UNIT 8 : BIOENERGETICS

Structure

8.1 INTRODUCTION

In the previous block you have studied about the cell structure, function and the biomolecules present in it. You know that cell has a well defined structure built from complex organic molecules. This is made possible by a continuous flux of energy, through the living system and its transformation for various processes. These energy transformations in living systems are governed by the laws of thermodynamics. The study of such transformations comes under the purview of bioenergetics which includes the generation of energy by the breakdown of nutrients, its storage and utilisation in performing various functions of the cell. Further, bioenergetics attempts to unravel the complex network of biochemical pathways involving a large number of interwoven biochemical reactions. Here one tries to apply the principles of thermodynamics to rationalise the biochemical reactions in terms of free energies of various species.

In this unit we would first recapitulate the fundamentals of thermodynamics and then would evolve a criterion for the spontaneity of biochemical reactions in terms of thermodynamic quantities. Living organisms differ markedly in their appearance yet they have a striking similarity in that they all use the 'wonder molecule', ATP, as the carrier of energy. We would study its formation during phosphorylation and photosynthesis and its utilisation in various energy requiring functions like synthesis of organic molecules, etc. In this unit we would also discuss the validity of application of thermodynamic principles to the living system. In the coming units you would study the biosynthesis and breakdown of some biomolecules which were described in Blocks 1 and 2.

Objectives

After studying this unit you should be able to :

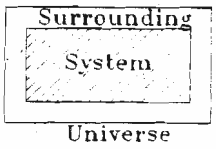
- predict the directions in which the various biochemicals reactions should proceed under the physiological conditions, calculate the energy input or output accompanying these reactions,

- describe the significance, formation and utilisation of ATP in living systems, and
- justify the applicability of the thermodynamic principles to living systems.

8.2 THERMODYNAMICS OF BIOCHEMICAL REACTIONS

Thermodynamics, also known as 'energetics', is better known in terms of some laws which have evolved on the basis of human experience. Before describing the laws and their applications to the biochemical reactions it would be worthwhile to get acquainted with the jargon of the terms used in thermodynamics. These are as follows:

Energetics in context of biological systems is called **bioenergetics.**



System: In thermodynamics any portion of the universe which we intend to study constitutes a system and everything else is its surroundings. A system is separated from its surroundings by a boundary (real or imaginary). A thermodynamic system and its surroundings can mutually exchange matter and energy. A system which permits the exchange of matter as well as energy is called an **open** system. On the other hand a system which permits the flow of energy but not of matter is called a **closed** system, whereas the one which does not permit the exchange of either energy of matter, is called an **isolated** system.

State of a System: In thermodynamics the state of a system is defined by assigning the value of thermodynamic variables namely, pressure, temperature and composition for example, 100 cm^3 of water at 298K and 1 bar pressure defines a state. Change in any of the parameters produces a new state.

Spontaneous Process: In thermodynamics, spontaneity refers to the tendency, of the change or, a chemical reaction to take place on it own accord. It has no relationship to the rate of the change. For example, when solid ammonium chloride is added to water, it goes into solution. Similarly when an acid and an alkali are mixed, they neutralise each other. These are examples of spontaneous processes. The reverse processes in these cases are not spontaneous, e.g., the formation of an acid and an alkali from aqueous solution of a salt. An ordinarily nonspontaneous process may be brought about under special conditions involving input of energy, e.g., electrolysis of salts. Some spontaneous processes may proceed exceedingly slow and the rate of reaction may be close to zero unless the reaction mixture is heated or a suitable catalyst is added. For example, the oxidation of glucose to carbon dioxide and water is a spontaneous process but does not proceed at a measurable rate unless glucose is heated or is acted upon by biological catalysts.

You know that a catalyst can only alter the rate of a spontaneous reaction. It cannot bring about a reaction which will not proceed spontaneously.

Now we shall state the laws of thermodynamics and see the thermodynamic quantities which emerge out of these. We would attempt to understand their significance and assess them as a criterion of spontaneity.

8.2.1 The First Law of Thermodynamics

A function depending on only the initial and final states is called the state function.

The first law of thermodynamics states that the total energy of a system and its surroundings, i.e., of the universe, is constant. If a system undergoes a change from state A to state B and in doing so it exchanges energy with its surroundings and performs some work, then according to the first law of thermodynamics,

$$E_B - E_A = \Delta E = Q - W \qquad \ldots (8.1)$$

Internal energy, E, includes the translational, rotational, vibrational and electronic energy of the molecule.

Where E_A and E_B represent the energy of the system, called internal energy of the system, in state A and B, respectively, Q stands for the heat absorbed by the system from its surroundings and W is the work done by the system on its surroundings. Note that work is also a form of energy.

You should note that although transformation of energy may take place, e.g., heat into work or vice versa, but the total energy of the universe remains constant. Therefore, the

first law of thermodynamics is also referred to as the **law of conservation of energy.** Note further that the energy change of the system, ΔE, depends only on the initial and final states of the system and is independent of the pathway of the change, or the number of steps involved. This is an important corollary of the first law.

Each system and indeed each of its constituents, is endowed with certain energy. In Eq. 8.1 E_A and E_B refer to the energy of the system, i.e., sum of the energies of all its constituents in state A and B, respectively. In most cases, we do not need to know these absolute values but only the changes in the energy (ΔE) as a result of any process or reaction, e.g., dissolution of ammonium chloride in water or neutralisation of an acid with alkali. This quantity can be experimentally determined by measuring the values of Q and W.

Most of the chemical reactions normally are carried out at a constant pressure. If we assume that the work involved in these is of expansion type then we may rewrite Eq. 8.1 as,

$$\Delta E = Q_p - P\Delta V$$

where the subscript p denotes the constancy of pressure. Rearranging the above equation we may write,

$$Q_p = (E_B + PV_B) - (E_A + PV_A)$$

Under these conditions a new function called enthalpy, H, defined below; is more pertinent for describing the chemical and biochemical reactions,

$$H = E + PV; \quad \Delta H = \Delta E + P\Delta V$$

Like internal energy, enthalpy is also a state function. We can only determine the change in enthalpy during a reaction, and not the absolute enthalpies of reactants or products. Normally the volume change is not large and the internal energy (E) and enthalpy (H) are only slightly different, more so for biochemical systems where the $P\Delta V$ term is negligiblv small.

So far we have defined two thermodynamic properties namely E and H. Let us see if these can provide a criterion for spontaneity. There are examples of spontaneous reactions in which for some the ΔE is –ve, while for others it is + ve. Clearly ΔE cannot be a criterion for spontaneity.

For quite some time it was believed that ΔH could be used as a criterion for spontaneity, since most of the spontaneous reactions at constant pressure are accompanied by evolution of heat i.e., are exothermic. But in course of time it became evident that there are many reactions that proceed spontaneously though accompanied by + ve ΔH. Both LiCl and $(NH_4)_2 SO_4$ dissolve readily i.e., spontaneously, in water. The former releases heat, while the latter absorbs heat. Thus enthalpy change also fails to be a criterion for spontaneity.

We see that the two thermodynamic properties obtained from the first law do not provide a sufficient criterion for the spontaneity of a reaction. We need to have an additional law that may provide the suitable criterion we are looking for. Let us see if the second law does the job.

8.2.2 The Second Law of Thermodynamics

This law can be stated in a number of equivalent ways. According to the statement most relevant to the biochemical processes, a change will take place spontaneously if it leads to an increase in randomness (or disorder) in the universe, i.e. of the system and its surroundings. The extent of randomness is represented by the thermodynamic function called **entropy** and denoted by S. Thus, the entropy of the universe must increase in any spontaneous process. In other words, S (system) + S (surroundings) should be greater than zero for a spontaneous change. We have got entropy change as a criterion for spontaneity but application of this statement to predict the spontaneity of a chemical reaction is not always possible. It is so because entropy changes are not easily measurable. Further, the law requires measurement of entropy changes not only of the

On microscopic level entropy is an index of the number of different ways that a system could be arranged without changing its energy. If there are Ω different ways then the absolute entropy (S) per mole of substance is,
$S = R \ln \Omega$, Where R is the gas constant.

systems but of its surroundings also. A convenient function for predicting the direction of a reaction was discovered by J. Gibbs. It is called **free energy** (G) and defined as.

$$G = H - TS; \quad \Delta G = \Delta H - T\Delta S \qquad \ldots (8.2)$$

Where ΔG is the change in the free energy of the system undergoing the chemical reaction, or any other process, at constant temperature T and pressure. ΔH and ΔS stand for the changes in the enthalpy and entropy, respectively of the system alone, and excludes the surroundings. Of the total enthalpy changes in a process, or a chemical reaction, a certain part (equal to $T\Delta S$) goes to increase the extent of randomness in the system and only the remainder amount i.e., $\Delta H - T\Delta S = \Delta G$ is available for performing useful work, which may be mechanical work, work of chemical synthesis or work due to transport of substances against the concentration gradient, etc. The concept of free energy and its applications are best illustrated with biochemical reactions, as will be seen with examples described later in this unit.

Chemical reactions with negative free energy changes are termed **exergonic**. Reactions with positive standard free energy changes are termed **endergonic**.

According to Gibbs, a reaction (or a process) will take place spontaneously if it is accompanied by a decrease in the free energy of the system. In other words, ΔG **(system) must be negative for a spontaneous reaction.** Note that application of this criterion for spontaneity does not require any measurements with the surroundings. Only the reacting system is to be considered.

