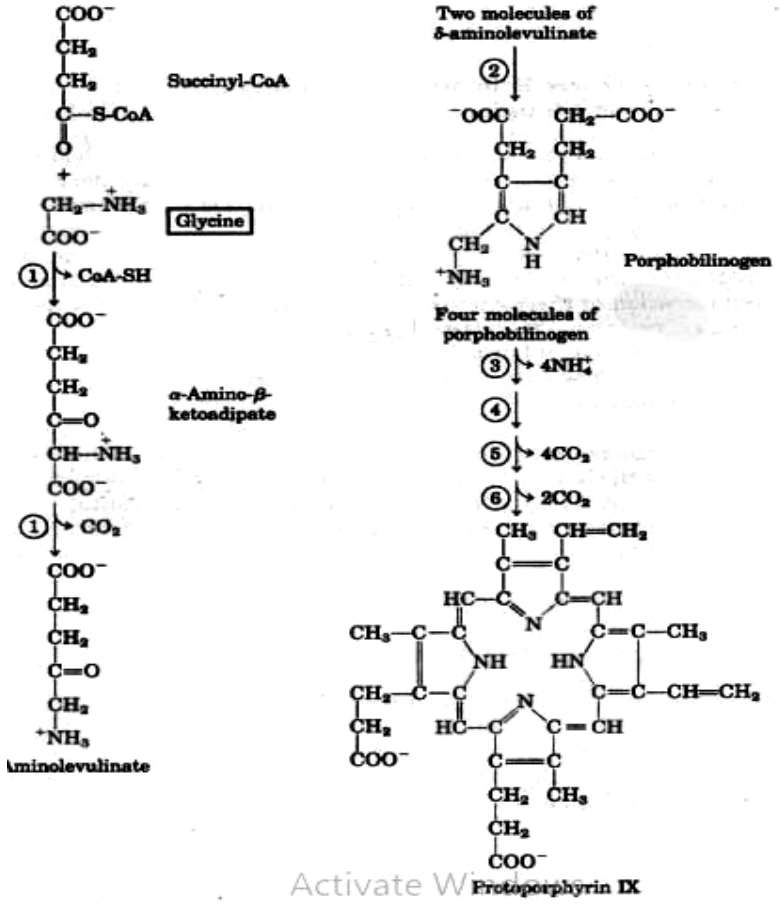

Fig. 6.5: Biosynthesis of protoporphyrin IX, the porphyrin of hemoglobin and myoglobin. The atoms furnished by glycine are shown in

red. The remaining carbon atoms are derived from the succinyl group of succinyl-CoA. Pathway enzymes are: 1 : d-aminolevulinate synthase, 2 : porphobilinogen synthase, 3 : uroporphyrinogen synthase, 4 : uroporphyrinogen III cosynthase, 5 : uroporphyxznogex decarboxylase, and 6 : coproporphyrinogen oxidase This pathway occurs in mammals; in bacteria and plants, glutamate is the precursor of 8-aminolevulinate.

6.4.2 Degradation of heme yields bile pigments

The iron-porphyrin or heme group of haemoglobin, released from dying erythrocytes in the spleen, is degraded to yield free Fe^{3+} and ultimately bilirubin, a linear (open) tetrapyrrole derivative. Bilirubin binds to serum albumin and is transported to the liver, where it transformed into the bile pigment bilirubin diglucuronide, which sufficiently water soluble to be secreted with other components of bi into the small intestine. Impaired liver function or blocked bile secretion causes bilirubin to leak into the blood, resulting in a yellowing the skin and eyeballs, a general condition called jaundice. Determination of bilirubin concentration in the blood is useful in diagnosing underlying liver disease.

6.5. Amino acids are required for the biosynthesis of creatine and glutathione

Phosphocreatine, derived from creatine, is an important energy reservoir in skeletal muscle. Creatine is derived from glycine and arginine (Fig. 6.6), and methionine plays an important role (as S-adenosylmethionine) as donor of a methyl group.

Glutathione (GSH) is a tripeptide derived from glycine, glutamate, and cysteine (Fig. 6.6). The first step in its synthesis is a condensation of the y-carboxyl group of glutamate with the a-amino group of cysteine. The carboxyl group is first activated by ATP to form an acyl phosphate intermediate, which is then attacked by the cysteine amino group. The second step is similar, with the carboxyl group of cysteine activated to an acyl phosphate to permit condensation with glycine.

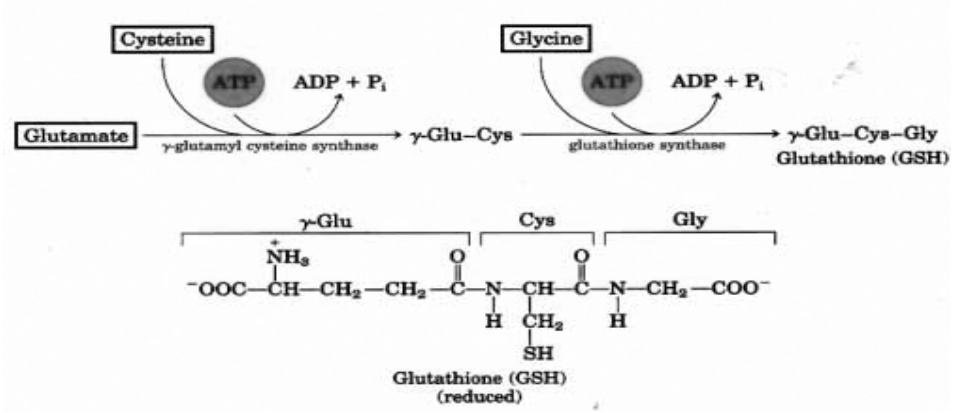

Fig. 6.6: Biosynthesis and structure of glutathione. The oxidised form of glutathione is also shown.

