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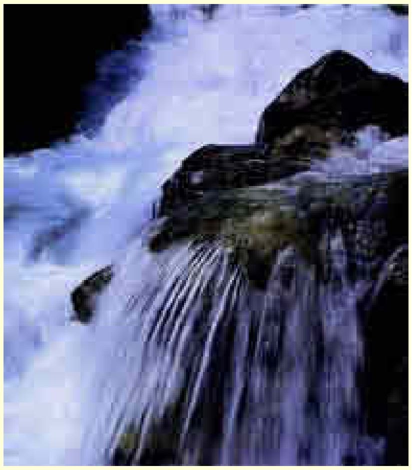

The potential energy of the water at the top of a waterfall is transformed into kinetic energy in spectacular fashion.

CHAPTER

20

Bioenergetics

COUPLING

Thermodynamics is the branch of physical chemistry that deals with the energy changes. And **biochemical thermodynamics** (or **biochemical energetics** or **bioenergetics**, as it is also called) is the field of biochemistry concerned with the transformation and use of energy by living cells. The chemical reactions occurring in living beings (or biochemical reactions) are associated with the liberation of energy as the reacting system moves from a higher to a lower energy level. *Most often, the energy is liberated in the form of heat*. In nonbiologic systems, heat energy may be transformed into mechanical or electrical energy. Since the biologic systems are isothermic, the heat energy cannot be used to drive the vital processes (such as synthesis, active transport, nerve conduction, muscular contraction etc.) obtain energy by chemical linkage (or coupling) to oxidation reactions. The simplest type of coupling (Fig. 20–1) may be represented by the equation.

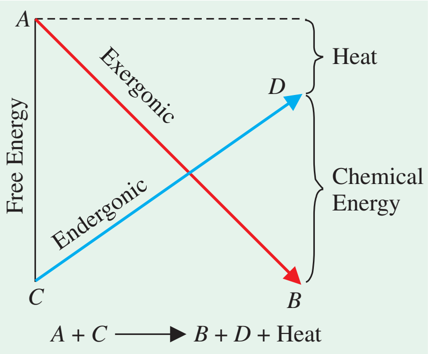

Fig. 20–1. Coupling of an exergonic to an endergonic reaction

$$A + C \longrightarrow B + D + \text{Heat}$$

The conversion of metabolite A to metabolite B occurs with the release of energy. It is coupled to another reaction, wherein energy is required to convert metabolite C to metabolite D.

CONCEPT OF ENERGY

Energy is defined as the capacity to do work, which is the product of a given force acting through a given distance:

$$\text{Work} = \text{Force} \times \text{Distance}$$

It is one of the fundamental components of any system. Energy exists in a variety of forms, such as electrical, mechanical, chemical, heat and light energy. These different forms of energy are interconvertible, for example, an electric motor converts electric into mechanical energy, a battery changes chemical into electrical energy and a steam engine transforms heat into mechanical energy. Some of the common examples of transformations of energy in biological systems are given in Fig. 20–2. Besides, these various forms of energy are also interrelated quantitatively, for example,

$$1.0 \text{ calorie of heat energy} \equiv 4.185 \times 10^{7} \text{ ergs of mechanical energy}$$


(*a*) (*b*) (*c*)

Fig. 20–2. Transformation of energy in biological systems

(*a*) The running horse represents conversion of chemical energy to mechanical energy.
(*b*) The electric fish (*Torpedinidae*) converts chemical energy to electrical energy.
(*c*) The phosphorescent bacteria convert chemical energy into light energy.

Furthermore, during conversion of one form of energy to the other, there is always some loss. As an example, when an electric motor transforms electric into mechanical energy, the output of useful energy is always less than the input. This is due to friction in the motor which generates heat. The heat, in turn, is dissipated in the environment and is no longer useful. Thus, when a work is done or when one form of energy is changed to the other, there is a loss of useful energy.

THERMODYNAMIC PRINCIPLES

Many quantitative observations made on the interconversion of various forms of energy have led scientists to the formulation of two fundamental laws of thermodynamics, the first and second. These laws help us understanding :

(*a*) the direction of a reaction, whether from left to right or vice versa,
(*b*) the accomplishment of work, whether useful or not, and
(*c*) whether the energy for driving a reaction must be delivered from an external source.

The First Law : Principle of Conservation of Energy

In thermodynamics, a *system* is a matter within a defined region. The matter in the rest of the universe is called the *surroundings*. Thus, the system plus the surroundings constitute the universe (Fig. 20–3), which literally includes the entire earth, rather even the outer space. Some physical

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or chemical processes can be made to take place in isolated systems which are unable to exchange energy with their surroundings. But in the biological world, the reacting system do exchange energy, and often matter also with their surroundings.

The principle of conservation of energy was first formulated by V. Mayer in 1841 as a result of a study of energy transformation in the inanimate world, but it is equally applicable to living systems. The first law of thermodynamics states that *the total amount of energy in the universe (i.e., the system + surroundings) remains constant*. Paraphrased, it says that energy cannot be created or destroyed. To date, there is no known exception to this law. Thus, whenever energy is used to do work or is converted from one to the other form, the total amount of energy is unchanged. The mathematical expression of the first law is :

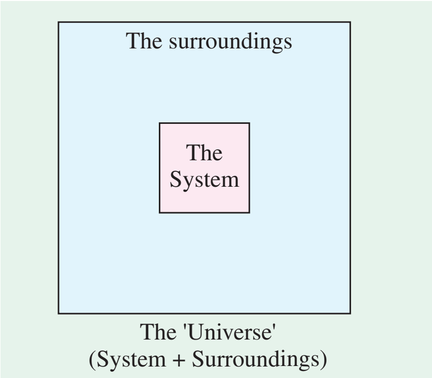

Fig. 20–3. Schematic representation of a reacting system and its surroundings

Energy can be exchanged between the system and the surroundings but only according to the laws of thermodynamics.

$$\Delta E = E_B - E_A = Q - W \qquad \text{... (1)}$$

where, ΔE = change in internal energy
ΔE_A = energy of a system at the start of a process
ΔE_B = energy of a system at the end of a process
Q = heat absorbed by the system
W = work done by the system.

A noteworthy point about equation 1 is that the change in energy of a system depends only on the initial and the final stages and not on the path of transformation.

The Second Law

The first law of thermodynamics cannot be used to predict whether a reaction can occur spontaneously, although ΔE is positive. In such cases, the system absorbs heat from its surroundings so that the sum of the energies of the system and its surroundings remains the constant. It is evident that a function other than ΔE is required. One such function is *entropy*, which is denoted by the symbol S. *Entropy is energy in a state of randomness or disorder*. It is unavailable, useless energy. In other words, entropy is a measure of the degree of randomness or disorder of a system. The entropy of a system increases (*i.e.*, ΔS is positive) when it becomes more disordered. Entropy becomes maximum in a system as it approaches equilibrium. When equilibrium is attained, no further change can occur spontaneously unless additional energy is supplied from outside the system.

The term **entropy** was first used in 1851 by Rudolf Clasius of Germany, one of the propounders of the second law of thermodynamics. The importance of entropy was pronounced by **John A. Wheeler** in following words : "No one is considered scientifically literate today who does not know what a Gaussian distribution is, or the meaning and scope of the concept of entropy."

Thus, according to the second law, at each transfer or transformation of energy, part of that energy assumes a form that cannot be passed on any further because it is randomly dispersed, often as heat. And it is this randomly dispersed energy which is called *entropy.* Ultimately closed systems run down. Open systems, such as ecosystems, however,with their constant input and outflow, maintain a steady state in spite of the second law of thermodynamics. Biological systems, thus, do not seem to conform to the second law, for the tendency of life is to produce order out of disorder, to decrease rather than increase entropy.

Although it is true that energy is conserved in the universe, the first law of the thermodynamics does not account for many physical and chemical processes. A simple example may help make this clear. Suppose a full gas cylinder is connected to an empty cylinder by a tube with value (Fig 20-4. If the valve is opened, gas flows from the full to the empty cylinder until the gas pressure is equal on both sides. Energy has not only been redistributed but also conserved. The expansion of gas is explained by the second law of thermodynamics and a condition of matter called entropy which, as already stated, is a measure of the randomness or disorder of a system. The greater the disorder of a system, the greater is its entropy. The second law states that physical and chemical processes proceed in such a way that the randomness or disorder of the universe (the system and its surroundings) increases to the maximum possible. Gas will always expand into an empty cylinder.