The value of ΔG depends only on the free energy of the system before and after the chemical reaction, i.e.,

$$\Delta G = G \text{ (products)} - G \text{ (reactants)}$$

Therefore, ΔG is independent of the mechanism or the pathway of the reaction. This property of ΔG is useful in many calculations as will be discussed later. Further, it must be kept in mind that ΔG provides no information about the rate at which the chemical reaction will proceed. Thus, we have got a working criterion for spontaneity. Since it is an important function, it would be worthwhile to explore its relationship with other important characteristics of different types of reactions.

Free Energy Changes in Reversible Reactions

In a chemical reaction,

$$A + B \rightleftharpoons C + D$$

the free energy change, ΔG for the conversion of A and B into C and D is given by the following relationship:

$$\Delta G = \Delta G^\circ + RT \ln \frac{[C][D]}{[A][B]} \qquad \ldots (8.3)$$

where ΔG° is a constant which is characteristic of the reaction, R is the molar gas constant, T the absolute temperature in Kelvin and the quantities within square brackets represent the concentrations, more precisely the activities, of the respective products and reactants. Significance of the constant ΔG° becomes clear if we presume that the reaction is carried out under standard conditions, i.e., where concentrations of all reaction partners (reactants and products) are maintained at unity (one mole per dm^3 for solutions and one bar pressure for gases). Under these conditions, $\Delta G = \Delta G^\circ$, because the second term on the right hand side of Eq. 8.3 becomes zero. Therefore, ΔG° is referred to as the **standard free energy change** (or simply the standard free energy) of the reaction. Note that the actual free energy change will depend on this value and the prevailing concentrations of reactants and products as in Eq. 8.3. The distinction between ΔG and ΔG° is very important. The values of ΔG° have been tabulated for many reactions. However, it is necessary to calculate the actual ΔG value of a chemical reaction under the prevailing concentration conditions with the help of Eq. 8.3. The criterion for spontaneity and the direction in which a reversible reaction will proceed is ΔG and not ΔG°. As stated earlier also the reaction will proceed in the direction of negative ΔG.

The standard free energy of a reversible reaction, ΔG°, is related to its equilibrium constant, K_{eq}. Under equilibrium conditions, the free energy of the system is minimum and ΔG of the reaction is equal to zero. Therefore,

$$\Delta G = 0 = \Delta G^\circ + RT \ln \frac{[C]_{eq}\,[D]_{eq}}{[A]_{eq}\,[B]_{eq}} \qquad \ldots (8.4)$$

where the terms within square brackets represent concentrations under equilibrium conditions. Since,

$$K_{eq} = \frac{[C]_{eq}\,[D]_{eq}}{[A]_{eq}\,[B]_{eq}}$$

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

$$\Delta G^\circ = -2.303 \times R \times T \times \log K_{eq} \qquad \ldots (8.5)$$

As a matter of convention, temperature is fixed at 298 K. Values of K_{eq} of various reactions at this temperature are used to compute their standard free energies. For example, if K_{eq} is equal to 0.1 at 298 K,

$$\Delta G^\circ = -2.303 \times 8.314 \times 298 \times (\log 0.1)$$

$$= -2.303 \times 8.314 \times 298 \times (-1)$$

$$= 5705.8 \text{ J mol}^{-1}$$

$$= 5.706 \text{ kJ mol}^{-1}$$

The ΔG° values for reactions having K_{eq} equal to 1.0 and 10 can be similarly computed and are found to be equal to zero and –5.706 kJ mol^{-1}, respectively. A comparison of these values shows that a ten fold change in the equilibrium constant brings about a change of 5.706 kJ mol^{-1} in the standard free energy of a reaction at 298 K.

Oxidation is the loss of electrons while reduction is the gain of electrons.

Standard reduction potential of an oxidant/reductant pair is the measure of the tendency of the reductant to get reduced, i.e., to accept electrons. It is related to the observed potential and the concentration ratio of the electron - donor and electron-acceptor species by Nernst equation which is :

$$E = E^o + \frac{2.303RT}{nF} \log \frac{[\text{electron- acceptor}]}{[\text{electron- donor}]}$$

Free Energy Change for Redox Reactions

For redox reactions, i.e., the ones involving simultaneous oxidation and reduction, the value of standard free energy and hence that of the equilibrium constant can be obtained from the difference in the standard reduction petentials of the oxidant/reductant pair involved.

The difference in standard reduction potential ΔE° is related to the standard free energy change by the following relation:

$$\Delta G^\circ = -nF\Delta E^\circ \qquad \ldots (8.6)$$

Where, n = number of electrons transferred

F = Faraday, it is the free energy change when one mole of electrons drops through a potential of one volt (96.485 k J $volt^{-1}$ mol^{-1})

ΔG° (in volts) = the difference in the standard reduction potentials of the two reaction pairs

For example, for the reaction given below, involved in electron transfer chain from glucose metabolites to molecular oxygen in aerobic organisms.

(cytochrome c)–Fe^{II} + (cytochrome f)–Fe^{III} → (cytochrome c)–Fe^{III} + (cytochrome f) -Fe^{II}

the value of ΔE° is 0.11V which gives

$$\Delta G^\circ = -1 \times 96.485 \times 0.11 = -10.61 \text{ kJ mol}^{-1}$$

SAQ 1

Tick √ mark for the correct answer.

The free energy change, ΔG,

i) is equal to zero at equilibrium

ii) is directly proportional to the standard free energy change

iii) is equal to $-RT \ln K_{eq}$

iv) can be calculated only when the reactants and products are present at 1 M concentrations

8.2.3 Conventions in Biochemical Energetics

For biochemical reactions two conventions are very important. The first one concerns the standard state and hence the standard free energy change. You would recall that the standard state refers to a situation where all the reactants and products are at unit molar concentration which means that in a reaction involving H^+ ions the standard state would have $[H^+] = 1M$ or $pH = 0$. However, biochemical reactions take place at a pH of about 7, i.e., $[H^+] = 10^{-7}M$ and the above mentioned standard state of $pH = 0$ is not of much relevance. Consequently, a different standard state is adopted for biochemical reactions in which the concentration of H^+ ions is $10^{-7}M$ (and $pH = 7.0$); while that of all other species is 1 M, as with non-biochemical reactions. Further the corresponding standard free energy is also given a new symbol i.e., $\Delta G^{o\prime}$ as against ΔG^o corresponding to the physical chemistry standard state.

For a reaction where H^+ ions are released,

$$A + B = C + nH^+$$

the two standard free energies are related as

$$\Delta G^o = \Delta G^{o\prime} + nRT \ln 1/10^{-7}$$

For $n = 1$ and $T = 298\,K$

$$\Delta G^o = \Delta G^{o\prime} + 40.0\,kJ\ mol^{-1}$$

i.e., for the reaction producing H^+, ΔG^o is greater than $\Delta G^{o\prime}$ by about 40 k J mol^{-1}. On the other hand for reactions involving H^+ as reactant,

$$C + nH^+ \rightarrow A + B$$

for $n = 1$ and $T = 298\,K$

$\Delta G^{o\prime}$ is greater than ΔG^o by about 40 k J mol^{-1}

for reactions which do not involve H^+ ions

$$\Delta G^o = \Delta G^{o\prime}$$

You may recall that the criterion for the spontaneity of a reaction is its ΔG value and not ΔG^o or $\Delta G^{o\prime}$. The value of ΔG remains the same irrespective of the standard state chosen.

Hence, proper care should be taken while using the ΔG values (given in the standard tables) for biochemical reactions.

Another convention used in biochemical energetics is that the activity of water molecules in aqueous solutions is taken to be unity although their concentration is about 55 M.

The values of standard free energy changes obtained for different steps of a reaction can be added. The sum value of the ΔGs for individual reaction steps will determine whether the reaction will proceed in forward direction or not. You will study the significance of this additive property of ΔG in the following subsection.

8.2.4 Additivity of ΔG Values — Coupling Reactions

The values of ΔE, ΔH and ΔG depend on the initial and final states of the system only. When a system is transformed from state A to the state B, then

$$\Delta G \text{ (for A} \rightarrow \text{B)} = G_B - G_A$$

ΔG° for the oxidation of glucose to carbon dioxide and water will be the same whether it is brought about by combustion in a bomb calorimeter (one step) or via a number of steps as in a living cell.

The value of ΔG is independent of the pathway of transformation or the number of steps involved in going from state A to state B.

This change may take place in a single step,

$$A \rightarrow B \; ; \; \Delta G_{(B-A)}$$

Alternatively, the reaction may take place through an intermediate (I) as given below:

$$A \rightarrow I \; ; \; \Delta G_{(I-A)}$$

$$I \rightarrow B \; ; \; \Delta G_{(B-I)}$$

Since ΔG depends on the initial and final states only, it follows that

$$\Delta G_{(B-A)} = \Delta G_{(I-A)} + \Delta G_{(B-I)}$$

In other words, the thermodynamic equations can be added or subtracted like algebraic equations. This is a very useful relationship. It helps us in calculating ΔG (or ΔG° or $\Delta G^{\circ\prime}$) values for reactions where direct determination may not be conveniently carried out.