Glutathione is present in virtually all cells, often at high levels, and can be thought of as a kind of redox buffer. It probably helps maintain the sulfhydryl groups of proteins in the reduced state and the iron of heme in the ferrous (Fe^{2+}) state, and it serves as a reducing agent for glutaredoxin. Its redox function can also be used in removing toxic peroxides that form in the course of growth and metabolism under aerobic conditions:

2 GSH + R-O-O-H ~ GSSG + H20 + R-OH

This reaction is catalyzed by glutathione peroxidase, a remarkable enzyme in that it contains a covalently bound selenium (Se) atom in the form of selenocysteine. The selenium is essential for the enzyme's activity. The oxidized form of glutathione (GSSG) contains two molecules of glutathione linked by a disulfide bond.

6.5.1 D-Amino acids are found primarily in bacteria

Although n-amino acids do not generally occur in proteins, they do serve some special functions in the structure of bacterial cell walls and peptide antibiotics. The peptidoglycans of bacteria contain both n-alanine and n-glutamate. n-Amino acids arise directly from the r. isomers by the action of amino acid racemases, which have pyridoxal phosphate as a required cofactor. Amino acid racemization is uniquely important to bacterial metabolism, and enzymes such as alanine racemase represent prime targets for pharmaceutical agents. One such agent,L-fluoroalanine, is being tested as an antibacterial drug. Another, cycloserine, is already used to treat urinary tract infections and tuberculosis. Both inhibitors also affect some other PLP-requiring enzymes.

6.5.2 Aromatic amino acids are precursors of many plant substances

Phenylalanine, tyrosine, and tryptophan are converted into a variety of important compounds in plants. The rigid polymer lignin is derived from phenylalanine and tyrosine. It is second only to cellulose in abundance in plant tissues. The structure of lignin is complex and not well understood. Phenylalanine and tyrosine also give rise to many commercially significant natural products, including tannins that inhibit oxidation in wines; alkaloids such as morphine that have potent physiological effects; and flavor components of products such as cinnamon oil, nutmeg, cloves, vanilla, and cayenne pepper. Tryptophan gives rise to the plant growth hormone, indole-3acetate or auxin (Fig. 6.7). This molecule has been implicated in the regulation of a wide range of biological processes in plant cells.

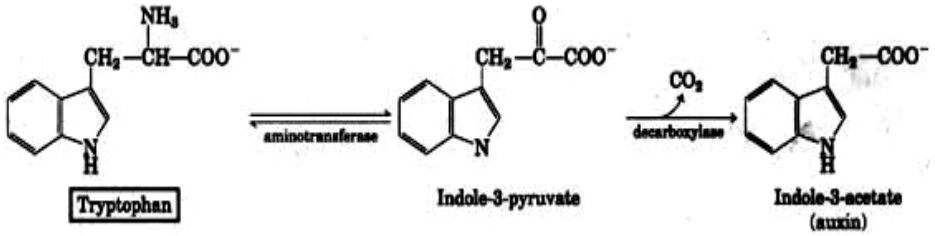

Fig. 6.7: Biosynthesis of indole-3-acetate (anxin).

6.5.3 Amino acids are converted to biological amines by decarboxylation

Many important neurotransmitters are primary or secondary amines derived from amino acids in simple pathways. In addition, some polyamines that are complexes with DNA are derived from the amino acid ornithine. A common denominator of many of these pathways is amino acid decarboxylation, another reaction involving pyridoxal phosphate. The synthesis of some neurotransmitters is illustrated in Fig. 6.8 Tyrosine gives rise to a family of catecholamines that includes dopamine, norepinephrine, and epinephrine. Levels of catecholamines are correlated with (among other things) changes in blood pressure in animals. The neurological disorder Parkinson's disease is associated with an underproduction of dopamine, and it has been treated by administering L-dopa. An overproduction of dopamine in the brain is associated with psychological disorders such as schizophrenia. Glutamate decarboxylation gives rise to y-aminobutyrate (GABA), an inhibitory neurotransmitter. Its underproduction is associated with epileptic seizures. GABA is used pharmacologically in the treatment of epilepsy and hypertension. Another important neurotransmitter, serotonin, is derived from tryptophan in a two-step pathway.

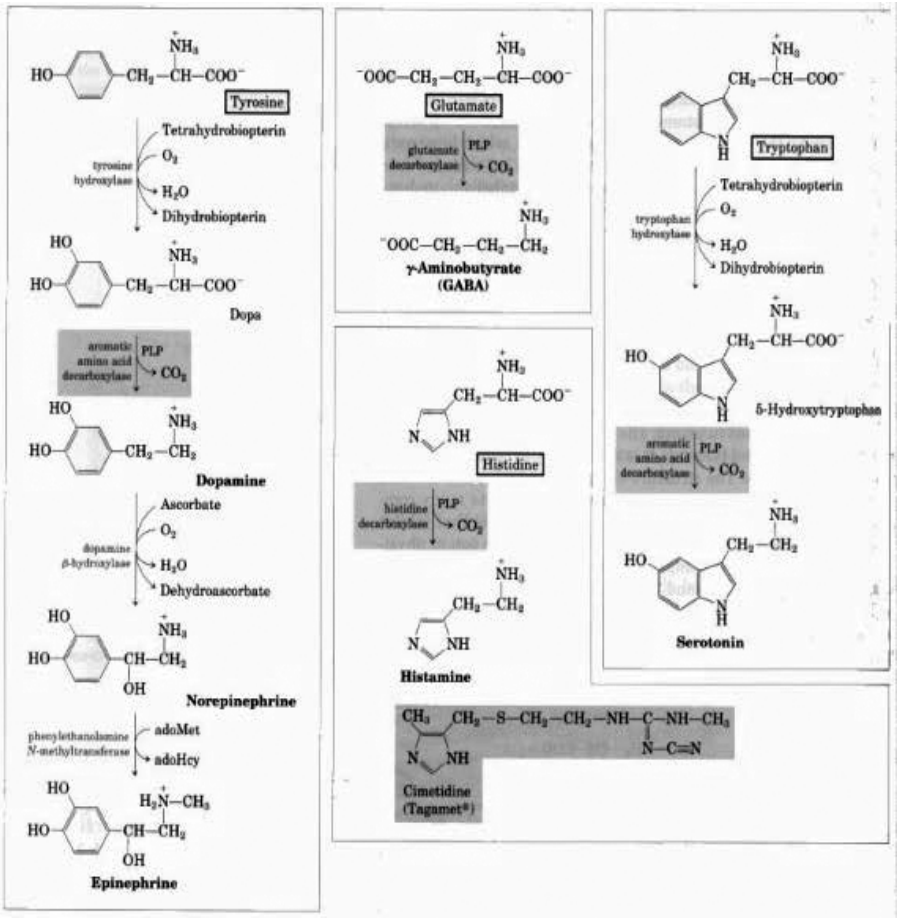

Fig. 6.8: Some neurotransmitters derived from amino acids. The key biosynthetic step is the same in each case: a PLP-dependent

decarboxylation. Cimetidine (shaded beige), a histamine analog, is used to treat duodenal ulcers.