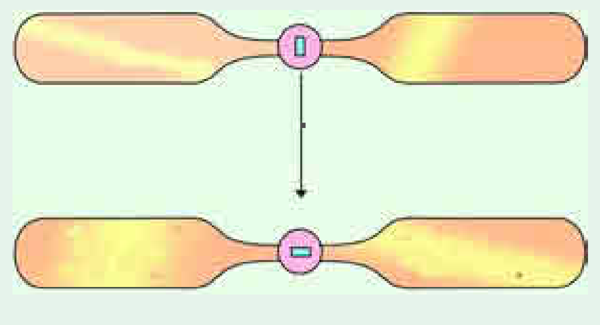

Fig. 20–4. A second law process

The expansion of gas into an empty cylinder simply redistributes the gas molecules until equilibrium is reached. The total number of molecules ramains unchanged.

The second law of thermodynamics states that a process can occur spontaneously only if the sum of the entropies of the system and its surroundings increases. This can be represented as:

$$(\Delta S_{\text{system}} + \Delta S_{\text{surroundings}}) > 0 \quad \text{for a spontaneous process} \quad \ldots \quad (2)$$

Thus, the total entropy of a system must increase if a process is to occur spontaneously. However, the entropy of a system can decrease even during a spontaneous process, provided the entropy of the surroundings increases to such extent that their sum is positive. When water freezes, its entropy decreases because the water molecules of ice exist in a more ordered state with less freedom of movement than in a liquid state. The decrease of entropy is particularly apparent in the formation of a snowflake (Fig. 20–5).

The difference between the first and second laws of thermodynamics is that the *first law* is concerned with the accounting of the various kinds of energy involved in a given process, whereas the *second law* is concerned with the availability of the energy of a given system for doing useful work.

Fig. 20–5. When water freezes, its entropy decreases which becomes particularly apparent in the formation of a snowflake.

(*Courtesy : Nuridsany and Perennou*)

Combining the Two Laws

Since the entropy changes of chemical reactions are not readily measurable, the entropy is not used as a criterion whether a biochemical process can occur spontaneously or not. Furthermore, for spontaneity, both the entropy changes (that of the surroundings and of the system) should be known. These difficulties are alleviated by using a different thermodynamic function, called the *free energy*, which is denoted by the symbol G (In older literature, however, the symbol F is used in its place).

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In 1878, Gibbs created the free energy function by combining the first and second laws of thermodynamics in the form of following equation :

$$\Delta G = \Delta H - T\Delta S \qquad \text{... (3)}$$

where, ΔG = the change in free energy of a reacting system,

ΔH = the change in heat content or enthalpy of this system,

T = the absolute temperature at which the process is taking place, and

ΔS = the change in entropy of this system.

JOSIAH WILLARD GIBBS

(LT, 1839-1903)

Gibbs was an American physical chemist, who single-handedly created a large portion of chemical thermo-dynamics. The symbol G is given to free energy in his honour. His work is the basis of biochemical thermodynamics, and he is considered by some to have been the greatest scientist born in the United States.

(*Courtesy: The Bettman Archive*)

In fact, this equation represents quantitative interrelationship between the changes in free energy, heat and entropy in chemical reactions at constant temperature (T) and pressure (P), the conditions prevailing in biological systems.

The term $T\Delta S$ is that fraction of ΔH which cannot be put to useful work. The ΔG indicates the free energy change or the theoretically available useful work. Naturally, in most cases the system is 'inefficient' and not all of the theoretically available work can be utilized. However, the properties of the surroundings do not enter into this equation.

The enthalpy change, ΔH is given by the following equation :

$$\Delta H = \Delta E + P\Delta V \qquad \text{...(4)}$$

where, ΔE = the change in internal energy of a reaction, and

ΔV = the change in volume of this reaction.

As the volume change, ΔV is small for nearly all biochemical reactions, hence ΔH is nearly equal to the change in internal energy, ΔE. Therefore, equation 3 modifies as follows :

$$\Delta G \cong \Delta E - T\Delta S \qquad \text{...(5)}$$

Thus, the change in free energy of a reaction, ΔG depends both on the change in internal energy and on the change in entropy of the system. The ΔG is a valuable criterion in determining whether a reaction can occur spontaneously. Thus,

(*a*) If ΔG is negative in sign, the reaction proceeds spontaneously with loss of free energy, *i.e.*, it is **exergonic**. If, in addition, ΔG is of great magnitude, the reaction goes virtually to completion and is essentially irreversible.

(*b*) If, however, ΔG is positive, the reaction proceeds only if free energy can be gained, *i.e.*, it is **endergonic**. If, in addition, ΔG is of high magnitude, the system is stable with little or no tendency for a reaction to occur.

(*c*) If ΔG is zero, the reaction system is at equilibrium and no net change takes place.

With regard to the free energy change, ΔG of a reacting system two more points need to be emphasized :

I. Firstly, the ΔG of a reaction depends only on the free energy of the products minus that of the reactants. *The ΔG of a reaction is independent of the path of transformation.* Obviously, the mechanism of a reaction has no effect on ΔG. As an instance, the value of ΔG is the same for the oxidation of glucose to CO_2 and H_2O whether it takes place by combustion or by a series of enzyme-catalyzed reactions.

II. Secondly, *the value of ΔG provides no information about the rate of a reaction.* A negative ΔG indicates that a reaction can occur spontaneously, but it does not signify that it will occur at a perceptible rate. As already pointed out, the rate of a reaction rather depends on the free energy of activation ($\Delta G^{\neq}$), which is unrelated to ΔG.

To get a feeling for the magnitude of changes in various forms of energy, an actual example of aerobic oxidation of glucose may be cited. The living cells carry out oxidation of glucose in the presence of oxygen to CO_2 and H_2O at constant temperature and pressure.

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

Assuming that the temperature is 25°C (or 298 K) and the pressure is 1.0 atm. (or 760 mm Hg), which are standard conditions in thermodynamic calculations, the following energy changes take place per molecule of glucose oxidized :

ΔG = – 686,000 cal/mol (*i.e.*, the free energy of the reacting moleculeshas decreased)

ΔH = – 673,000 cal/mol (*i.e.*, the reacting molecules have released heat)

The equation 3 may also be written as :

$$\Delta S = \frac{\Delta H - \Delta G}{T} \qquad \text{...(6)}$$

Substituting the above values in equation 6, we get :

$$\Delta S = \frac{-673{,}000 - (-686{,}000)}{298}$$

= 44 cal/deg (*i.e.*, the entropy of the universe has increased)

RELATIONSHIP BETWEEN STANDARD FREE ENERGY CHANGE AND EQUILIBRIUM CONSTANT

In a model reaction,

$$A + B \rightleftharpoons C + D \qquad \text{...(7)}$$

The free energy change, ΔG of this reaction is given by

$$\Delta G = \Delta G^\circ + RT \log_e \frac{[C][D]}{[A][B]} \qquad \text{...(8)}$$

where, ΔG° = Standard free energy change,

R = Gas constant,

T = Absolute temperature, and

$[A]$, $[B]$, $[C]$ and $[D]$ = Molar concentrations (*i.e.*, activities) of the reactants

To elaborate, ΔG° is the free energy change for this reaction under standard conditions, *i.e.*,when each of the reactants *A, B, C* and *D* is present at a concentration of 1.0 M. Thus, the ΔG of a reaction depends on the nature of the reactants (expressed in ΔG° term) and on their concentrations (expressed in logarithmic terms), as shown in equation 8.