An important corollary of the additivity of free energy values is that certain unfavourable reactions (ΔG + ve) can be made to proceed by suitably coupling them with highly favourable reactions. Coupling of the reactions can take place in different ways. One of these is with the aid of suitable enzymes. For example, in the glycolysis (one of the metabolic pathways to be studied in the next unit), first step is the conversion of glucose to glucose-6-phosphate.

$$\text{glucose} + P_i \rightleftharpoons \text{glucose-6-phosphate}; \quad \Delta G^{\circ\prime} = 13.8 \text{kJ mol}^{-1}$$

As you can see, it is an endergonic process and would not be spontaneous. However this reaction can be driven by coupling it with a highly exergonic reaction i.e., the hydrolysis of ATP.

$$(\text{ATP} + H_2O \rightarrow \text{ADP} + P_i + H^+ \; ; \; \Delta G^{\circ\prime} = -30.5 \text{ kJ mol}^{-1})$$

The overall reaction is,

$$\text{ATP} + \text{glucose} \rightarrow \text{glucose-6-phosphate} + \text{ADP}; \quad \Delta G^{\circ\prime} = -17.2 \text{ kJ mol}^{-1}$$

It may however be noted that above two reactions would not couple if they are occurring independently. This reaction is catalysed by an enzyme called hexokinase which facilitates the transfer of phosphate group from ATP to glucose.

The favourable reaction either precedes or follows the unfavourable reaction. If it precedes then it provides the reactant for the unfavourable reaction in large amount to push it ahead. If it follows the unfavourable reaction then it continuously removes the product of first reaction thereby effectively pulling the reaction through.

This mode of coupling is essentially a manifestation of law of mass action.

For an unfavourable reaction B → C, highly favourable reactions like A → B or C → D can act as suitable coupling reactions where the former would have to precede and latter would have to follow the reaction B→C. For example, in the following sequence of reaction,

$$\text{Acetyl-CoA} + \text{oxaloacetate} \xrightarrow{A} \text{citryl-CoA} \xrightarrow{B} \text{citrate} + \text{CoA}$$

The $\Delta G^{o\prime}$ for step A is close to zero (but –ve) while the step B is highly exergonic and the overall reaction becomes quite favourable and proceeds in the right direction to a good extent. The energy carrier in living systems for various energy requiring processes (Sec. 8.5) is adenosine triphosphate (ATP) about which you will study in the next section. Before that try to answer the following SAQ.

SAQ 2

a) You have learnt about the similar nature of ΔG^{o} and $\Delta G^{o\prime}$. State when will ΔG^{o} and $\Delta G^{o\prime}$ be actually different?

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.....

b) For a reaction; A → B, will ΔG^{o} value be able to indicate its feasibility?

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8.3 ATP: THE ENERGY CARRIER IN BIOLOGICAL ENERGY TRANSFORMATIONS

A human being with a sedentary occupation needs about 8000 kJ per day, while somebody carrying out manual work requires upto 16000 kJ per day.

ATP is not a 'store' of energy but rather the link between exergonic and endergonic reactions.

You would recall that free energy change under isothermal conditions is the energy available for useful work. Since living cells function isothermally, these must utilise free energy for performing various energy requiring functions. A key function of cellular metabolism lies in transferring of free energy between the systems producing it and those consuming it. It was pointed out before that virtually all organisms make use of adenosine triphosphate, ATP, for this purpose. We would now make an attempt to understand how does ATP act as a carrier of free energy and what makes it the molecule of choice for the purpose.

8.3.1 Hydrolysis of ATP

The triphosphate component of ATP is responsible for its role as an energy carrier.

You have studied in Unit 4 that adenosine triphosphate is a nucleotide consisting of a purine base, adenine, a ribose sugar and a triphosphate unit. Fig. 8.1 gives the structure of ATP and its hydrolysis products namely, adenosine diphosphate, ADP; adenosine monophosphate, AMP, and the inorganic phosphates. A large amount of free energy is

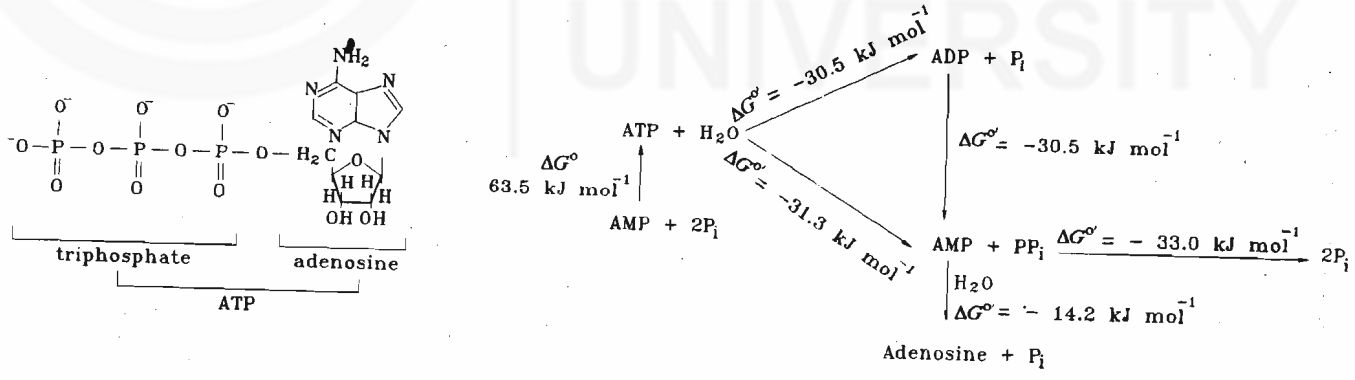


Fig.8.1 : Structure and hydrolysis of ATP and its components

generated on the hydrolysis of ATP as detailed in the figure.

The actual free energy change (ΔG) for the hydrolysis of ATP to ADP and phosphate ion (P_i) in a living cell is considerably different from $\Delta G^{o\prime}$ given in Fig. 8.1 and depends on the intracellular concentrations of the reaction partners, which are far from unity and are unequal. For example, the concentrations of ATP, ADP and P_i in human erythrocytes (red cells of the blood) are 2.25, 0.25 and 1.65 mM, respectively. Presuming the pH of blood to be equal to 7 and a temperature of 298 K (standard

temperature and pH), the actual ΔG value for the hydrolysis of ATP in these cells can be calculated with the help of Eq. 8.3.

$$\Delta G = \Delta G^{o\prime} + \left(2.303 \times R \times T \times \log \frac{[ADP]\,[P_i]}{[ATP]}\right)$$

$$= -30.5 + \left(2.303 \times 8.314 \times 298 \times \log \frac{(2.5 \times 10^{-4})\,(1.65 \times 10^{-3})}{(2.25 \times 10^{-3})}\right)$$

$$= -30.5 - 21.31$$

$$= -51.8\ \text{kJ mol}^{-1}$$

he numbers pertaining to $\Delta G^{o\prime}$ given above will be useful when we try to rationalise ıe choice of ATP as energy carrier. For just now, it should suffice to note that a large mount of energy is available on hydrolysis of ATP. Let us see how this energy is ıarnessed i.e. how does ATP work?

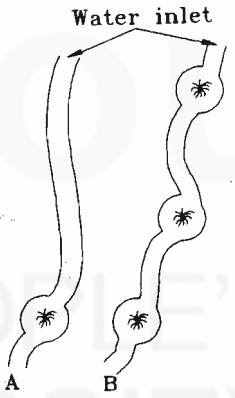
8.3.2 Role of ATP in Biochemical Energy Transformations

The free energy available from the hydrolysis is used to drive a number of reactions which require an input of free energy. This is done by suitably coupling the appropriate reaction. You would recall the example given in subsection 8.2.4. In this process of driving an energy requiring process, ATP gets hydrolysis to ADP. For the sustenance of life we need to have some mechanism of regenerating ATP, ADP and P_i by taking energy from external source.

Chemotrophs are the organisms using chemical source of energy.

In chemotrophs metabolic breakdown of nutrients takes place in such a way that the released free energy is utilised to drive the endergonic (free energy requiring) synthesis of ATP from ADP and P_i (positive $\Delta G^{o\prime}$). Similarly during photosynthesis the light energy is converted into chemical energy in the form of ATP (besides producing some reducing substances).

Thus, ATP is continuously formed and consumed in the living systems. It acts as the energy coinage or **"energy currency"** of the living cells. An analogy of the role of ATP in living cells is seen in exchange of currency when you visit a foreign country. In that country, you have to first get your money changed into the local currency in order to be able to pay for various services and merchandise. Similarly, all energy received by the living cells (chemical energy of the nutrients of light energy during photosynthesis) is converted into its own energy currency, i.e. ATP, before it can be utilised to drive the various energy requiring functions.



An analogous situation occurs in electricity generation from a large water fall when the only available turbine is of small and limited capacity. Since the turbine placed at the bottom cannot produce power more than it capacity (say 100 MW), the rest of the energy of the fall will go waste (dissipated as heat). On the other hand if several turbines are placed at appropriate heights, the energy of the water fall can be more efficiently harnessed. You can compare Figs. A and B for this. Similarly the large amount of free energy made available on oxidation of a glucose molecule can be better conserved if it is released in several packets representing the steps of metabolism. Many more molecules of ATP can be synthesised.

The role of ATP as an energy transducing agent, or vehicle, suggests that it should be rapidly "turned over", i.e., rapidly and repeatedly broken down to ADP and P_i and resynthesised in the living cells. An idea of the rapid turnover of ATP can be had from the fact that a resting adult human being "turns over" 30-40 kg ATP in one day, i.e., converts it into ADP and P_i and then back to ATP, The relative concentrations of ATP, ADP and P_i remain constant.

Before we go into the chemistry of ATP-ADP interconversion, it is important to compare the free energy change involved in this process with that obtainable from the oxidation of a common nutrient, namely, glucose.