6.6. Nitrogen excretion and the urea cycle

The urea cycle is a series of reactions that converts toxic ammonium into the non- toxic nitrogen excretion production urea. The urea cycle is regulated by substrate availability and by the enzyme carbamoyl phosphate synthetase I, which is regulated by N-acetylglutamate. The amount of nitrogen ingested is balanced by the excretion of an equivalent amount of nitrogen. About 80% of excreted nitrogen is in the form of urea. The activity of urea cycle is regulated at two levels: Prolonged starvation ∀ breaks down muscle proteins. Ammonia is a toxic product of nitrogen metabolism which should be removed from the body. The urea cycle or ornithine cycle converts excess ammonia into urea in the mitochondria of liver cells. The urea forms, then enters the blood stream, is filtered by the kidneys and is ultimately excreted in the urine (Fig 6.9).

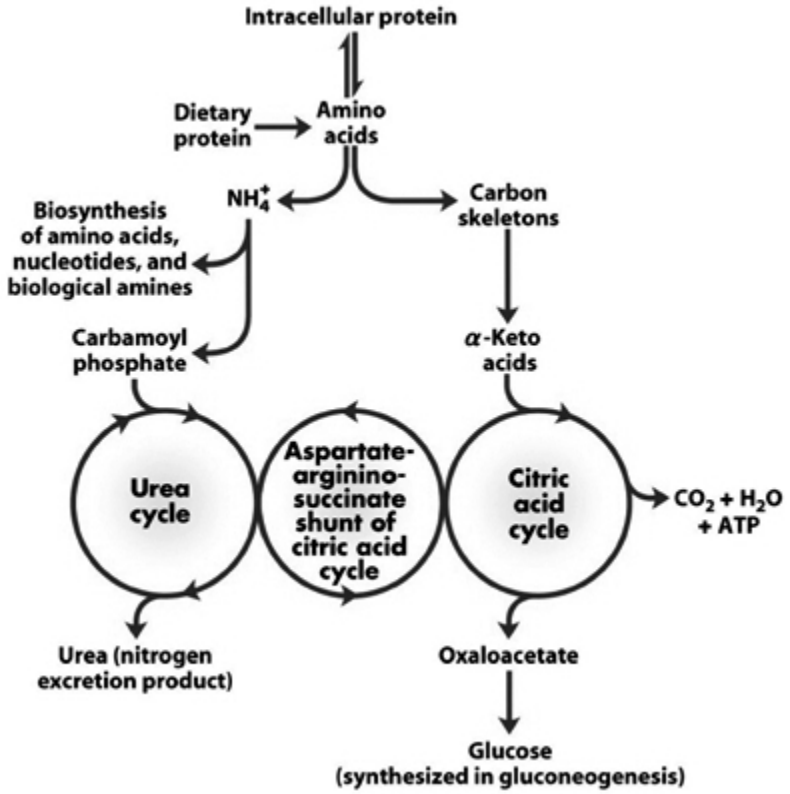

Fig. 6.9: A summary of the points of entry of the standard amino acids into the citric acid cycle. (The boxes around the amino acids are shaded of the catabolic pathways, here and in fig. throughout the rest of this chapter).

Some amino acids are listed more than once; these are broken down to yield different fragments, each of which enters the citric acid cycle at a different point. This scheme represents the major catabolic

pathways in vertebrates, but there are minor variations from organism to organism. Threonine, for instance, is degraded into acetyl-CoA via pyruvate in some organisms.

Most aquatic species, such as the bony fishes, excrete amino nitrogen as ammonia and are thus called ammonotelic animals; most terrestrial animals excrete amino nitrogen in the form of urea and are thus ureotelic; and birds and reptiles excrete amino nitrogen as uric acid and are called uricotelic. Plants recycle virtually all amino groups, and nitrogen excretion occurs only under very unusual circumstances. There is no general pathway for nitrogen excretion in plants. In ureotelic organisms, the ammonia in the mitochondria of hepatocytes is converted to urea via the urea cycle. This pathway was discovered in 1932 by Hans Krebs (who later also discovered the citric acid cycle) and a medical student associate, Kurt Henseleit. Urea production occurs almost exclusively in the liver, and it represents the fate of most of the ammonia that is channeled there.

6.6.1. Urea is formed in the liver

Using thin slices of liver suspended in a buffered aerobic medium, Krebs and Henseleit found that the rate of urea formation from ammonia was greatly accelerated by adding any one of three α-amino acids: ornithine, citrulline, or arginine. Each of these three compounds stimulated urea synthesis to a far greater extent than any of the other common nitrogenous compounds tested, and their structures suggested that they might be related in a sequence. From these and other facts Krebs and Henseleit deduced that a cyclic process occurs (Fig. 6.10), in which ornithine plays a role resembling that of oxaloacetate in the citric acid cycle. A molecule of ornithine combines with one molecule of ammonia and one of CO_2 to form citrulline. A second amino group is added to citrulline to form arginine, which is then hydrolyzed to yield urea, with regeneration of ornithine. Ureotelic animals have large amounts of the enzyme arginase in the liver. This enzyme catalyzes the irreversible hydrolysis of arginine to urea and ornithine. The ornithine is then ready for the next turn of the urea cycle. The urea is passed via the bloodstream to the kidneys and is excreted into the urine.