At equilibrium, $\Delta G = 0$. Equation 8 then becomes,

$$O = \Delta G^\circ + RT \log_e \frac{[C][D]}{[A][B]} \qquad ...(9)$$

or

$$\Delta G^\circ = -RT \log_e \frac{[C][D]}{[A][B]} \qquad ...(10)$$

The equilibrium constant under standard conditions, K'_{eq} for the reaction $A + B \rightleftharpoons C + D$, is given by

$$K'_{eq} = \frac{[C][D]}{[A][B]} \qquad ...(11)$$

However, in reactions in which more than one molecule of any reactant or product participates, the general reaction“

$$a\text{A} + b\text{B} \rightleftharpoons c\text{C} + d\text{D}$$

where a, b, c and d are the number of molecules of A, B, C and D participating, the equilibrium constant is given by

$$K'_{eq} = \frac{[C]^c\,[D]^d}{[A]^a\,[B]^b} \qquad ...(12)$$

Substituting equation 11 into equation 10 gives

$$\Delta G^\circ = -RT \log_e K'_{eq} \qquad ...(13)$$

or

$$\Delta G^\circ = -2.303\ RT \log_{10} K'_{eq} \qquad ...(14)$$

or

$$K'_{eq} = 10^{-\Delta G^\circ/(2.303RT)} \qquad ...(15)$$

Substituting $R = 1.98 \times 10^{-3}$ kcal mol^{-1} degree^{-1} and $T = 298$°K (corresponding to 25°C) gives

$$K'_{eq} = 10^{-\Delta G^\circ/1.36} \qquad ...(16)$$

when ΔG° is expressed in kcal/mol. Thus, the standard free energy, $\Delta G^\circ$and the equilibrium constant, K'_{eq} are related by a simple expression. For example, a change in equilibrium constant by a factor of 10 results in a change in standard free energy of –1.36 kcal/mol at 25°C (refer Table 20–1). At 37°C, however, the change in standard free energy would be of 1.42 kcal/mol. Values of ΔG° may be expressed in joules or calories per mole.

Table 20–1. Numerical relationship between equilibrium constants of chemical reactions and their standard free energy changes at 25°C

K'_{eq}	ΔG° (kcal/mol)
10^{-5} or 0.00001	+ 6.82
10^{-4} or 0.0001	+ 5.46
10^{-3} or 0.001	+ 4.09
10^{-2} or 0.01	+ 2.73
10^{-1} or 0.1	+ 1.36
1	0
10^{1} or 10	– 1.36
10^{2} or 100	– 2.73
10^{3} or 1,000	– 4.09
10^{4} or 10,000	– 5.46
10^{5} or 1,00,000	– 6.82

$\Delta G°$ may also be defined as the difference between the free energy content of the reactants and that of the products under standard conditions (*i.e.*, 298 K pressure and 1.0 atmospheric pressure) when the reactants and products are present in their standard concentrations, namely 1.0 *M*. When $\Delta G°$ is negative (*i.e.*, the products contain less free energy than the reactants), the reaction will proceed to form the products under standard conditions, since all chemical reactions will tend to go in that direction resulting in decrease in the free energy of the system. When $\Delta G°$ is positive (*i.e.*, the products of the reaction contain more free energy than the reactants), the reaction will tend to go in the reverse direction if we start with 1 *M* concentration of all components. To be more explicit, reactions with a negative $\Delta G°$ proceed forward in the direction written when they start with all reactants and products at 1.0 *M*, until they reach equilibrium. Reactions with a positive $\Delta G°$ will proceed in the reverse of the direction written when they start with all components at 1.0 *M*. Table 20–2 summarizes these relationships.

Table 20–2. Relationship among K'_{eq} , $\Delta G°$ and the direction of chemical reactions under standard conditions

When K'_{eq} is :	*$\Delta G°$ is :*	*Starting with 1 M components, the reaction :*
> 1.0	Negative	proceeds forward
1.0	Zero	remains at equilibrium
< 1.0	Positive	proceeds backward

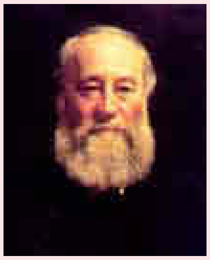
Two more points need be emphasized :

I. Biochemical reactions take place near *p*H 7.0. Hence, *p*H 7.0 is conventionally designated the standard pH in biochemical energetics. The standard free energy change at *p*H 7.0 in biochemical energetics is designated by the symbol, $\Delta G°'$, which shall be used in further discussions.

II. The basic SI (Systéme International) unit of energy is joule (J). However, in biology and medicine, heat and energy measurements are equally expressed in calories (cal). The interrelationship between colories and joules is :

$$1.000 \text{ cal} = 4.184 \text{ J}$$

A **calorie (cal)** is equivalent to the amount of heat needed to raise the temperature of 1 gram of water from 14.5 to 15.5°C. A **kilocalorie (kcal)** is equal to 1,000 cal. Also, 1 kcal = 4.184 kJ.



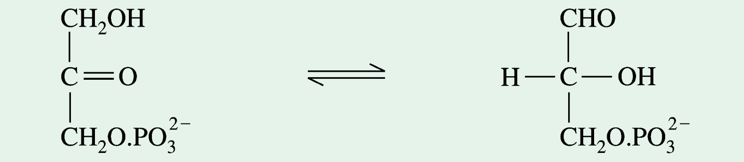
JAMES PRESCOTT JOULE

(LT, 1818-1889)

James Prescott Joule was a British engineer who experimentally proved the first law of thermodynamics. He determined the mechanical equivalent of heat in 1843. The unit Joule is named after him. A **Joule (J)** is the amount of energy required to apply a 1 newton force over a distance of 1 metre. A **kilojoule (kJ)** is equal to 1,000 J. Faraday's belief in the unity of nature was vindicated in work by Joule, Thomson, Helmholtz, Clasius and Maxwell.

STANDARD FREE ENERGY CHANGES AT pH 7.0 OR $\Delta G°'$

To calculate $\Delta G°'$, an example of isomerization of dihydroxyacetone phosphate (DHAP) to glyceraldehyde 3-phosphate (G-3-P) may be taken. This is one of the reactions of glycolysis.



Dihydroxyacetone phosphate Glyceraldehyde 3-phosphate

At equilibrium, the ratio of glyceraldehyde 3-phosphate to dilydroxyacetone phosphate is 0.0475 at 25°C (298°K) and pH 7.0. Hence,

$$K'_{eq} = 0.0475$$

The standard free energy change for this reaction is, then, calculated from equation 14, as follows :

$$\begin{aligned}\Delta G^\circ &= -2.303\ RT \log_{10} K'_{eq}\\ &= -2.303 \times 1.98 \times 10^{-3} \times 298 \times \log_{10} (0.0475)\\ &= +1.8 \text{ k cal/mol}\end{aligned}$$

When the initial concentration of DHAP is $2 \times 10^{-4}\ M$ and the initial concentration of G-3-P is $3 \times 10^{-6}\ M$, the ΔG can be calculated from equation 8, as follows :

$$\begin{aligned}\Delta G &= \Delta G^\circ + RT \log_e \frac{[C]\,[D]}{[A]\,[B]}\\ &= 1.8 \text{ kcal/mol} + 2.303\ RT \log_{10} \frac{3\times10^{-6}\ M}{2\times10^{-4} M}\\ &= 1.8 \text{ kcal/mol} - 2.5 \text{ kcal/mol}\\ &= -0.7 \text{ kcal/mol}\end{aligned}$$

The negative value for ΔG points out that isomerization of dihydroxyacetone phosphate to glyceraldehyde 3-phosphate can occur spontaneously, when these compounds are present at concentrations mentioned above. Although ΔG is negative for this reaction, the value of $\Delta G^{\circ\prime}$ is positive. It is important to note that the magnitude of ΔG for a reaction (whether smaller, larger or the same as $\Delta G^{\circ\prime}$) depends on the concentrations of the reactants .The criterion of spontaneity for a reaction is ΔG and not $\Delta G^{\circ\prime}$. Table 20–3 lists the standard free energy changes for some chemical reactions.

A perusal of the table (given on the next page) indicates that hydrolysis of esters, amides, peptides and glycosides, as well as rearrangements, and elimination proceed with relatively small standard free energy changes. Hydrolysis of acid anhydrides proceeds with relatively large decreases in standard free energy, whereas oxidation of organic compounds to CO_2 and H_2O takes place with rather huge decreases in standard free energy.

Difference Between ΔG and ΔG°′

The difference between the free energy change, ΔG and the standard free energy change, $\Delta G^{\circ\prime}$ of a chemical reaction should be clearly understood. The actual free energy change, ΔG of a chemical reaction is a function of the conditions of concentration, *p*H and temperature under which the reaction is taking place. Moreover, the ΔG of an ongoing chemical process is always negative, becomes smaller (*i.e.*, less negative) as the reaction proceeds and is zero at the point of equilibrium, indicating that no more work can be done by the reaction. The value of ΔG declines with time as the reaction proceeds because the actual concentrations of the reactants will be getting smaller and those of the resultants getting larger.