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O \;;\; \Delta G^{o\prime} = -3085.7\ \text{kJ mol}^{-1} \quad \ldots (8.7)$$

You see that a large amount of free energy becomes available on complete oxidation of glucose into carbon dioxide and water. For an efficient utilisation of this energy for the synthesis of ATP, it is necessary that it should be liberated in several smaller packets, each of which should be adequate to drive the synthesis of ATP (see the margin).

Thus, the oxidation of glucose must take plac via a large number of steps and, therefore, involve an equal number of intermediates, called **metabolites**. It is indeed found to be so and this is a common feature of all metabolic pathways. Therefore, the metabolism is commonly referred to as **"Intermediary Metabolism"**. Some examples of metabolic pathways will be discussed in Units 9 and 10.

8.3.3 Structural Basis for the Role of ATP

We have seen before that the $\Delta G^{o\prime}$ value for the hydrolysis of ATP, is much larger than that for AMP. It is, in fact, found to be larger than that for the hydrolysis of all simple phosphate esters, e.g., glycerol-1-phosphate, glucose-6-phosphate, etc., Table 8.1.

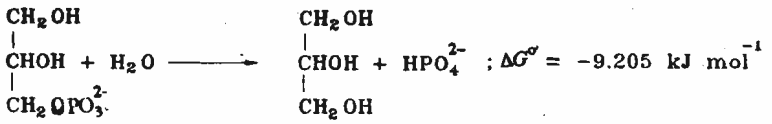
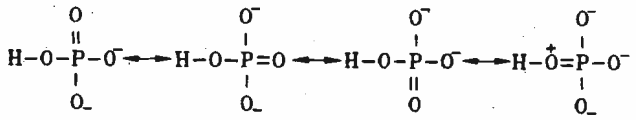


Table 8.1 : Standard Free Energy of Hydrolysis of Some Compounds of Biological Interest at pH 7

Sl.No.	Compound (Reaction)	$\Delta G^{o\prime}$ (kcal mol^{-1})	$\Delta G^{o\prime}$ (kJ mol^{-1})
1.	Phosphoenol pyruvate	−14.8	−61.92
2.	Carbamoyl phosphate (→ carbamate + P_i)	−12,3	−51.46
3.	1,3-Diphosphoglycerate (→ phosphoglycerate + P_i)	−11.8	−49.37
4.	Phosphocreatine	−10.3	−43.1
5.	Acetyl phosphate	−10.3	−43.1
6.	Pyrophosphate ion (PP_i + H_2O → $2P_i$)	−7.9	−33.0
7.	ATP (→ AMP + PP_i)	−7.5	−31.38
8.	ATP (→ ADP + P_i)	−7.3	−30.5
9.	ADP (→ AMP + P_i)	−7.3	−30.5
10.	AMP (→ adenosine + P_i)	−3.4	−14.23
11.	Glucose-1-phosphate	−5.0	−20.92
12.	Fructose-6-phosphate	−3.8	−15.90
13.	Glucose-6-phosphate	−3.3	−13.80
14.	Glycerol-1-phosphate	−2.2	−9.20
15.	Acetyl-AMP (→ acetate + AMP)	−13.3	−55.65
16.	Acetyl-SCoA (→ acetate + CoA-SH)	−7.5	−31.38
17.	Carboxylic esters (→ carboxylate ion + alcohol)	−4.0	−16.73

Since $\Delta G^{o\prime}$ values are related to the respective equilibrium constants of these reactions, it follows that ATP has a higher tendency to transfer its terminal phosphate group to water as compared to glycerol-1-phosphate or other simple phosphate esters. Therefore, it is said to have a higher **phosphate group transfer potential** than the phosphate esters.

The higher phosphate group transfer potential of ATP can be readily explained on the basis of its structure and that of ADP and phosphate ion at pH 7. ATP has four negative charges situated close to one another at this pH. There is strong force of repulsion between them. This repulsion is lesser in ADP which has three negative charges. Furthermore, at pH 7 phosphate ion is resonance stabilised due to delocalisation of its electrons.



This leads to a lower value of free energy of the products and, therefore, to a numerically larger $\Delta G^{o\prime}$ value for the reaction, which is equal to the difference between the free energy of the products and the reactants. No such resonance stabilisation is possible for ATP^{4-}.

Similar arguments are also applicable for explaining the large $\Delta G^{o\prime}$ for the hydrolysis of ADP to AMP and P_i, but not for the hydrolysis of AMP, glycerol-1-phosphate or other phosphate esters.

ATP and ADP have been referred to as **high energy phosphate compounds** and the phosphoanhydride, or pyrophosphate, bond between phosphate groups of these compounds has often been referred to as a **high energy bond.** The latter term must be understood to merely indicate the site of hydrolytic cleavage and not as suggesting that the free energy is somehow localised in that bond. It is always necessary to consider the complete structures of reactants and products.

A compound is referred to as high energy compound if its standard free energy of hydrolysis is negative and numerically larger than 29.2 k J mol^{-1}, i.e., it must be more negative than 29.2 k J mol^{-1}.

There are several other "high energy phosphate compounds", which participate in the metabolic processes, Table 8.1. The phosphate group transfer potentials of some of these are higher than that of ATP. This intermediate. position of ATP, with respect to phosphate group transfer potential is very significant.

Some compounds, other than phosphate derivatives, also exhibit large $\Delta G^{o\prime}$ values for hydrolysis, Table 8.1. These are also referred to as "high energy compounds". As we will see, some of these function as intermediates in the utilisation of ATP for biosynthetic work. It may be noted that classification as high energy compounds is based entirely on the $\Delta G^{o\prime}$ value for the hydrolytic cleavage.

Let us now take up the question why ATP is the molecule of choice for its role as energy carrier for different life processes. In this context the exergonic nature of its hydrolysis is of prime importance. We have already seen that the hydrolysis of ATP provides a large amount of free energy, obvious question is that if the hydrolysis of ATP to ADP or AMP is exergonic to similar extent ($\Delta G^{o\prime}$ for both is $\simeq -30.5$ k J mol^{-1}) then why do we have ATP/ADP pair as the vehicle of free energy and not ATP/AMP.

The answer to the above lies in the fact that the hydrolysis of ATP to AMP gives a molecule of pyrophosphate (PP_i). This molecule gets further hydrolysed giving an additional large amount of free energy $\Delta G^{o\prime} = -33.0$ kJ mol^{-1}. As a consequence the effective $\Delta G^{o\prime}$ for the hydrolysis to AMP and inorganic phosphate becomes large which means that the reaction would be almost irreversible. This would make the regeneration of ATP (from AMP) very difficult. On the other hand in case of ADP the regeneration of ATP would require moderate amount of free energy.

Further the intermediate position of ATP with respect to phosphate group transfer potential enables it to function as an efficient carrier of phosphate group in various metabolic reactions. This is because ATP is able to transfer its phosphate group to a number of metabolites and also a number of molecules are available (with higher phosphate group transfer potential) which can help regenerate ATP.

Another aspect in which ATP scores a point is that, it itself and its hydrolysis products are reactants or products for a number of biochemical reactions which makes it an efficient choice for coupling. You would recall that the available free energy can be utilised only if it is possible to suitably couple the reaction.

The purine base and ribose sugar (i.e., the nucleoside component) of AMP, ADP and ATP contribute towards interaction of these with enzymes. It is of importance in regulating enzyme activity. The above mentioned qualities besides the stability of ATP are responsible for its role.

In the process of study of role and mode of action of ATP we have realised that ATP has to be rapidly turned over. Let us now see how is this job accomplished. Before that try the following SAQ.

SAQ 3

Choose the correct answer. Each of the following contains a high energy phosphate group except

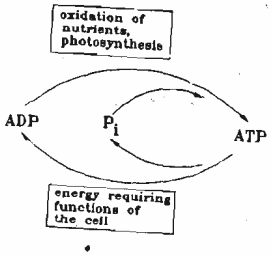
i) ATP

ii) ADP

iii) AMP

iv) Pyrophosphate ion

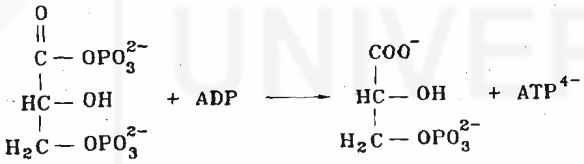
8.4 FORMATION OF ATP



The replenishment of ATP takes place in a number of ways. In this section we would take up two major routes for the synthesis of ATP. These routes include its synthesis during breakdown of nutrients and photosynthesis. In the breakdown of nutrients, some ATP is formed by the reaction of ADP with other phosphorylated metabolites having a higher phosphate group transfer potential. This method is called **substrate level phosphorylation**. In aerobic organisms, substrates are oxidised by transferring their electrons to coenzymes. The reduced coenzymes so obtained, (NADH, $FADH_2$, etc.) transfer their electrons eventually to oxygen. The latter reaction is accompanied by the synthesis of several molecules of ATP. This is the major source of cellular ATP in aerobic organisms and is referred to as **oxidative phosphorylation** or **electron transport chain**. Let us study these ATP forming processes in detail.

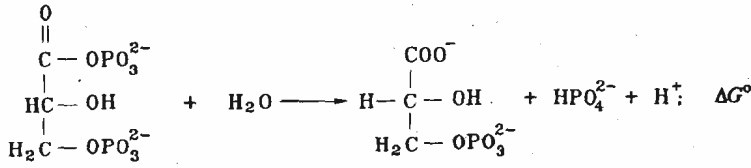
8.4.1 Substrate Level Phosphorylation

In the conversion of glucose and other sugars to pyruvate (glycolysis) in muscles, two reactions bring about the formation of ATP. You will study about them in Unit 9 also. These are the reactions of ADP with 1,3-diphosphoglycerate (DPG) and with phosphoenol pyruvate (PEP).