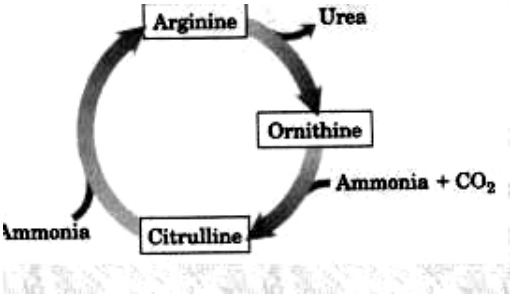

Fig. 6.10: The urea cycle. The three amino acids found by Krebs and Henseleit to stimulate urea formation from ammonia in liver slices are boxed. As shown, ornithine and citrnlline can serve as successive

precursors of arginine. Note that citrulline and ornithine are nonstandard amino acids that are not found in proteins.

6.6.2. The production of urea from ammonia involves five enzymatic steps

The urea cycle begins inside the mitochondria of hepatocytes, but three of the steps occur in the cytosol; the cycle thus spans two cellular compartments (Fig. 6.11). The first amino group to enter the urea cycle is derived from ammonia inside the mitochondria, arising by the multiple pathways described above. Some ammonia also arrives at the liver via the portal vein from the intestine, where it is produced by bacterial oxidation of amino acids. Whatever its source, the NH4+ generated in liver mitochondria is immediately used, together with HCO3- produced by mitochondrial respiration, to form carbamoyl phosphate in the matrix. This ATP-dependent reaction is catalyzed by the enzyme **carbamoyl phosphate** synthetase I. The mitochondrial form of the enzyme is distinct from the cytosolic (II) form, which has a separate function in pyrimidine biosynthesis. **Carbamoyl phosphatesynthetase** I is a regulatory enzyme; it requires N-acetylglutamate as a positive modulator (see below). Carbamoyl phosphate may be regarded as an activated carbamoyl group donor.

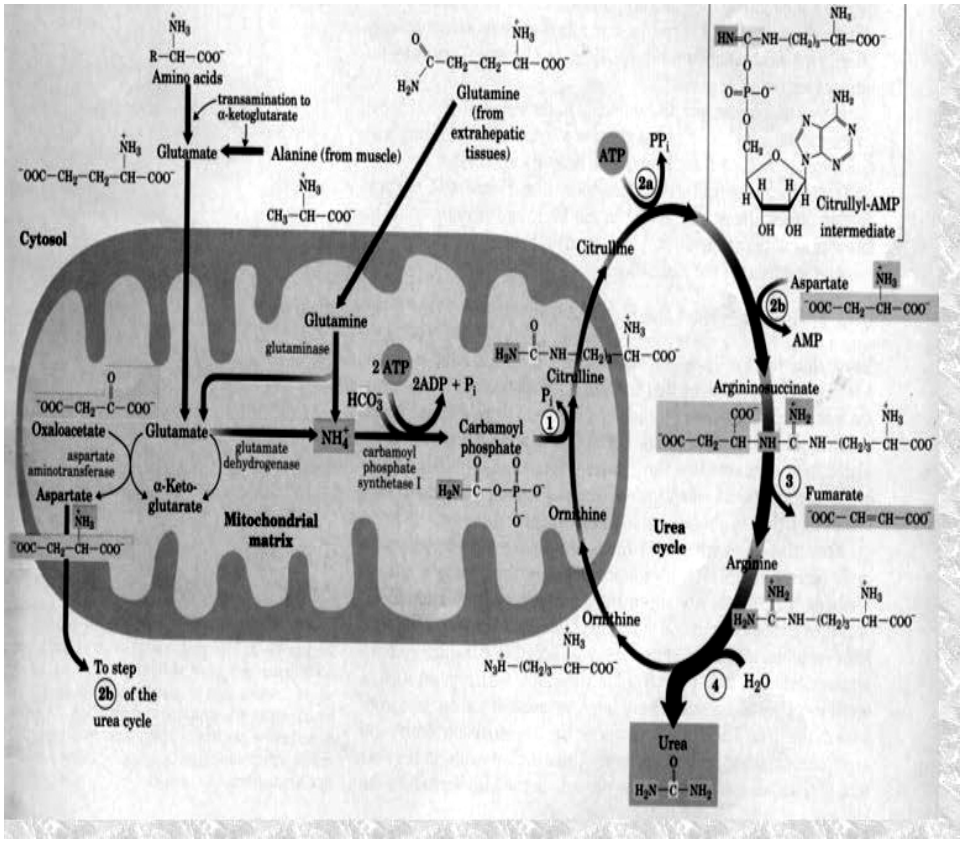

Fig. 6.11: The urea cycle and the reactions that feed amino groups into it. Note that the enzymes catalyzing these reactions (named in the text) are distributed between the mitochondrial matrix and the cytosol.

One amino group enters the urea cycle from carbamoyl phosphate (step 1), formed in the matrix; the other (entering at step 2) is derived from aspartate, also formed in the matrix via transamination of oxaloacetate and glutamate in a reaction catalyzed by aspartate aminotransferase.

The urea cycle itself consists of four steps: (l) Formation of citrulline from ornithine and carbamoyl phosphate. Citrulline passes into the cytosol. (2) Formation of argininosuccinate through a citrullyl-AMP intermediate. (3) Formation of arginine from argininosuccinate. This reaction releases fumarate, which enters the citric acid cycle. (4) Formation of urea. The arginase reaction also regenerates the starting compound in the cycle, ornithine.

The carbamoyl phosphate now enters the urea cycle, which entails four enzymatic steps. Carbamoyl phosphate donates its carbamoyl group to ornithine to form citrulline and release Pi (Fig. 6.11, step l) in a reaction catalyzed by ornithine transcarbamoylase. The citrulline is released from the mitochondrion into the cytosol.