Table 20–3. Standard free energy changes for representative chemical reactions under standard conditions (*i.e.*, at 25°C and pH 7.0)

Reaction Types and Reactions	*$\Delta G^{\circ\prime}$ (in kcal/mol)*
Elimination of Water	
Malate $\longrightarrow$ Fumarate + H_2O	+ 0.75
Rearrangements	
Glucose 1-phosphate $\longrightarrow$ Glucose 6-phosphate	–1.74
Fructose 6-phosphate $\longrightarrow$ Glucose 6-phosphate	–0.40
Hydrolysis	
Esters:	
Ethyl acetate + H_2O $\longrightarrow$ Ethanol + Acetate	–4.7
Glucose 6-phosphate + H_2O $\longrightarrow$ Glucose + Phosphate	–3.3
Amides and peptides:	
Glutamine + H_2O $\longrightarrow$ Glutamate + NH_{4+}	–3.4
Glycylglycine + H_2O $\longrightarrow$ 2 Glycine	–2.2
Glycosides:	
Maltose + H_2O $\longrightarrow$ 2 Glucose	–3.7
Lactose + H_2O $\longrightarrow$ Glucose + Galactose	–3.8
Acid anhydrides:	
Acetic anhydride + H_2O $\longrightarrow$ 2 Acetate	–21.8
ATP + H_2O $\longrightarrow$ ADP + Phosphate	–7.3
Oxidations with Molecular Oxygen	
Glucose + $6O_2$ $\longrightarrow$ $6CO_2$ + $6H_2O$	–686
Palmitic acid + $23O_2$ $\longrightarrow$ $16CO_2$ + $16H_2O$	–2338

Thus, in every spontaneous chemical or physical process, the free energy of the reacting system always decreases, *i.e.*, ΔG is negative. On the contrary, the value of standard free energy change, $\Delta G^{\circ\prime}$ for a chemical reaction is characteristic and unchanging, and may be positive, negative or zero, depending on the equilibrium constant of the reaction. $\Delta G^{\circ\prime}$ is, thus, an immutable constant and tells us in which direction and how far a given reaction will go in order to reach equilibrium when it occurs under standard conditions, *i.e.*, when the initial concentration of all components is 1.0 *M*, *p*H is 7.0 and the temperature is 25°C.

Standard Free Energy Value of Chemical Reactions are Additive.

A noteworthy thermodynamic fact is that the overall free energy change for a series of reactions is equal to the sum of the free energy changes of the individual steps. Consider the two consecutive reactions where each reaction has its own equilibrium constant and a characteristic standard free energy change, $\Delta G_1^{\circ\prime}$ and $\Delta G_2^{\circ\prime}$.

$$\text{A} \longrightarrow \text{B} \qquad \Delta G_1^{\circ\prime} = -\,8 \text{ kcal/mol}$$
$$\text{B} \longrightarrow \text{C} \qquad \Delta G_2^{\circ\prime} = +\,5 \text{ kcal/mol}$$

Since the two reactions are sequential, the intermediate product *B* cancels out and the overall reaction with its standard free energy change, $\Delta Gs^{\circ\prime}$ may be written as :

$$\text{A} \longrightarrow \text{C} \qquad \begin{aligned} \Delta Gs^{\circ\prime} &= \Delta G_1^{\circ\prime} + \Delta G_2^{\circ\prime} \\ &= -8 + (+5) \\ &= -3 \text{ kcal/mol} \end{aligned}$$

Under standard conditions, *A* can be spontaneously converted into *B* because ΔG is negative.

However, the conversion of *B* into *C*, under standard conditions is thermodynamically not feasible. But as the free energy changes are additive, the conversion of *A* into *C* has a $\Delta G^{\circ\prime}$ value of –3 kcal/mol, which obviously means that A can be converted into C spontaneously under standard conditions. Thus, the above two sequential reactions are coupled by the intermediate product, *B*. In other words, *a thermodynamically unfavourable reaction can be driven by a thermodynamically favourable reaction.*

Two sequential steps from glycogen breakdown in muscles will illustrate this fact more clearly.

$$\text{Glucose 1-phosphate} \xrightarrow[\text{mutase}]{\text{Phosphogluco-}} \text{Glucose 6-phosphate} \quad \Delta G_1^{\circ\prime} = -\ 1.74 \text{ kcal/mol}$$

$$\text{Glucose 6-phosphate} \xrightarrow[\text{isomerase}]{\text{Glucose phosphate}} \text{Fructose 6-phosphate} \quad \Delta G_2^{\circ\prime} = +\ 0.40 \text{ kcal/mol}$$

On adding the two reactions, we get :

$$\text{Glucose 1-phosphate} \longrightarrow \text{Fructose 6-phosphate}$$

This has a standard free energy change value,

$$\begin{aligned} \Delta Gs^{\circ\prime} &= \Delta G_1^{\circ\prime} + \Delta G_2^{\circ\prime} \\ &= -1.74 + (+\ 0.40) \\ &= -1.36 \text{ kcal/mol} \end{aligned}$$

Because $\Delta Gs^{\circ\prime}$ is negative, glucose 1-phosphate is converted into fructose 6-phosphate in the muscles.

ATP AS UNIVERSAL CURRENCY OF FREE ENERGY IN BIOLOGICAL SYSTEMS

The living objects require a continuous supply of free energy mainly for the following 4 purposes :

(*a*) to synthesize macromolecules from simpler and smaller precursors,
(*b*) to transport molecules and ions across membranes against gradients,
(*c*) to perform mechanical work, as in the muscle contraction, and
(*d*) to ensure fidelity of information transfer.

The free energy in these processes is derived from the environment. The phototrophs obtain this energy by trapping light energy from the sun. On the other hand, the chemotrophs obtain it by the oxidation of foodstuffs. This free energy (derived from light or from the oxidation of foodstuffs) is partly transformed into a special form before it is used for biosynthesis, transport, motion and fidelity. This special carrier of free energy is adenosine triphosphate (ATP). ATP plays a central role in the transference of free energy from the exergonic (= *energy-yielding*) to the endergonic (= *energy-requiring*) processes in the cells. During breakdown of energy-rich foodstuffs or fuel molecules, some of the free energy is harnessed to make ATP from adenosine diphosphate (ADP) and inorganic phosphate (P_i), a process that requires input of free energy. ATP then donates much of its chemical energy to energy-requiring processes (biosynthesis, transport etc.) by undergoing a breakdown to ADP and P_i (Fig. 20–6).

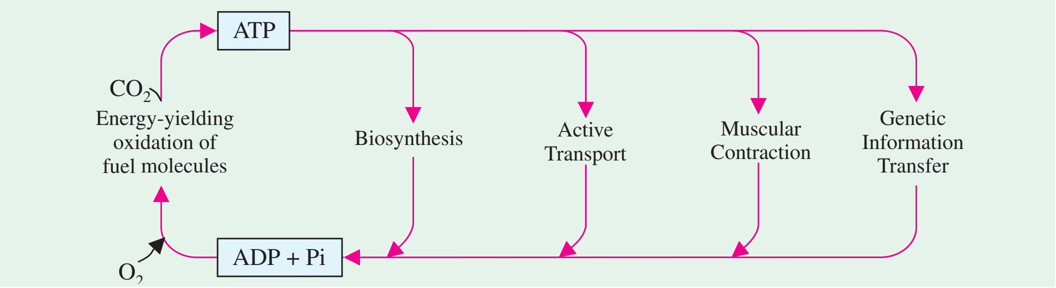


Fig. 20–6. The ATP cycle in cells

FRITZ ALBERT LIPMANN (LT, 1899–1986)

Lipmann was a German-born American biochemist. After obtaining M.D. degree, he started research career in 1927 as an unpaid graduate student under the famous biochemist Otto Meyerhof. He was awarded **1953 Nobel Prize in Medicine or Physiology** for his discovery of coenzyme A and revealing its importance in intermediary metabolism, along with Sir Hans Adolf Krebs of Great Britain. Lipmann is popularly called as the **'father' of ATP cycle.** He introduced the "*squiggle*" notation (~) to designate the energy-rich bonds of biomolecules such as ATP and ADP. He was professionally active at Rockefeller University until his death at age 87. His famous quote (1949) reads as follows :

"It seems that in the field of biosynthesis we have a rare example of progress leading to simplification."