(DPG) ... (8.8)

The $\Delta G^{\circ\prime}$ value for this reaction can be calculated from the standard free energies of hydrolysis of DPG and ATP, Table 8.1.



... (8.9)

$$ADP^{3-} + HPO_4^{2-} + H^+ \rightarrow ATP^{4-} + H_2O; \Delta G^{\circ\prime} = +30.5\,kJ\,mol^{-1} \quad \ldots (8.10)$$

Adding Eq. 8.9 and 8.10, we get the $\Delta G^{o\prime}$ value for the reaction of Eq. 8.8, which is equal to –18.8 k J mol^{-1}. Thus, this reaction is exergonic. It should, therefore, proceed spontaneously from left to right. The equilibrium constant for this reaction can be calculated as follows.

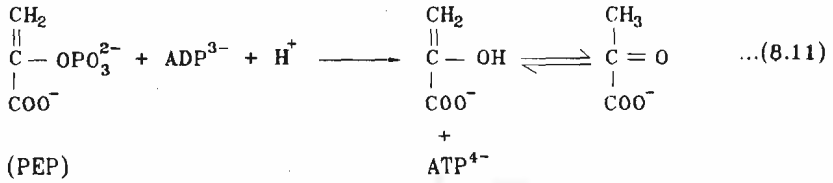
$$\Delta G^{o\prime} = - RT \ln K'_{eq} = - 2.303 \times R \times T \log K'_{eq}$$

Thus,
$$\log K'_{eq} = \frac{-18800}{-(2.303 \times 8.314 \times 298)} = 3.3$$

and
$$K'_{eq} = 1.99 \times 10^3 \text{ at pH 7 and 298K}$$

Note that the K'_{eq} value of the reaction heavily favours the formation of ATP. This is achieved by "spending" a much larger amount of free energy than is just adequate for the synthesis of ATP.

The second substrate level phosphorylation reaction is that between ADP and phosphoenol pyruvate (PEP), Eq.8.11.



...(8.11)

(PEP)

The $\Delta G^{o\prime}$ value for this reaction can be computed from the data given in Table 8.1 in a similar manner as explained for the reaction of Eq. 8.8.

SAQ 4

Calculate the $\Delta G^{o\prime}$ value for the reaction of Eq. 8.11 using the data given in Table 8.1.

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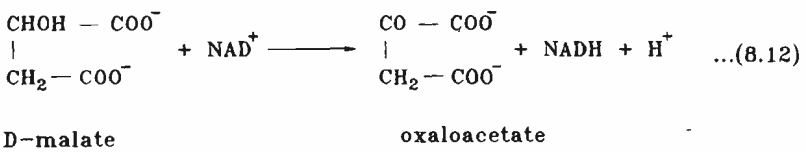
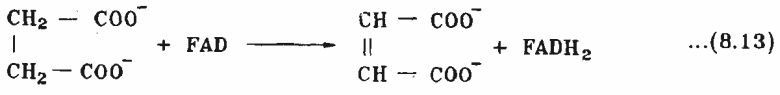
8.4.2 Oxidative Phosphorylation

It was mentioned earlier that oxidative phosphorylation involves reactions with reduced coenzymes. Two typical reactions producing the reduced coenzymes are given below:



...(8.12)

D–malate oxaloacetate



...(8.13)

succinate fumarate

The reduced coenzymes, NADH and $FADH_2$, are subsequently reoxidised by a series of reactions called **electron transport chain**, in which molecular oxygen is the ultimate electron acceptor. The pathway is also called **oxidative phosphorylation**, because under normal physiological conditions the electron transport is tightly coupled to the synthesis of several molecules of ATP from ADP and P_i. The steps of electron transport chain

Coenzyme is an accessory substance, not a protein, necessary to the protein part of an enzyme to work and is noncovalently bound to a protein. You have studied the structures of nicotinamide adenine dinucleotide, NAD and flavin adenine dinucleotide. FAD in unit 7 of this course.

The requirement for oxygen in electron transport process results in its another name, the **respiratory chain.**

are shown in Fig. 8.2 in which the sites of ATP synthesis are indicated. The reactions of Fig. 8.2 and the coupled ATP synthesis are brought about by the enzymes located on the inner membrane of mitochondria (see Unit 1 for the structure of mitochondria). With the exception of coenzyme Q, all the other members of this chain are associated with protein.

In the first step of the electron transport chain NAD^+ is reduced to NADH by a dehydrogenase which removes two hydrogen atoms from its substrate. NADH carries a proton and a hydride ion and transfers them to an enzyme complex, NADH dehydrogenase. This complex has a cofactor, i.e., flavin mononucleotide (FMN) which

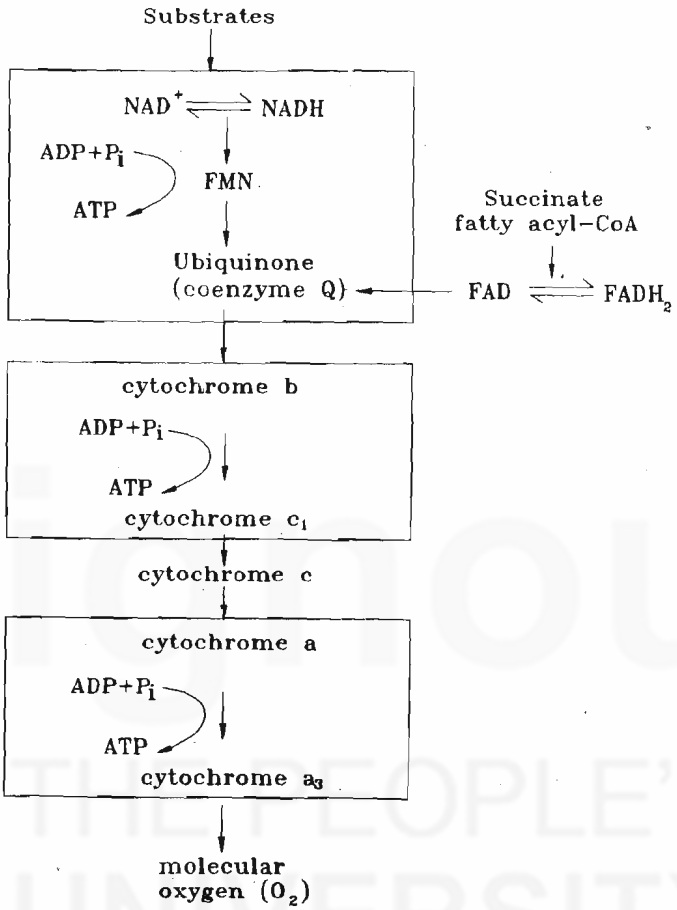


Fig. 8.2 : Electron transport chain showing the direction of flow of electrons and the sites of coupling with ATP synthesis (in boxes)

gets converted to $FMNH_2$ by accepting the two hydrogen atoms. The hydrogen atoms are next transferred to ubiquinone known as coenzyme Q. The remaining members of the chain are cytochromes. Electrons are passed down the chain to cytochromes b, c_1, c a and a_3. Cytochrome a_3 is the only cytochrome that can react directly with molecular oxygen. It is at this site that the transported electrons, molecular oxygen and free protons are brought together to produce water.

We had earlier seen (Sec. 8.2.3) that in oxidation-reduction reactions, the standard free energy change and, therefore, the equilibrium constant of the reaction can be calculated from the difference in the standard reduction potentials of the oxidant-reductant pairs involved. The overall standard free energy change in the oxidation of NADH by molecular oxygen (O_2) (Fig. 8.2) can be calculated from the standard reduction potentials of O_2/H_2O and NAD^+ / NADH pairs as per the following reactions.

$$1/2\,O_2 + 2\,H^+ + 2\,e^- \rightarrow H_2O\,;\ \Delta E^{\circ\prime} = +\,0.82\,V$$

$$NAD^+ + H^+ + 2e^- \rightarrow NADH\,;\ \Delta E^{\circ\prime} = -\,0.32\,V$$

$E^{\circ\prime}$ indicates standard potential at pH 7, i.e., at biochemical standard state.

Subtracting and rearranging the above reactions, we get,

$$NADH + H^+ + 1/2\,O_2 \rightarrow NAD^+ + H_2O\,; \quad \Delta E^{o\prime} = 1.14\,V$$

Substituting the values of n (= 2) and $\Delta E^{o\prime}$ (1.14 volts) in Eq. 8.6 we can calculate the value of $\Delta G^{o\prime}$ for the oxidation of NADH by O_2.

$$\Delta G^{o\prime} = -2 \times 96.48 \times 1.14 = -219.97\,kJ\,mol^{-1}$$

Note a large drop in the standard free energy of the system. If harnessed properly, this free energy can be utilised to drive the synthesis of several molecules of ATP ($\Delta G^{o\prime} = +30.5\,kJ\,mol^{-1}$) as indeed it happens in various steps of electron transport in the living cell, Fig. 8.2.

The above discussion shows that thermodynamically the synthesis of ATP may be coupled to specific steps of electron transport chain where the $\Delta E^{o\prime}$ may be adequate to provide the necessary free energy input. However, it does not explain as to how the coupling is brought about (as is the case with all thermodynamic calculations).