The second amino group is introduced from aspartate (generated in the mitochondria by transamination (Fig. 6.11) and transported to the cytosol) by a condensation reaction between the amino group of aspartate and the ureido (carbonyl) group of citrulline to form argininosuccinate (step 2). This reaction, catalyzed by argininosuccinate synthetase of the cytosol, requires ATP and proceeds through a citrullyl-AMP intermediate. The argininosuccinate is then reversibly cleaved by argininosuccinatelyase to form free arginine and fumarate (step 3), which enters the pool of citric acid cycle intermediates. In the last reaction of the urea cycle the cytosolic enzyme arginase cleaves arginine to yield urea and ornithine (step 4). Ornithine is thus regenerated and can be transported into the mitochondrion to initiate another round of the urea cycle. The product of one enzyme is often channeled directly to the next enzyme in the pathway. In the urea cycle, mitochondrial and cytosolic enzymes appear to be clustered in this way. The citrulline transported out of the mitochondria is not diluted into the general pool of metabolites in the cytosol. Instead, each molecule of citrulline is passed directly into the active site of a molecule of argininosuccinate synthetase. This channeling continues for argininosuccinate, arginine, and ornithine. Only the urea is released into the general pool within the cytosol.

6.7. The citric acid and urea cycles are linked

The fumarate produced in the argininosuccinate lyase reaction is also an intermediate of the citric acid cycle. Fumarate enters the mitochondria, where the combined activities of fumarase (fumarate hydratase) and malate dehydrogenase transform fumarate into oxaloacetate (Fig. 6.12). Aspartate, which acts as a nitrogen donor in the urea cycle reaction catalyzed by argininosuccinate synthetase in the cytosol, is formed from oxaloacetate by transamination from glutamate; the other product of this transamination is α-ketoglutarate, another

intermediate of the citric acid cycle. Because the reactions of the urea and citric acid cycles are inextricably intertwined, together they have been called the "Krebs bicycle."

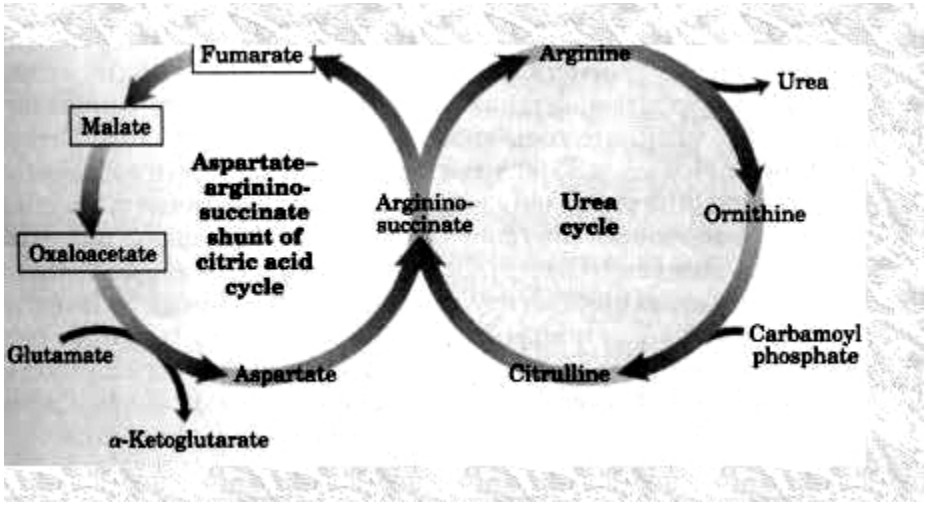

Fig. 6.12: The "Krebs bicycle," composed of the urea cycle on the right, which meshes with the aspartate-argininosuccinate shunt of the citric acid cycle on the left.

Fumarate produced in the cytosol by argininosuccinate lyase of the urea cycle enters the citric acid cycle in the mitochondrion and is converted in several steps to oxaloacetate. Oxaloacetate accepts an amino group from glutamate by transamination, and the aspartate thus formed leaves the mitochondrion and donates its amino group to the urea cycle in the argininosuccinate synthetase reaction. Intermediates in the citric acid cycle are boxed.

6.8. The activity of the urea cycle is regulated

The flux of nitrogen through the urea cycle varies with the composition of the diet. When the diet is primarily protein, the use of the carbon skeletons of amino acids for fuel results in the production of much urea from the excess amino groups. During severe starvation, when breakdown of muscle protein supplies much of the metabolic fuel, urea production also increases substantially, for the same reason.

These changes in demand for urea cycle activity are met in the long term by regulation of the rates of synthesis of the urea cycle enzymes and carbamoyl phosphate synthetase I in the liver. All five enzymes are synthesized at higher rates during starvation or in animals on very high-protein diets than in well-fed animals on diets containing primarily carbohydrates and fats. Animals on protein-free diets produce even lower levels of the urea cycle enzymes.

On a shorter time scale, allosteric regulation of at least one key enzyme is involved in adjusting flux through the cycle. The first enzyme in the pathway, carbamoyl phosphate synthetase I, is allosterically

activated by N-acetylglutamate, which is synthesized from acetyl-CoA and glutamate (Fig. 6.13). N-Acetylglutamate synthase is in turn activated by arginine, a urea cycle intermediate that accumulates when urea production is too slow to accommodate the ammonia produced by amino acid catabolism.