Adenosine triphosphate (ATP) was discovered in extracts of skeletal muscles by Karl Lohmann in Germany and by Cyrus Fiske and Yellapragada Subbarow in the United States, simultaneously in 1929. Later, it was found to be present in all types of cells— animal, plant and microbial. Although first thought to be concerned with muscular contraction only, ATP has now been assigned many cell activities. In 1941, Fritz A. Lipmann postulated that ATP is the primary and universal carrier of chemical energy in cells. He adequately stated that the small chemical units from which the material of organisms is built "are glued together by an enormously versatile condensing reagent, ATP." He also first proposed the ATP cycle, shown in a present-day form in Fig. 20–6.

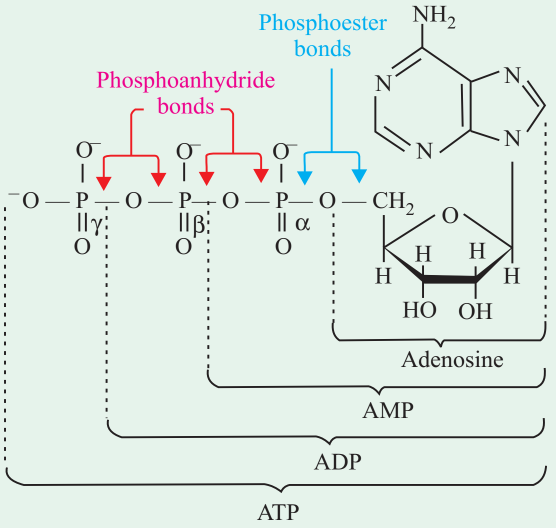


Fig. 20–7. The structure of ATP, indicating its relationship to ADP, AMP and adenosine

The phosphoryl groups of ATP, starting with that on AMP, are designated as α, β and γ phosphates. At pH 7.0, the phosphate groups are almost completely ionized, hence so shown here. The terminal phosphate group (*i.e.*, γ) can be enzymatically transferred to various phosphate acceptors. Note the differences between phosphoester and phosphoanhydride bonds.

Adeosine triphosphate (ATP) and its successive hydrolysis products, adenosine diphosphate (ADP) and adenosine monophosphate (AMP) are nucleotide, consisting of an adenine, a ribose and a 3, 2 or 1 phosphate group(s) respectively (Fig. 20–7). ATP, ADP and AMP occur not only in cell cytosol but also in mitochondria and the nucleus. In normal respiring cells, ATP makes up about 75% or more of the sum of all 3 adenine ribonucleotides (refer Table 20–4).

Contents

Table 20–4. **Concentrations (in mM) of 3 adenine ribonucleotides, phosphate (Pi) and phosphocreatine (PCr) in some cells***

Cells type	*ATP*	*ADP*	*AMP*	*Pi*	*PCr*
Rat muscle	8.05	0.93	0.04	8.05	28
Rat liver	3.38	1.32	0.29	4.80	0
Rat brain	2.59	0.73	0.06	2.72	4.7
Escherichia coli	7.90	1.04	0.82	7.90	0
Human erythrocytes	2.25	0.25	0.02	1.65	0

* These data are for the entire cell contents except in the case of erythrocytes where the concentrations are those of cytosol, since erythrocytes are absent from nucleus and mitochondria both.

(*Adapted from Lehninger AL, 1984*)

At *p*H 7.0, the phosphate group(s) of ATP, ADP and AMP are almost fully ionized, so that they occur as multiple charged anions ATP^{4-} ADP^{3-} and AMP^{2-}. But as the cell fluid contains high concentrations of Mg^{2+}, the ATP and ADP both exist largely as Mg ATP^{2-} and $MgADP^{-}$ complexes (Fig. 20–8). In fact, in phosphate transfer reactions, ATP participates as its complex form. However, ATP can also form complex with Mn^{2+}.

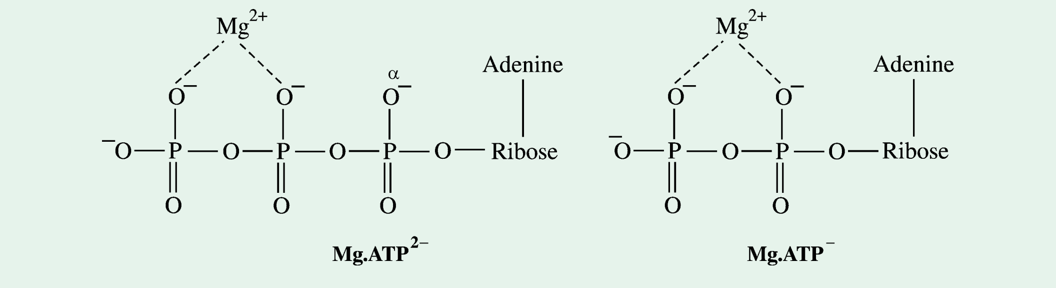


Fig. 20–8. **Magnesium ion complexes of ATP and ADP**

ATP serves as the principal immediate donor of free energy in biological systems rather than as a storage form of energy. In a typical cell, an ATP molecule is consumed within a minute of its formation. *The turnover of ATP is very high.* For instance, a resting human consumes about 40 kg ATP in a day. During strenuous labour, the ATP is consumed at the rate of even 0.5 kg per minute. The endergonic processes such as biosynthesis, active transport etc., can occur only if ATP is continuously regenerated from ADP (refer Fig. 20–9). Phototrophs harvest the free energy in light to regenerate ATP whereas chemotrophs form ATP by the oxidation of foodstuffs.

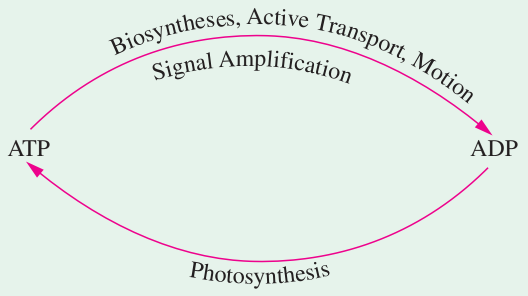


Fig. 20–9. **The ATP-ADP cycle**

The cycle is the fundamental mode of energy exchange in biological systems.

FREE ENERGY OF HYDROLYSIS OF ATP AND OTHER ORGANOPHOSPHATES

When ATP is hydrolyzed, it loses its terminal γ phosphate group to form ADP and orthophosphate or inorganic phosphate (Pi).

$$\text{ATP} + H_2O \longrightarrow \text{ADP} + \text{P}i$$

The standard free energy change, $\Delta G°'$ for this reaction is –7.3 kcal/mol. Standard free energy changes have also been determined for the hydrolysis of other phosphorylated compounds or organophosphates (Table 20–5). Some phosphates yield more and some yield less free energy than ATP upon hydrolysis, under standard conditions. This intermediate position enables ATP to function efficiently as a carrier of phosphoryl groups. Thus, with respect to the $\Delta G°'$ value of hydrolysis of ATP, two classes of organo *phosphates* are recognized : high energy phosphates exemplified by enolphosphates (*e.g.*, phosphoenolpyruvate), phosphoguanidines (*e.g.*, creatine phosphate and arginine phosphate) etc., which have $\Delta G°'$ values larger than that of ATP and low energy phosphates, exemplified by ester phosphates found in the intermediates of glycolysis, which have $\Delta G°'$ values smaller than that of ATP. However, the designations 'high' and 'low' do not clearly indicate that there are 3 classes of phosphates. Such phosphates as phosphoenolpyruvate, creatine phosphate etc., whose $\Delta G°'$ values are higher than that of ATP, should better be designated as 'super' high energy phosphates, the ATP then be designated as 'high' energy phosphate and the ester phosphates as 'low' energy phosphates.

Table 20–5. **Standard free energy of hydrolysis of some phosphorylated compounds**

	Compound	$\Delta G°'$ *(kcal/mol)*
1.	Phosphoenolyruvate*	–14.8
2.	Carbamoyl phosphate	–12.3
3.	3-phosphoglyceroyl phosphate or 1, 3-diphosphoglycerate (→ 3-phosphoglycerate + P*i*)	–11.8
4.	Creatine phosphate or phosphocreatine	–10.3
5.	Acetyl phosphate	–10.1
6.	Arginine phosphate	–8.0
7.	ATP (→ AMP + PPi)	–7.7
8.	ATP (→ ADP + Pi)	–7.3
9.	ADP (→ AMP + Pi)	–7.3
10.	Pyrophosphate (→ 2Pi)	–6.9
11.	Glucose 1-phosphate	–5.0
12.	Fructose 6-phosphate	–3.8
13.	AMP (→ Adenosine + Pi)	–3.4
14.	Glucose 6-phosphate	–3.3
15.	Glycerol 3-phosphate	–2.2

*Note that phosphoenolpyruvate has the highest phosphate group transfer potential of the compounds listed.