Mechanism of oxidative phosphorylation — Chemiosmotic Hypothesis

Mechanism of coupling of electron transport and ATP synthesis has been a subject of research for many years. A suggestion was made that an intermediate with a high phosphate group transfer potential might be formed during the electron transport and that it might then transfer its phosphate group to ADP. The reaction between ADP and the postulated intermediate would be similar to the reactions of ADP with DPG and PEP (Eq. 8.8 and 8.11). However, all experiments designed to detect such an intermediate ended in failures. No such intermediate has been detected so far.

It has been found that a complex enzyme assembly located in the inner mitochondrial membrane is responsible for the synthesis of ATP. This enzyme was first discovered through its catalytic action in bringing about the hydrolysis of ATP, i.e., the reverse of synthesis. Accordingly, it was named as an **ATPase**. Specifically, it is called H^+ – ATPase or F_1–F_0 –ATPase. These names were assigned, respectively, on the basis of the role of protons in its activity and the subunit structure of the enzyme. The physiological role of this enzyme is to catalyse the synthesis of ATP from ADP and P_i. Therefore, it is also referred to as **ATP-synthase.**

A novel suggestion was made by Peter Mitchell in 1961 to explain the role of ATP-synthase and the coupling of electron transport to ATP synthesis. According to him, the free energy released in specific steps of electron transport is utilised to "pump" protons from the mitochondrial matrix, i.e., from inside the inner membrane, to the intermembrane space, i.e., to outside the inner membrane, as represented in Fig. 8.3.

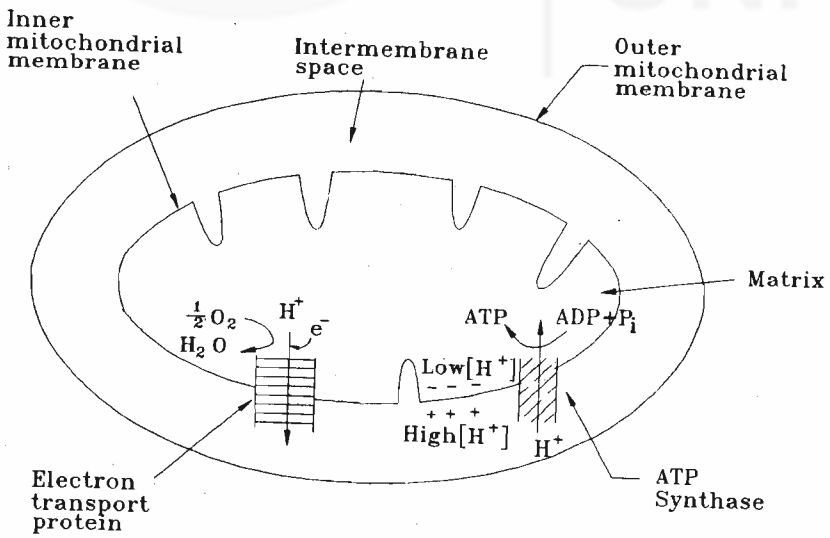


Fig. 8.3 : Oxidative phosphorylation on the inner mitochondrial membrane. Electron transport brings about "pumping" of protons from the matrix into the inner membrane space. As the protons "flow" down the gradient through ATP synthase, they drive the endergonic synthesis of ATP.

The unidirectional proton pumping is a consequence of the unique vectorial, i.e., directional, location of the electron transport proteins across the membrane. Since the inner mitochondrial membrane is not freely permeable to protons, this results in storage of some free energy in the form of a pH gradient with the outside becoming more acidic than the inside and a membrane potential where the outside has more positive charges than the inside. Together, the two gradients constitute a **proton motive force.** The excess protons "flow" back into the matrix through the ATP-synthase. This enzyme is located in the membrane and provides a channel for the flow of protons through an otherwise impermeable membrane. The free energy stored in the proton motive force drives the synthesis of ATP from ADP and P_i. Mitchell's hypothesis has been referred to as the **chemiosmotic hypothesis.** This simple and elegant hypothesis is supported by a variety of experimental results which are as follows :

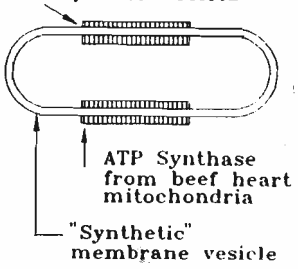
- It has been demonstrated that a proton gradient is established across the inner mitochondrial membrane during electron transport.
- ATP synthesis is found to take place when a pH gradient is imposed on the inner mitochondrial membrane even when no electron transport is taking place.
- According to this hypothesis, a closed compartment (or a vesicle) is necessary for the coupling of electron transport and ATP synthesis because otherwise the protons can move freely on both sides and no proton gradient can be established. Consistent with this prediction, no ATP synthesis is observed when electron transport is catalysed by fragments of the inner membrane. The liberated free energy is dissipated as heat.
- Some substances help transport protons by providing an alternative proton channel through the membrane. Such substances do not allow any proton gradient to be established. In their presence, electron transport proceeds unhindered but ATP is not synthesised. Presumably, the proton flow through the channels provided by these compounds is more facile than that through ATP-synthase. Such compounds are referred to as **uncouplers**. In their presence also, the free energy released on electron transport is dissipated as heat. 2,4-dinitrophenol is a commonly employed uncoupler.

8.4.3 Photosynthesis

Photosynthesis is the process in which plants use the energy of light to synthesise carbohydrates from the simple molecules of carbon dioxide and water.

As will be discussed in Unit 12, photosynthesis can be shown to proceed in a "light reaction" and a "dark reaction". In the light reaction, photo energy is utilised to produce a reduced coenzyme, NADPH, and ATP, which are required in the dark reaction for fixation and conversion of carbon dioxide into carbohydrates. We will discuss the synthesis of ATP only. Other details will be discussed in Unit 12.

Photosynthesis takes place in plants and some photosynthetic bacteria. In plants the process is brought about in chloroplasts. The latter contain flattened membrane-enclosed vesicles called **thylakoids**. These are the loci of photosynthesis. Chemiosmotic hypothesis applies also to ATP synthesis during photosynthesis. The inner compartment of the thylakoids becomes more acidic on irradiation. The resultant proton gratient then drives the synthesis of ATP in a similar manner as discussed above for the corresponding process in the mitochondria. Here also the results of some simple and straightforward experiments support the chemiosmotic hypothesis which are:



- Isolated chloroplasts were soaked in a buffer of pH 4 to permit a slow equilibration and lowering of pH in the inner compartment. ADP and P_i were added and the pH of the outer medium was suddenly raised to 8, thus setting up an artificial pH gradient where the interior of chloroplasts was more acidic than outside. ATP synthesis was found to take place in dark concomitant with the dissipation of the pH gradient. This shows that a pH gradient represents an energy rich state which can drive ATP synthesis without input of any other kind of energy.
- According to the chemiosmotic hypothesis, the proton pumping across the membrane and ATP synthesis are two separate reactions. These are catalysed by different enzymes and are coupled by virtue of their catalysts being located in a vectorial manner on the same membrane. This has been tested with a simple experiment by taking the proton pumping part from one source and ATP-synthase

from a different source. The former was the purified bacteriorhodopsin isolated from photosynthetic halobacteria and the latter was ATP synthase isolated from beef heart mitochondria (a nonphotosynthetic system). The two were incorporated into membranes which formed closed vesicles shown in the margin. Such "artificial" or "reconstituted" vesicles were found to bring about the synthesis of ATP on being irradiated with light. This observation supports the hypothesis and shows that the mechanism of ATP synthesis by photophosphorylation is essentially the same as in oxidative phosphorylation.

Details of how the light energy is harnessed and transformed into chemical energy are fascinating and will be discussed in Unit 12.

Like ATP, a proton gradient is also a readily accessible form of stored free energy which is put to various uses by different organisms. These include active transport of ions, rotation of flagella, ATP synthesis and also heat production during hibernation.

SAQ 5

Complete with the correct answer.

In oxidative phosphorylation, dinitrophenol

i) acts to inhibit the electron transport.

ii) allows electron transport to proceed without ATP synthesis.

iii) inhibits the cytochrome action.

8.5 UTILISATION OF ATP FOR ENERGY REQUIRING FUNCTIONS

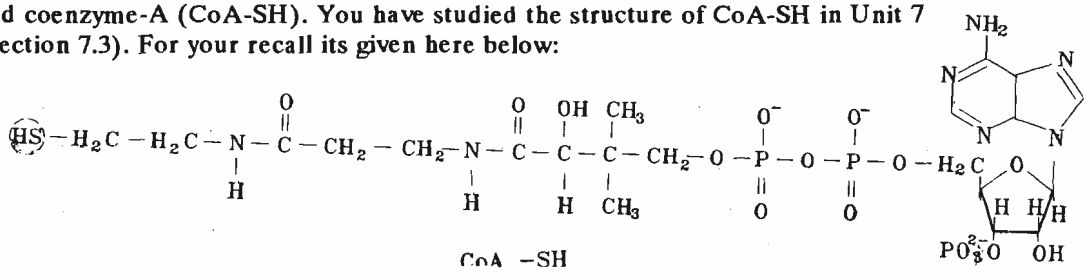
In the preceding section, we have discussed some examples of the transmutation of various forms of energy input, chemical nutrients or light into ATP which represents the chemical form of the energy currency of the living cells. We will now study, in brief, a few examples of the utilisation of the chemical energy of ATP for some energy requiring functions of the cells.

8.5.1 Synthesis of Organic Molecules

Let us consider the reversible reaction of acids and alcohols.