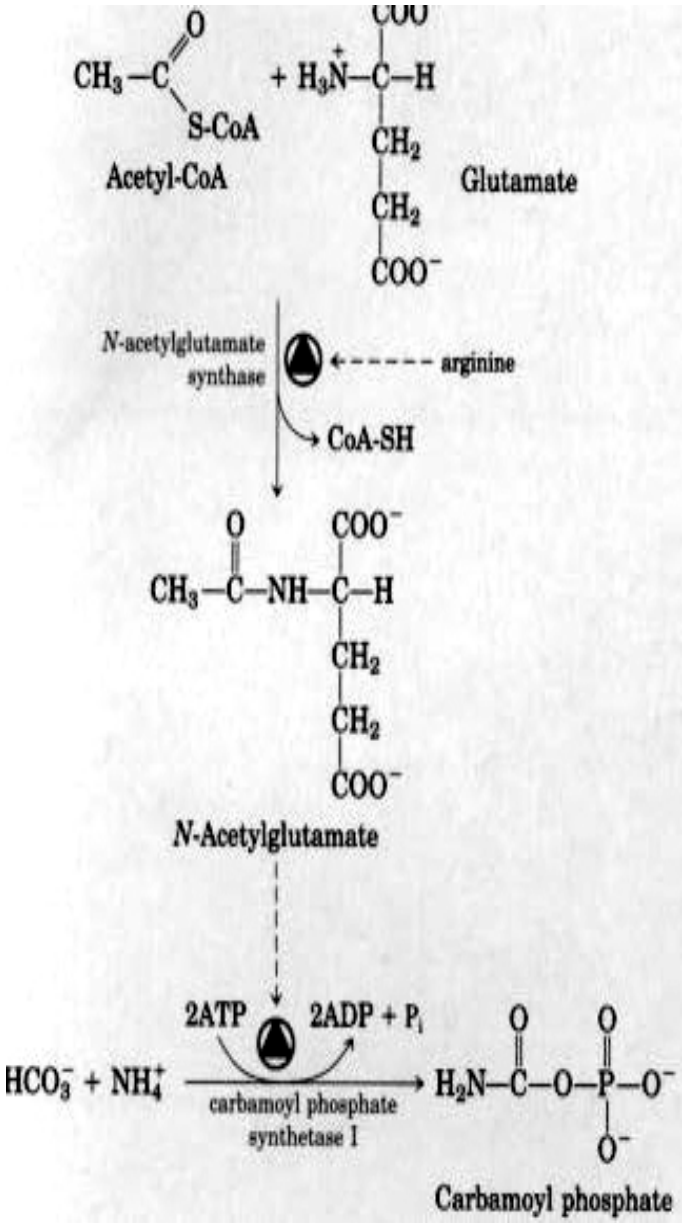

Fig. 6.13: Synthesis of N-acetylglutamate, the allosteric activator of carbamoyl phosphate synthetase I, is stimulated by high concentrations of arginine. Increasing arginine levels signal the need for more flux through the urea cycle

6.9. The Urea cycle is energetically expensive

The urea cycle brings together two amino groups and HCO3- to form a molecule of urea, which diffuses from the liver into the

bloodstream, thence to be excreted into the urine by the kidneys. The overall equation of the urea cycle is

$$2NH_4^+ + HCO_3^- + 3ATP^{4-} + H_2O \longrightarrow \text{urea} + 2ADP^{3-} + 4Pi^{2-} + AMP^{2-} + 5H^+$$

The synthesis of one molecule of urea requires four high-energy phosphate groups. Two ATPs are required to make carbamoyl phosphate and one ATP is required to make argininosuccinate. In the latter reaction, however, the ATP undergoes a pyrophosphate cleavage to AMP and pyrophosphate, which may be hydrolyzed to yield two Pi.

It has been estimated that, because of the necessity of excreting nitrogen as urea instead of ammonia, ureotelic animals lose about 15% of the energy of the amino acids from which the urea was derived. This loss is counterbalanced by metabolic adaptations in some ruminant animals. The cow transfers much urea from its blood into the rumen, where microorganisms use it as a source of ammonia to manufacture amino acids, which are then absorbed and used by the cow. Urea is sometimes added to cattle feed as an inexpensive nitrogen supplement. The recycling of urea not only reduces the net investment of chemical energy, it also reduces the requirements for protein intake and urine production. This can be important for ruminants that must subsist on a low-protein grass diet in a dry environment. The camel, by transferring urea into its gastrointestinal tract and recycling it like the cow, greatly reduces the water loss connected with the urinary excretion of urea. This is one of several biochemical and physiological adaptations that enables the camel to survive on a very limited water intake.

6.10. Genetic defects in the urea cycle can be life-threatening

People with genetic defects in any enzyme involved in the formation of urea have an impaired ability to convert ammonia to urea. They cannot tolerate a protein-rich diet because amino acids ingested in excess of the minimum daily requirements for protein synthesis would be deaminated in the liver, producing free ammonia in the blood. As ammonia is toxic and causes mental disorders, retarded development and in high amounts, coma and death. Humans, however, are incapable of synthesizing half of the 20 standard amino acids, and these essential amino acids (Table 6.2) must be provided in the diet. Patients with defects in the urea cycle are often treated by substituting in the diet the α-keto acid analogs of the essential amino acids, which are the indispensable parts of the amino acids. The α-keto acid analogs can then accept amino groups from excess nonessential amino acids by aminotransferase action (Fig. 6.14). In this way the essential amino acids are made available for biosynthesis and nonessential amino acids are kept from delivering their amino groups to the blood in the form of ammonia.

Table 6.1: Non essential and essential amino acids for human and the albino rats.

Nonessential	Essential
Alanine	Arginine*
Asparagine	Histidine
Aspartate	Isoleucine
Cysteine	Leucine
Glutamate	Lysine
Glutamine	Methionine
Glycine	Phenylalanine
Proline	Threonine
Serine	Tryptophan
Tyrosine	Valine

Table Nonessential and essential amino acids for humans and the albino rat Nonessential Essential Alanine Arginine* Asparagine Histidine Aspartate Isoleucine Cysteine Leucine Glutamate Lysine Glutamine Methionine Glycine Phenylalanine Proline Threonine Serine Tryptophan Tyrosine Valine * Essential in young, growing animals but not in adults.

6.10.1. Habitat determines the molecular pathway for nitrogen excretion

Urea synthesis is not the only, or evens the most common, pathway among organisms for excreting ammonia. The basis for differences in the molecular form in which amino groups are excreted lies in the anatomy and physiology of different organisms in relation to their usual habitat. Bacteria and free-living protozoa simply release ammonia to their aqueous environment, in which it is diluted and thus made harmless. In the bony fishes (ammonotelic animals), ammonia is rapidly cleared from the blood at the gills by the large volume of water passing through these respiratory structures. Although quite sensitive to NH_3, fish are relatively tolerant of NH_4^+. Liver is also the primary site of amino acid catabolism in fish, and NH_4^+ produced by transdeamination is simply released from the liver into the blood for transport to the gills and excretion. The bony fishes thus do not require a complex urinary system to excrete ammonia.