A persual of Table 20–5 indicates that when ADP is hydrolyzed to AMP and inorganic phosphate, the $\Delta G°'$ value of this reaction is the same as that of the reaction, ATP ⟶ ADP + P*i*, that is –7.3 kcal/mol. Thus, the two terminal phosphate groups of ATP (β and γ) are both high energy groups. On the contrary, the $\Delta G°'$ value of hydrolysis of AMP to yield adenosine and phosphate is much lower, that is only –3.4 kcal/mol. Thus, the phosphate group of AMP (*i.e.*, the a phosphate group of ATP) is in the low energy class. The hydrolysis of ATP to yield AMP plus PPi proceeds with $\Delta G°'$ –7.7 kcal/mol, slightly greater than the $\Delta G°'$ for the hydrolysis of the terminal or γ phosphate bond.

$$\text{ATP} + H_2O \longrightarrow \text{AMP} + \text{PP}i$$

The inorganic pyrophosphate is subsequently hydrolyzed by the enzyme pyrophosphatase to yield 2 moles of inorganic orthophosphate. The $\Delta G°'$ value of this reaction is –6.9 kcal/mol.

$$\text{PPi} + H_2O \longrightarrow 2\text{Pi}$$

The overall reaction has a $\Delta G^{\circ\prime}$ value –14.6 kcal/mol which is the sum of the $\Delta G^{\circ\prime}$ values of the two sequential component reactions.

$$\text{ATP} + 2\ \text{H}_2\text{O} \longrightarrow \text{AMP} + 2\text{P}i$$

It is noteworthy that the $\Delta G^{\circ\prime}$ of the overall reaction is exactly twice the $\Delta G^{\circ\prime}$ of the terminal phosphate groups of ATP and ADP.

Some biosynthetic reactions are, however, driven by nucleotides that are analogous to ATP, namely guanosine triphosphate (GTP), cytidine triphosphate (CTP) and uridine triphosphate (UTP). They are present in all cells but in much lower concentrations than ATP. Also found in the cells in low concentrations are the corresponding deoxyribonucleoside 5′-triphosphates denoted as *d*ATP, *d*GTP, *d*CTP and *d*TTP. Although ATP is the mainstream carrier of phosphate groups in the cell, the other types of nucleotides serve certain specific biosynthetic pathways. They acquire their terminal phosphate groups mainly from ATP in reactions catalyzed by Mg^{2+}- dependent enzymes called *nucleoside diphosphokinases*. These enzymes promote the following types of reversible reactions :

$$\text{ATP} + \text{GDP} \rightleftharpoons \text{ADP} + \text{GTP}$$
$$\text{ATP} + \text{CDP} \rightleftharpoons \text{ADP} + \text{CTP}$$
$$\text{ATP} + \text{UDP} \rightleftharpoons \text{ADP} + \text{UTP}$$
$$\text{GTP} + \text{UDP} \rightleftharpoons \text{GDP} + \text{UTP}$$
$$\text{ATP} + d\text{CDP} \rightleftharpoons \text{ADP} + d\text{CTP}$$

The energy contained in ATP may be channelized into different biosynthetic pathways via different nucleoside and deoxynucleoside triphosphates (refer Fig. 20–10).

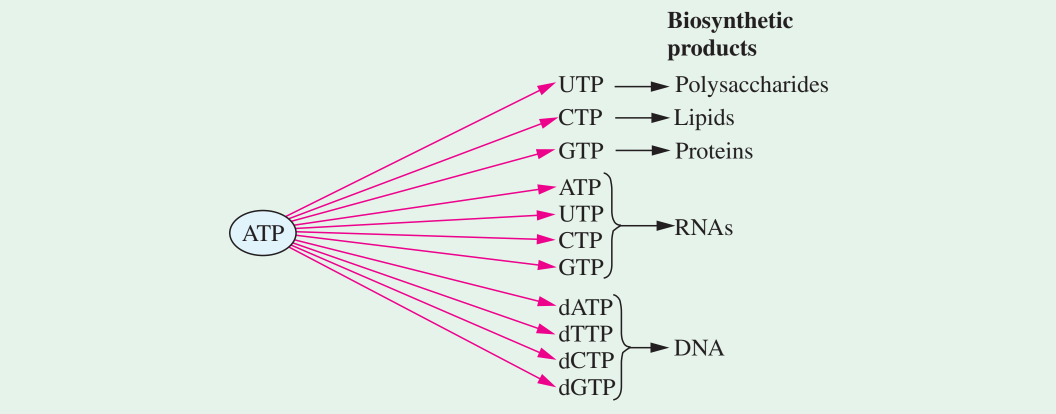


Fig. 20–10. Channeling of ATP energy into various biosynthetic pathways.

STRUCTURAL BASIS OF THE HIGH GROUP TRANSFER POTENTIAL OF ATP

The ATP molecules deliver considerably more free energy on hydrolysis of the terminal phosphate group than the hydrolysis of ester phosphates, say glucose 6-phosphate. The $\Delta G^{\circ\prime}$ values of both these reactions are –7.3 kcal/mol and –3.3 kcal/mol respectively (refer Table 20–5). This means that ATP has a stronger tendency to transfer its terminal phosphate group to water than does glucose 6-phosphate. In other words, *ATP has a higher phosphate group transfer potential than glucose 6-phosphate*.

The standard free energy change is a measure of the difference in the free energy content of the reactants and the products. Therefore, the reason for the high $\Delta G^{\circ\prime}$ value for ATP should be sought in the properties of both the substrate and products. There are 3 main reasons of high $\Delta G^{\circ\prime}$

value (or high group transfer potential) for ATP :

1. Degree of ionization. At pH 7.0, ATP is almost completely ionized as the ATP^{4-} ion. This, on hydrolysis, yields 3 products, namely ADP^{3-}, HPO^{2-} and H^+.

$$ATP^{4-} + H_2O \longrightarrow ADP^{3-} + HPO_4^{2-} + H^+$$

At pH 7.0 (the standard *p*H for $\Delta G^{\circ\prime}$ hydrolysis), the hydrogen ion concentration is only 10–7 M. This means that by the law of mass action, the equilibrium for ATP hydrolysis tends to be pulled far to the right, since $[H^+]$ at pH 7.0 is very small as compared with the standard concentration of 1.0 M. In contrast, when glucose 6–phosphate is hydrolyzed at pH 7.0, no significant extra H^+ ion is formed and, therefore, no such pulling force takes place.

$$\text{Glucose 6-phosphate}^{2-} + H_2O \longrightarrow \text{Glucose} + HPO_4^{2-}$$

2. Electrostatic repulsion. At *p*H 7.0, the ATP molecule carries 4 negative charges. These charges repel each other strongly because they are in close proximity. However, when ATP is hydrolyzed, the electrostatic repulsion between these negatively-charged groups is reduced because of the separation of the negatively-charged products, ADP^{3-} and HPO_4^{2-}. These products have relatively little tendency to approach each other to react in the backward direction to form ATP again. On the contrary, when glucose 6-phosphate is hydrolyzed, two products glucose and HPO_4^{2-} are formed. Because glucose has no charge, the two products do not repel each other and tend to recombine more readily.

3. Resonance stabilization. The two products of ATP hydrolysis, ADP^{3-} and HPO_4^{2-} are resonance hybrids, *i.e.*, special stable forms in which certain electrons are in a configuration having much less energy than in their original positions in the ATP molecule. When ATP is hydrolyzed, the electrons in the two products (ADP^{3-} and HPO_4^{2-}) can sink to lower energy levels than in the nonhydrolyzed ATP. This causes ADP^{3-} and HPO_4^{2-} ions to contain less free energy than when they were still combined as ATP^{4-}.

ADP and Pi enjoy greater resonance stabilization than does ATP. For example, orthophosphate (Pi) has a number of resonance forms of similar energy (refer Fig. 20–11), whereas the terminal phosphate portion of ATP has fewer resonance forms per phosphate group.