The equilibrium for this reaction lies predominantly towards the left hand side of the equation, i.e., hydrolysis of esters is favoured. This is especially so in aqueous solutions where the concentration of water molecules is exceedingly large (approx. 55M). Thus, aqueous suspensions of esters, e.g., oils and fats, tend to yield solutions of acids and alcohols. A familiar example is the rancidification of butter. Formation of esters, therefore, requires special conditions in order to overcome the unfavourable equilibrium constant. In the laboratory, this is achieved by carrying out the reaction of an acid and alcohol under anhydrous conditions and by continuous removal of water produced, e.g., in the presence of concentrated sulphuric acid. The living systems utilise a different approach. They "spend" free energy in order to "drive" this otherwise unfavourable endergonic process in aqueous environments. The free energy is provided by net hydrolysis of a molecule of ATP. This is explained as follows.

In the biological systems, the acid molecule is first "activated" by reaction with ATP and coenzyme-A (CoA-SH). You have studied the structure of CoA-SH in Unit 7 (Section 7.3). For your recall its given here below:



CoA –SH

$$R - COO^- + ATP \rightleftharpoons R - CO - AMP + PP_i$$
acyladenylate pyrophosphate

$$R - CO - AMP + CoA - SH \rightleftharpoons R - CO - SCoA + AMP$$

$$R - COO^- + ATP + CoA - SH \rightleftharpoons R - CO - SCoA + AMP + PP_i \quad \ldots(8.15)$$

The equilibrium constant for the above reaction is close to unity ($\Delta G^{o\prime}$ is close to zero). One "high energy bond" of ATP is broken and another bond of almost equal energy is established in acyl-SCoA (compare the $\Delta G^{o\prime}$ values for the hydrolysis of ATP and acetyl-CoA in Table 8.1). In the physiological systems, the reaction of formation of esters is driven to completion by removal of pyrophosphate ion (PP_i) product which is hydrolysed with the help of another enzyme, phyrophosphatase.

$$PP_i + H_2O \longrightarrow 2P_i \; ; \; \Delta G^{o\prime} = -33.0 \text{ kJ mol}^{-1}$$

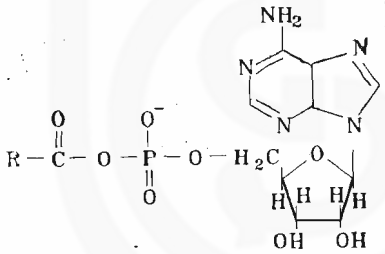
The overall reaction becomes practically irreversible due to large drop in the free energy.

$$RCOO^- + ATP + COA - SH + H_2O \rightarrow RCOSCoA + AMP + 2P_i \; ; \; \Delta G^{o\prime} = -30.5 \text{ kJ mol}^{-1}$$

Note the "expenditure" of two high energy pyrophosphate bonds for establishing one high energy thiol-ester linkage. This principle of driving an "uphill", i.e., an endergonic reaction, to completion by spending a much larger amount of free energy is observed in many biosynthetic pathways. Many of these are rendered irreversible by hydrolysis of pyrophosphate ion.

Acyl-CoA is an "activated" form of acyl moiety in the sense that it has a high acyl group transfer potential. The acyl group can be spontaneously transferred to various acceptors, e.g., alcohols, amines, water etc. Such acyl group transfer reactions proceed with a large free energy drop, i.e., $\Delta G^{o\prime}$ is large and negative.

$$R - CO - SCoA + R'OH \rightarrow R - CO - OR' + CoA - SH$$



(Acyl – AMP)

A slightly different but recurring motive observed in biosynthetic reactions requiring "activated" acyl groups utilises acyl-adenylate, also called acyl-AMP without the participation of coenzyme-A. For example, in the protein biosynthesis the carboxyl group of each amino acid is activated by the formation of aminoacyl-adenylate and the reaction is rendered irreversible by the hydrolysis of the resultant pyrophosphate ion.

8.5.2 Muscle Contraction

Let us now study how the free energy released on hydrolytic cleavage of ATP is used for mechanical work, namely muscle contraction.

The contractile muscle fibres consist of overlapping arrays of thick and thin filaments. The thick filaments are made up of bundles of parallel, rod shaped molecules of myosin with small "head" structures, which are in contact with the thin filaments. The latter consist of two strands of fibrous actin (F-actin) twisted around each other. In the muscle fibre, the thick and thin filaments are regularly arranged parallel to each other (inter-digitated) in repeating units, called sarcomeres (Fig. 8.4). During contraction, the thick filaments slide into the spaces between the thin filaments in each sarcomere, thereby causing a shortening of the entire muscle fibre. This is accompanied by hydrolysis of ATP, which provides the necessary free energy. It was observed by A. Szent-Gyorgyi (1940) that the actomyosin fibres contracted on the addition of ATP, but myosin or actin fibres taken separately did not.

Myosin of the thick filaments possesses ATPase activity, i.e., it catalyses the hydrolysis of ATP into ADP and P_i. The actomyosin complex hydrolyses ATP faster than myosin alone. The binding of ATP to actomyosin dissociates the complex, i.e., the contact between actin and myosin is broken. ATP is hydrolysed in the myosin-ATP complex, but the products are not released into the medium. The resulting myosin-ADP-P_i complex binds actin again, which accelerates the dissociation of the products (ADP and P_i) from myosin. The actomyosin so formed is now ready to bind another molecule of

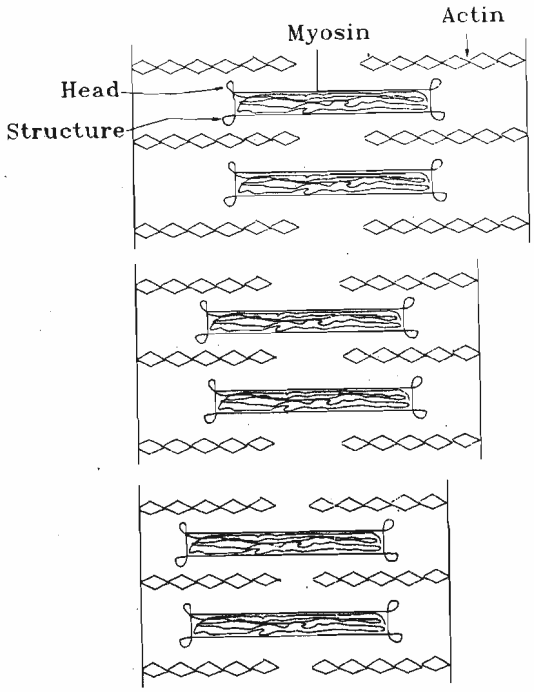


Fig. 8.4 : Thick(myosin) and thin (actin) filaments of a muscle 'sarcomere' in different stages of contraction

ATP (Fig. 8.5). When actin binds to the myosin-ADP-P_i complex, the linkage is not at the same site as in the initial actomyosin complex, but to a neighbouring site as depicted in Fig. 8.4. This is due to a conformational change in the "head" part of the myosin molecule on binding ATP. This results in a rowing type action, with the myosin head structure acting as an oar, which is responsible for the sliding motion of the thick filaments.

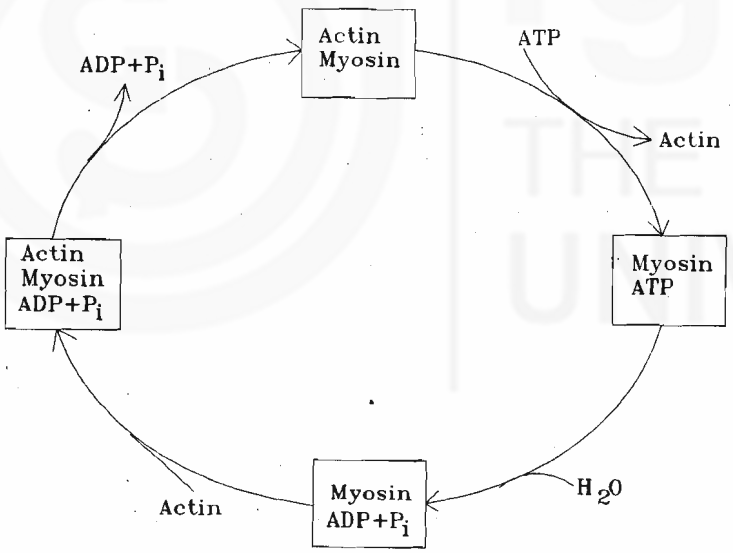


Fig. 8.5 : Hydrolysis of ATP by actomyosin complex

8.6 THERMODYNAMICS AND THE LIVING CELLS

In this unit, we have considered some isolated biochemical reactions and processes and the accompanying energy transductions in light of the principles of classical thermodynamics or equilibrium thermodynamics. These principles were earlier arrived at from considerations of closed systems at equilibrium in which all changes took place slowly and reversibly. The intact living cells, on the other hand, are open systems. Further, the concentrations of various cellular constituents are far from equilibrium

values and the metabolic reactions proceed quite fast. It may, therefore, be asked as to whether the laws of equilibrium thermodynamics are strictly applicable to the living cells. In this unit, as also in most textbooks of biochemistry, laws of thermodynamics have been applied for two purposes, namely, to predict the direction of spontaneous reactions and to calculate the free energy changes accompanying such reactions under the concentration conditions prevailing in the living cells. For these purposes, the laws of thermodynamics apply equally well to the living and nonliving systems.