Organisms that excrete ammonia could not survive in an environment in which water is limited. The evolution of terrestrial species depended upon mutations that conferred the ability to convert ammonia to nontoxic substances that could be excreted in a small volume of water.

Two main methods of excreting nitrogen have evolved: conversion to either urea or uric acid.

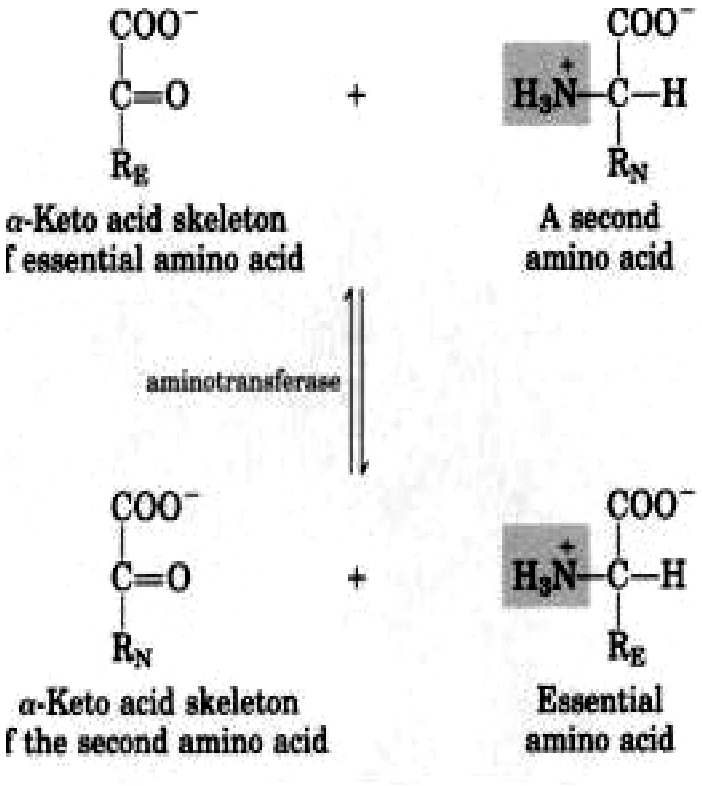

Fig. 6.14: The essential amino acids (those with carbon skeletons that cannot be synthesized by animals and must be obtained in the diet) can be synthesized from the corresponding a-keto acids by transamination. The dietary requirement for essential amino acids can therefore be met by the a-keto acid skeletons. RE and RN represent R groups of essential and nonessential amino acids, respectively.

The importance of the habitat in excretion of amino nitrogen is illustrated by the change in the pathway of nitrogen excretion as the tadpole undergoes metamorphosis into the adult frog. Tadpoles are entirely aquatic and excrete amino nitrogen as ammonia through their gills. The tadpole liver lacks the necessary enzymes to make urea, but during metamorphosis it begins synthesizing these enzymes and looses the ability to excrete ammonia. In the adult frog, which is more terrestrial in habit, amino nitrogen is excreted almost entirely as urea.

In birds and reptiles, availability of water is an especially important consideration. Excretion of urea into urine requires simultaneous excretion of a relatively large volume of water; the weight of the required water would impede flight in birds, and reptiles living in arid environments must conserve water. Instead, these animals convert amino nitrogen into uric acid, a relatively insoluble compound that is extracted as a semisolid mass of uric acid crystals with the feces. For the advantage of excreting amino nitrogen in the form of solid uric acid, birds and reptiles must carry out considerable metabolic work; uric acid is a purine, and the

biosynthesis of uric acid is a complex energy-requiring process that is part of the purine catabolic pathway.

On many islands off the coast of South America, which serve as immense rookeries for sea birds, uric acid is deposited in enormous amounts. These huge guano deposits are used as fertilizer, thus returning organic nitrogen to the soil, to be used again for the synthesis of amino acids by plants and soil microorganisms.

The molecular nitrogen that makes up 80% of the earth's atmosphere is unavailable to living organisms until it is reduced. Fixation of atmospheric N2 takes place in certain free-living soil bacteria and in symbiotic bacteria in the root nodules of leguminous plants, by the action of the complex nitrogenase system. Formation of ammonia by bacterial fixation of N_2, nitrification of ammonia to form nitrate by soil organisms, conversion of nitrate to ammonia by higher plants, synthesis of amino acids from ammonia by plants and animals, and conversion of nitrate to N_2 by some soil bacteria in the process of denitrification. The fixation of N_2 as NH_3 is carried out by a protein complex called the nitrogenase complex, in a reaction that requires ATP. The nitrogenase complex is very labile in the presence of O_2.

In living systems, reduced nitrogen is incorporated first into amino acids and then into a variety of other biomolecules, including nucleotides. The key entry point is the amino acid glutamate. Glutamate and glutamine are the nitrogen donors in a wide variety of biosynthetic reactions. Glutamine synthesize, which catalyzes the formation of glutamine from glutamate, is a key regulatory enzyme of nitrogen metabolism.

The amino acid and nucleotide biosynthetic pathways make repeated use of the biological cofactors pyridoxal phosphate, tetrahydrofolate and S-adenosylmethionine. Pyridoxal phosphate is required for transamination reactions involving glutamate and for a number of other amino acid transformations. One-carbon transfers are carried out using S-adenosylmethionine (at the -CH3 oxidation level) and tetrahydrofolate (usually at the -CHO and -CH2OH oxidation levels). Enzymes called glutamine amidotransferases are used in reactions that incorporate nitrogen derived from glutamine.

Mammals (e.g., humans and the albino rat) can synthesize 10 of the 20 amino acids of proteins. The remainder, which are required in the diet (essential amino acids), can be synthesized by plants and bacteria. Among the nonessential amino acids, glutamate is formed by reductive amination of α-ketoglutarate and is the precursor of glutamine, proline, and arginine.