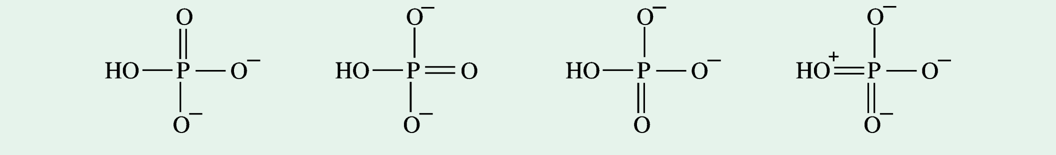


Fig. 20–11. Significant resonance forms of orthophosphate

To indicate the presence of high energy phosphate group, Lipmann introduced the symbol ~P indicating high energy phosphate bond. Although in long usage among biochemists, the term '*high energy phosphate bond*' is misleading since it wrongly suggests that the bond itself contains high amount of energy. What the symbol ~P indicates is that the group attached to the bond, on transfer to an appropriate acceptor, results in transfer of large amount of free energy. Hence, the term '*group transfer potential*' is preferred to '*high energy bond*'. In fact, the free energy released by hydrolysis of phosphate esters does not come from the specific bond that is broken but results from the fact that the products of the reaction have a smaller free energy content than the reactants. Therefore, the use of the term '*high energy phosphate compound*' be preferred when referring to ATP and other phosphate compounds having a large negative $\Delta G^{\circ\prime}$ value of hydrolysis. Thus, whereas ATP contains two high energy phosphate groups and one low energy phosphate group, ADP contains one high energy and one low energy phosphate groups. AMP, however, contains only one phosphate group of low energy type (Fig. 20–12).

Contents

Adenosine –P~P~P	Adenosine –P~P	Adenosine –P
ATP	**ADP**	**AMP**

Fig. 20–12. Structure of adenine nucleotides, showing the position and number of high energy bonds (~)

ATP HYDROLYSIS AND EQUILIBRIA OF COUPLED REACTIONS

For a better understanding of the role of ATP in energy coupling, the follownig reaction with a positive $\Delta G^{\circ\prime}$ value (say + 4 kcal/mol) is taken as an example :

$$\text{A} \rightleftharpoons \text{B} \qquad \Delta G^{0\prime} = +\,4 \text{ kcal/mol}$$

This reaction is thermodynamically unfavourable without an input of free energy. The equilibrium constant, K'_{eq} of this reaction at 25°C as per equation 16 is :

$$\frac{[B]_{eq}}{[A]_{eq}} = K'_{eq} = 10^{-\Delta G^{\circ\prime}/1.36} = 1.15 \times 10^{-3}$$

Thus, A cannot be spontaneously converted into B when the molar ratio of B/A is equal to or greater than 1.15×10^{-3}. However, if the reaction is coupled to the hydrolysis of ATP (whose $\Delta G^{\circ\prime}$ value is –7. 3 kcal/mol), A can be converted into B when $[B]/[A]$ ratio is higher than 1.15×10^{-3}. The new overall reaction shall be :

$$\text{A} + \text{ATP} + \text{H}_2\text{O} \rightleftharpoons \text{B} + \text{ADP} + \text{Pi} + \text{H+}$$

Because the $\Delta G^{\circ\prime}$ values of the sequential reactions are additive, the value of standard free energy change of the above reaction shall be :

$$\begin{aligned} \Delta G^{\circ\prime} &= \Delta G_1{}^{\circ\prime} + \Delta G_2{}^{\circ\prime} \\ &= +\,4 + (-7.3) \\ &= -3.3 \text{ kcal/mol} \end{aligned}$$

The equilibrium constant of this coupled reaction will be :

$$\begin{aligned} K'_{eq} &= \frac{[B]_{eq}}{[A]_{eq}} \times \frac{[ADP]_{eq}\,[P_i]_{eq}}{[ATP]_{eq}} \\ &= 10^{3.3/1.36} \\ &= 2.67 \times 10^{2} \end{aligned}$$

At equilibrium, the ratio $[B]/[A]$ will be :

$$\frac{[B]_{eq}}{[A]_{eq}} = K'_{eq} \times \frac{[ATP]_{eq}}{[ADP]_{eq}\,[P_i]_{eq}}$$

The $[ATP]/[ADP]\,[Pi]$ ratio in the cells generating ATP is maintained at a high level, typically of the order of 500. Thus,

$$\begin{aligned} \frac{[B]_{eq}}{[A]_{eq}} &= 2.67 \times 10^{2} \times 500 \\ &= 1.34 \times 10^{5} \\ &= 10^{8} \text{ approx.} \end{aligned}$$

This means that the hydrolysis of ATP enables A to be converted to B until the $[B]/[A]$ ratio reaches a value of 1.34×10^{5}. This equilibrium is quite different from the value of 1.15×10^{-3} that does not include ATP hydrolysis. In other words, the coupled hydrolysis of ATP has changed the equilibrium ratio of B to A by a factor of about 10^{8}. Thus, the cells maintain a high level of ATP by using light or substrates as sources of free energy. The hydrolysis of one ATP molecule then changes the equilibrium ratio of products to reactants of a coupled reaction by a very large factor of about 10^8. More generally, the hydrolysis of n ATP molecules changes the equilibrium ratio of a coupled reaction (or sequence of reactions) by factor of 10^{8n}. For example, the hydrolysis of 3 ATP molecules in a coupled reaction changes the equilibrium ratio by a factor of $10^{8\times3}$ or 10^{24}. Thus, a thermodynamically unfavourable reaction sequence can be converted into a favourable one by coupling it to the hydrolysis of a sufficient number of ATP molecules.

ROLE OF HIGH ENERGY PHOSPHATES AS THE 'ENERGY CURRENCY' OF THE CELL

ATP has a position midway down the list of standard free energies of hydrolysis of organophosphates (refer Table 20–5). Therefore, ATP acts as a donor of high energy phosphates to the compounds below it in the table.

Likewise, ADP can accept high energy phosphate, in the presence of enzyme, to form ATP from the compounds above ATP in the table.

Thus, an ATP/ADP cycle connects these two types of processes, *i.e.*, those which generate ~P and those which utilize ~P.

A. Processes which generate ~P. The processes that feed ~P into ATP/ADP cycle fall mainly within 4 groups :

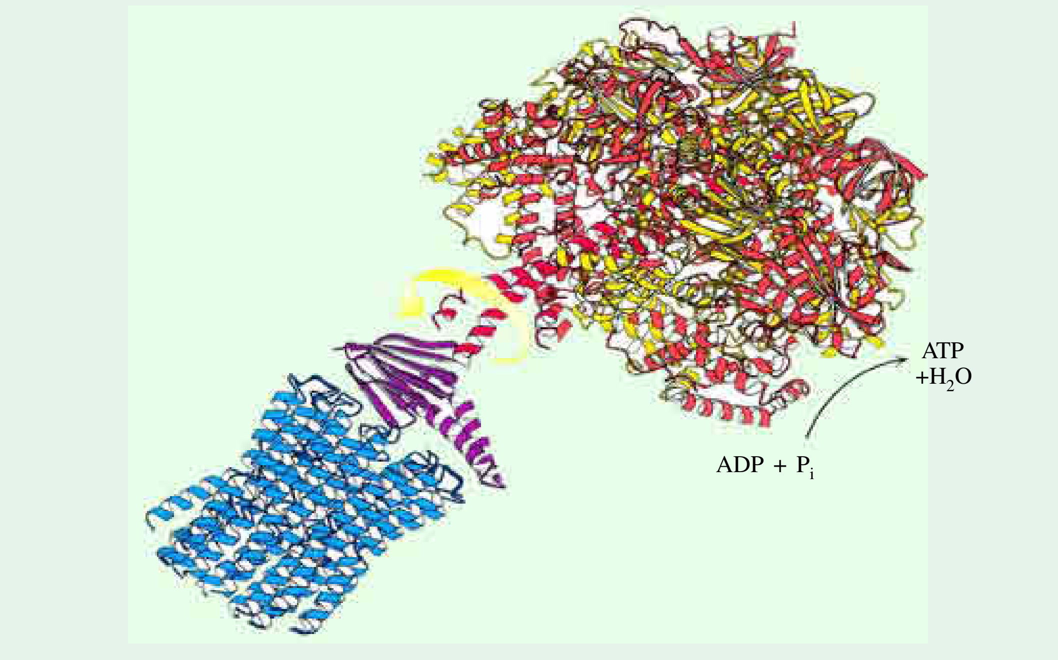


Fig. 20–13. Ribbon model of the X-ray structure of ATP synthetase

This enzyme is a molecular assembly that transduces the free energy associated with a proton gradient to the chemical energy associated with ATP. The proton gradient drives the rotation of one component of the assembly within the other. This rotational motion in turn drives the synthesis and release of ATP.