At any given time, a living cell is in a steady state, in which the rate of input of matter and energy is equal to the rate of output of matter and energy. In a steady state, the concentration of cellular constituents, e.g., ATP, remain constant over a period of time because the rate of their formation (and input, if any) exactly equals the rate of their utilisation. For such a system, it will be more appropriate to apply the concepts of nonequilibrium (or irreversible) thermodynamics which are much more complicated and beyond the scope of this course. However, two properties of an open system are relevant here and need to be emphasised: (1) An open system in steady state is capable of doing useful work only when its constituents are not at equilibrium with one another. A system at equilibrium is incapable of doing any useful work at constant temperature, because $\Delta G^{o\prime} = 0$ for such a system. (2) A steady state represents the most orderly state of an open system. Rate of entropy production in an open system is minimum in steady state and the system operates with maximum efficiency. Note that $\Delta G = (\Delta H - T\Delta S)$ is maximised when ΔS is minimised. This has been aptly summed up by A. Katchalsky, a pioneer in the application of non-equilibrium thermodynamics to the living systems. He wrote, "But since there is no possibility of escaping the entropic doom imposed on all nature phenomena under the second Law of thermodynamics living organisms choose the least evil - they produce entropy at a minimum rate by maintaining a steady state."

SAQ 6

Tick (√) on the correct statements and (X) on the wrong statements given below.

i) Living cells are always in equilibrium.

ii) The rate of formation of each component in a cell is exactly equal to its rate of utilisation.

iii) Thermodynamically speaking the living cells constitute a closed system.

iv) The myosin-ATP complex is responsible for muscle contraction.

v) Generally hydrolysis of ATP provides the energy required for the synthesis of organic molecules in biological systems.

8.7 SUMMARY

Living organisms are highly organised systems, constituted by cells with well defined structure built from complex organic molecules. The living cells depend on randomly distributed and unorganised raw materials. This apparently spontaneous creation of an organised system from unorganised surroundings, i.e., creating order out of chaos, is made possible by a continuous flux of energy through the living system. Exchange of energy, or more precisely of enthalpy (i.e., heat content), between a system and its surroundings is governed by the law of conservation of energy or the first law of thermodynamics. Out of the total enthalpy change in a system, a certain part goes to increase the extent of randomness or entropy and the remainder amount (change in free energy) is available for performing useful work at constant temperature. The living cells depend on the latter for capture and utilisation of energy. The changes in enthalpy, ΔH, entropy, ΔS and free energy, ΔG of system are related by the equation, $\Delta G = \Delta H - T\Delta S$.

A physical transformation or a chemical reaction will take place spontaneously, i.e., of its own accord, if the accompanying ΔG of the system is negative. An exergonic process where ΔG is positive, can be made to proceed spontaneously if it can be coupled with a more strongly exergonic process, so that the net ΔG of the total system is negative. ΔG is related to standard free energy change, ΔG°, a constant, by the following relationship,

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

In biochemical systems, all measurements are made at pH 7 which is held constant. The standard free energy change at pH 7 is denoted by $\Delta G^{\circ\prime}$.

All biological systems depend on the availability of free energy in one form or the other, e.g., chemical and light energy. The immediate source of free energy in living systems is adenosine triphosphate (ATP). The standard free energy of the hydrolytic cleavage of ATP into adenosine diphosphate (ADP) and phosphate ion (P_i) at pH 7 and 298 K, $\Delta G^{\circ\prime}$, is found to be equal to –30.5 kJ mol^{-1}. The externally supplied energy from nutrients or from light in photosynthesis is first converted into ATP, i.e., its net synthesis from ADP and P_i. This is subsequently released by a net hydrolytic breakdown of ATP into ADP and P_i and utilised to drive the various energy requiring (endergonic) processes of the living cell, e.g., chemical synthesis, mechanical work, transport of ions and molecules against osmotic gradient, etc. ATP has been referred to as a "high energy" compound. Due to its unique role, ATP is continuously and rapidly synthesised and broken down, i.e., "turned over" in all living systems.

Thermodynamically, the living cells are said to constitute an open system which exchanges matter and energy continuously with its surroundings. At any given time a living cell is in a steady state, i.e., the rate of input of matter and energy is equal to the rate of output of matter and energy. The entropy increase in minimum when an open system is in steady state.

8.8 TERMINAL QUESTIONS

Use the data from Table 8.1 for answering the following questions.

1) Predict the direction in which the following reversible reactions will proceed spontaneously at pH 7 and 298 K, if the concentration of each reaction partner is one mole per litre.

   a) ATP + creatine $\rightleftharpoons$ phosphocreatine + ADP

   b) ATP + glucose $\rightleftharpoons$ glucose-6-phosphate + ADP

   c) ATP + acetate $\rightleftharpoons$ acetyl phosphate + ADP

   d) ATP + pyruvate $\rightleftharpoons$ phosphoenol pyruvate + ADP

2) Calculate the ΔG° and equilibrium constan K'_{eq} values for the isomerisation of glucose-6-phosphate to glucose-1-phosphate.

3) For the reaction,
   ATP + 3 - phosphoglycerate $\rightleftharpoons$ 1.3-diphosphoglycerate + ADP

   a) Calculate the $\Delta G^{\circ\prime}$ and K_{eq} values.

   b) If ATP/ADP ratio is 10, what will be ratio of 1.3-diphosphoglycerate to 3-phosphoglycerate?

4) ATP is considered the energy currency of a cell. Justify this statement.

8.9 ANSWERS

Self Assessment Questions

1) i)

2) a) When H^+ is a reactant or product the two will be very different.

   b) $\Delta G^{\circ\prime}$ value will be able to indicate the feasibility only under the condition that this value is similar to the actual free energy change. This condition may not apply under different conditions of temp., pH or concentration from those of standard one.

3) (iii)

4) The reaction can be considered to be the sum of the following two reactions, for which the $\Delta G^{o\prime}$ values are known from Table 8.1.

phosphoenol pyruvate + $H_2O \rightleftharpoons$ pyruvate + P_i; $\Delta G^{o\prime}$ = -61.9 kJ mol^{-1}

ADP + $P_i \rightleftharpoons$ ATP + H_2O; $\Delta G^{o\prime}$ = 30.5 kJ mol^{-1}

Sum : Phosphoenolpyruvate + ADP → ATP + pyruvate; $\Delta G^{o\prime}$ = -31.4kJ mol^{-1}

5) ii)

6) i) X ii) √ iii) X iv) √ v) √

Terminal Questions

1) Each reaction will proceed spontaneously in the direction in which ΔG value is negative. Since the initial concentration of each reaction partner is unity and pH and temperature are 7 and 298 K, respectively, $\Delta G = \Delta G^{o\prime}$. The value of $\Delta G^{o\prime}$ for each reaction can be calculated from the data of Table 8.1 by breaking the reaction into appropriate steps and applying the principle of additivity of ΔG values.

For example, the reaction (a) can be considered to be the sum of the following two reactions:

ATP + H_2O → ADP + P_i; $\Delta G^{o\prime}$ = –30.5 kJ mol^{-1}

Creatine + P_i → Creatine – P + H_2O; $\Delta G^{o\prime}$ = + 43.0 k J mol^{-1}

Sum: ATP + Creatine → Creatine -P + ADP; $\Delta G^{o\prime}$ = + 12.5 k J mol^{-1}

Note that $\Delta G^{o\prime}$ value has a plus sign if the reaction proceeds from left hand side (LHS) to the right hand side (RHS). The value of $\Delta G^{o\prime}$ for the same reaction going from RHS to LHS will be –12.5 k J mol^{-1}. Answers for the reactions b, c and d can be similarly obtained. It is found that the direction in which these reactions will proceed spontaneously are as follows:

a) RHS ⟶ LHS

b) LHS ⟶ RHS

c) RHS ⟶ LHS

d) RHS ⟶ LHS

2) The reaction; Glucose-6-phosphate ⟶ Glucose-1-phosphate, can be considered to be sum of the following reactions:

Glucose-6-phosphate + H_2O ⟶ Glucose + P_i ; $\Delta G^{o\prime}$ = -13.8 k J mol^{-1}

Glucose + P_i ⟶ Glucose-1-phosphate + H_2O ; $\Delta G^{o\prime}$ = + 20.9 k J mol^{-1}

Glucose-6-phosphate ⟶ Glucose-1-phosphate; $\Delta G^{o\prime}$ = + 7.1 k J mol^{-1}

The value of K'_{eq} can be obtained from the relationship,

$$\Delta G^{o\prime} = -R\,T \ln K'_{eq}$$

$$+7100 = -2.303 \times 8.314 \times 298 \times \log K'_{eq}$$

$$\log K'_{eq} = +1.244$$

Therefore, $K'_{eq} = +5.69 \times 10^{-2}$

3) a) $\Delta G^{o\prime}$ and K'_{eq} can be calculated in a similar manner as explained for question No. 2 above.

$\Delta G^{o\prime}$ = + 18.8 k J mol^{-1}

$K'_{eq} = 5.01 \times 10^{-4}$

b) $$K'_{eq} = \frac{[\text{Disphosphoglycerate}] \times [\text{ADP}]}{[3-\text{phosphoglycerate}] \times [\text{ATP}]} = 5.01 \times 10^{-4}$$

$$\frac{[\text{Diphosphoglycerate}]}{[3-\text{phosphoglycerate}]} = 5.01 \times 10^{-4} \times \frac{[\text{ATP}]}{[\text{ADP}]}$$

$$= 5.01 \times 10^{-4} \times 10$$

$$= 5.01 \times 10^{-3}$$

4) Hint : You can answer this question from Sec. 8.3.