Alanine and aspartate (and thus asparagine) are formed from pyruvate and oxaloacetate, respectively by transamination. The carbon chain of serine is derived from 3-phosphoglycerate. Serine is a precursor of glycine; the β-carbon atom of serine is transferred to tetrahydrofolate. Cysteine is formed from methionine and serine by a series of reactions in which S-adenosylmethionine and cystathionine are intermediates. The

aromatic amino acids (phenylalanine, tyrosine, and tryptophan) are formed via a pathway in which the intermediate chorismate occupies a key branch point. Phosphoribosyl pyrophosphate is a precursor of tryptophan and histidine, both essential amino acids. The biosynthetic pathway to histidine is interconnected with the purine synthetic pathway. Tyrosine can also be formed by hydroxylation of phenylalanine, an essential amino acid. The pathways for biosynthesis of the other essential amino acids in bacteria and plants are complex. The amino acid biosynthetic pathways are subject to allosteric end-product inhibition; the regulatory enzyme is usually the first in the sequence. The regulation of these synthetic pathways is coordinated.

Many other important biomolecules are derived from amino acids. Glycine is a precursor of porphyrins; porphyrins, in turn, are degraded to form bile pigments. Glycine and arginine give rise to creatine and phosphocreatine. Glutathione, a tripeptide, is an important cellular reducing agent. D-amino acids are synthesized from L-amino acids in bacteria in racemization reactions requiring pyridoxal phosphate. The PLP-dependent decarboxylation of certain amino acids yields some important biological amines, including neurotransmitters. The aromatic amino acids are precursors of a number of plant substances.

The purine ring system is built up in a step by step fashion on 5-phosphoribosylamine. The amino acids glutamine, glycine, and aspartate furnish all the nitrogen atoms of purines. "Two ring-closure steps ensue to form the purine nucleus. Pyrimidines are synthesized from carbamoyl phosphate and aspartate. Ribose-5-phosphate is then attached to yield the pyrimidine ribonucleotides. Purine and pyrimidine biosynthetic pathways are regulated by feedback inhibition. Nucleoside monophosphates are converted to their triphosphates by enzymatic phosphorylation reactions. Ribonucleotides are converted to deoxyribonucleotides by the action of ribonucleotide reductase, an enzyme with novel mechanistic and regulatory characteristics. The thymine nucleotides are derived from the deoxyribonucleotides dCDP and dUMP. Uric acid and urea are the end products of purine and pyrimidine degradation. Free purines can be salvaged and rebuilt into nucleotides by a separate pathway. Genetic deficiencies in certain salvage enzymes cause serious genetic diseases such as Lesch-Nyhan syndrome and severe immunodeficiency disease. Another genetic deficiency results in the accumulation of uric acid crystals in the joints, causing gout. The enzymes of the nucleotide biosynthetic pathways are targets for an array of chemotherapeutic agents used to treat cancer and other diseases.

6.11. Summary

Ammonia is highly toxic to animal tissues. Ammonotelic animals (bony fishes, tadpoles) excrete amino nitrogen from their gills as ammonia. Ureotelic animals (adult terrestrial amphibians and all mammals) excrete amino nitrogen as urea, formed in the liver by the urea

cycle. Arginine is the immediate precursor of urea. Arginase hydrolyzes arginine to yield urea and ornithine and arginine is resynthesized in the urea cycle. Ornithine is converted to citrulline at the expense of carbamoyl phosphate, and an amino group is transferred to citrulline from aspartate, re-forming arginine. Ornithine is regenerated in each turn of the cycle. Several of the intermediates and byproducts of the urea cycle are also intermediates in the citric acid cycle and the two cycles are thus interconnected. The activity of the urea cycle is regulated at the levels of enzyme synthesis and allosteric regulation of the enzyme that forms carbamoyl phosphate. Uricotelic animals (birds and reptiles) excrete amino nitrogen in semisolid form as uric acid, a derivative of purine. The mode of nitrogen excretion is determined by habitat. The formation of the nontoxic urea and of solid uric acid has a high ATP cost. Genetic defects in enzymes of the urea cycle can be compensated for by dietary regulation.

6.12. Terminal questions

Q.1. For products of amino acid transamination draw the structure and give the name of the α-keto acid resulting when the following amino acids undergo transamination with α-ketoglutarate:

a. Aspartate

b. Alanine

c. Glutamate

d. Phenylalanine

Answer:----------

Q.2. In measurement of the alanine aminotransferase reaction rate the activity (reaction rate) of alanine aminotransferase is usually measured by including an excess of pure lactate dehydrogenase and NADH in the reaction system. The rate of alanine disapperance is equal to the rate of NADH disappearance measured spectrophotometrically. Explain how this assay works?

Answer:----------

Q.3. In the pathway of carbon and nitrogen in glutamate metabolism when [2-14C, 15N]glutamate undergoes oxidative degradation in the liver of a rat, in which atoms of the following metabolites will each isotope be found?

a. Urea

b. Citrulline

c. Succinate

d. Ornithine

e. Arginine

f. Aspartate

Answer:----------

Q.4. During ammonia intoxication resulting from an arginine-deficient diet conducted some years ago, cats were fasted overnight then given a single complete in amino acids but without arginine, within 2 h, blood ammonia levels increased from a normal level of 18 μg/L to 140 μg/L, and the cats showed the clinical symptoms of ammonia toxicity. A control group fed a complete amino acid diet or an amino acid diet in which arginine was replaced by ornithine showed no unusual clinical symptoms.

a. What was the role of fasting in the experiment ?

b. What caused the ammonia levels to rise? Why did the absence of arginine lead to ammonia toxicity? Is arginine an essential amino acid in cats? Why or why not?

Q.5. Why can ornithine be substituted for arginine?

Answer:----------

Q.6. Alanine and Glutamine in the Blood Blood plasma contains all the amino acids required for the synthesis of body proteins, but they are not present in equal concentrations. Two amino acids, alanine and glutamine, are present in much higher concentrations in normal human blood plasma than any of the other amino acids. Suggest possible reasons for their abundance.

Answer:----------

Q.7. Inhibitors of Nucleotide Biosynthesis Suggest mechanisms for the inhibition of (a) alanine racemase by L-fluoroalanine and (b) glutamine amidotransferases by azaserine.?

Answer:----------

6.13. Further readings

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Notes

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