I. *Oxidative phosphorylations.* The greatest quantitative source of ~P in aerobes is from reactions catalyzed by the enzyme *ATP synthetase* (Fig. 20–13.), which in fact reverses the hydrolysis of ATP. The free energy to drive this process is obtained from respiratory chain oxidation within mitochondria, often called oxidative phosphorylation.

Contents

II. *Catabolism of glucose to lactic acid.* Energy is also captured during catabolism of glucose to lactic acid in a sequence of reactions commonly called as Embden-Meyerhof-Parnas (E.M.P.) pathway of glycolysis. In this pathway, there is a net formation of 2 high energy phosphate groups which produce 2 ATP molecules from 2 ADP molecules. One ATP molecule is formed when 1, 3 diphosphoglycerate, one of the intermediates of the pathway, is enzymatically catalyzed to 3-phosphoglycerate.

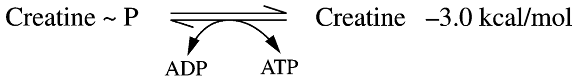
$$\text{1,3-diphosphoglyceric acid + ADP} \xrightarrow{\text{Phospholglycerate kinase}} \text{3-phosphoglyceric acid + ATP}$$

Another ATP molecule results when another intermediate, 2-phosphoenolpyruvate changes enzymatically to pyruvate.

$$\text{2-phosphoenolpyruvic acid + ADP} \xrightarrow{\text{Pyruvate kinase}} \text{Pyruvic acid + ATP}$$

III. *Oxidation of pyruvic acid.* Further energy capture occurs at the *succinyl thiokinase* step of the Krebs' Cycle (= citric acid cycle) wherein succinyl CoA, one of the intermediates of the cycle, transforms to succinate. Here again, one ATP molecule is formed from one ADP molecule.

IV. *The muscle compounds.* Another group of compounds acting as storage forms of high energy phosphate within muscles include creatine phosphate, occurring in vertebrate muscle and arginine phosphate, occurring within invertebrate muscle. A substantial free energy is lost in the reaction,



$$\text{Creatine} \sim \text{P} \underset{\text{ADP} \qquad \text{ATP}}{\rightleftharpoons} \text{Creatine} \quad -3.0\ \text{kcal/mol}$$

Under physiologic conditions, the above reaction allows ATP concentrations to be maintained in muscle whereas ATP is being used as a source of energy for muscular contraction. On the other hand, when ATP is in sufficient quantity, the reaction occurs in reverse direction and allows the concentration of creatine phosphate to increase substantially and thus acts as a store of high energy phosphate.

B. Processes which utilize ~P. When adenosine triphosphate (ATP) acts as a phosphate donor, the phosphate group is invariably converted to one with low energy, producing phosphoric esters of alcohols. Such reactions are catalyzed by kinases. Among them is *hexokinase*, which catalyzes transfer of phosphate group from ATP to D-glucose. This reaction proceeds by an *orthophosphate cleavage* of ATP, in which ATP loses a single orthophosphate group.

$$\text{D–glucose + Adenosine–P}\sim\text{P}\sim\text{P} \xrightarrow{\text{Hexokinase}} \text{D–glucose 6-phosphate + Adenosine–P}\sim\text{P}$$

Another enzyme is glycerol kinase, which catalyzes transfer of phosphate group from ATP to glycerol. This reaction also proceeds by an orthophosphate cleavage of ATP.

$$\text{Glycerol + Adenosine–P}\sim\text{P}\sim\text{P} \xrightarrow{\text{Glycerol kinase}} \text{Glycerol 3-phosphate + Adenosine–P}\sim\text{P}$$

In both the cases, a hydroxyl group of the acceptor molecule is phosphorylated to yield a phosphoric ester. Since the $\Delta G^{\circ\prime}$ values for the hydrolysis of glucose 6-phosphate and glycerol 3-phosphate are smaller than for ATP, the above reactions proceed to the right as written, if we begin with 1.0 M concentrations of the substrates and reactants. Glucose 6-phosphate and glycerol 3-phosphate may be considered as energized forms of glucose and glycerol respectively. These compounds can now undergo further enzymatic reactions in which they serve as activated building blocks for the synthesis of larger molecules, *i.e.*, glycogen and lipid respectively. Fig. 20–14 shows the flow sheet of enzymatic phosphate transfer reactions in the cell.

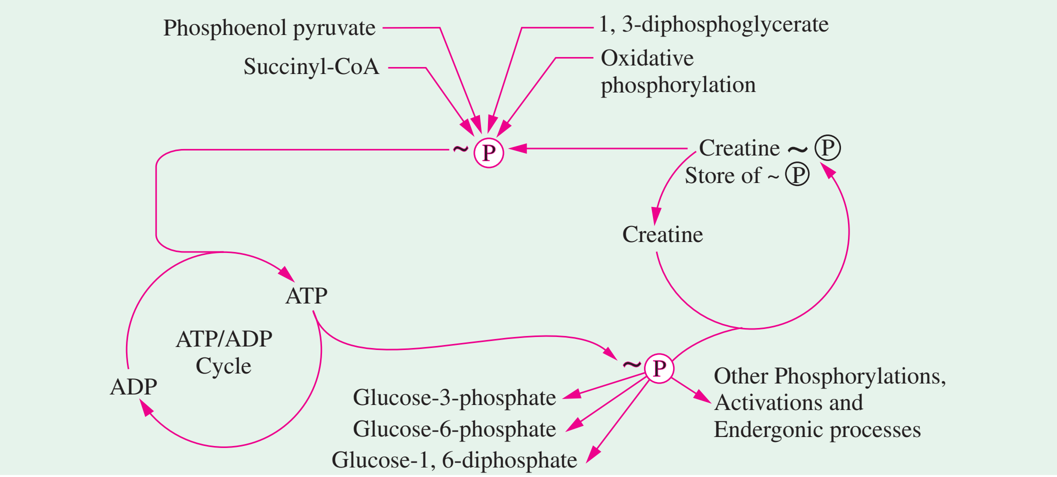


Fig. 20–14. Role of ATP/ADP cycle in transfer of high energy phosphate

An important feature of this cycle is that all super high-energy phosphate compounds must pass their phosphate groups *via* ATP to various acceptor molecules to form their low energy phosphate derivatives.

(*Adapted from Harper, Rodwell and Mayes, 1979*)

INTERCONVERSION OF ADENINE NUCLEOTIDES

The enzyme adenylate kinase (= myokinase) is present in most cells. It catalyzes the interconversion of ATP and AMP on the one hand and ADP on the other.

$$\underset{\text{(1 ATP)}}{\text{Adenosine–P\~P\~P}} + \underset{\text{(1 AMP)}}{\text{Adenosine–P}} \xrightarrow{\text{Adenylate kinase}} \underset{\text{(2 ADP)}}{2\ \text{Adenosine–P\~P}}$$

This reaction performs 3 main functions :

(*a*) It permits the high energy phosphate of ADP to be used in the formation of ATP.

(*b*) It is a means by which AMP can be rephosphorylated to form ADP.

(*c*) Under stress of ATP depletion, AMP concentration increases which acts as a metabolic signal to increase the rate of catabolic reactions so that more ATP molecules are regenerated.

When ATP reacts enzymatically to form AMP, inorganic pyrophosphate (symbolized PPi) is formed. An example is the enzymatic activation of a fatty acid to form its coenzyme A ester, a reaction that 'energizes' the fatty acid and converts it into an activated precursor, fatty acyl-CoA, for the biosynthesis of lipids. In this reaction, the two terminal groups of ATP (*i.e.*, β and γ groups) are removed in *one piece* as inorganic pyrophosphate, leaving adenosine monophosphate.

$$\text{ATP} + \underset{\text{(Fatty acid)}}{\text{R.COOH}} + \text{CoA–SH} \xrightarrow{\text{Thiokinase}} \text{AMP} + \text{PPi} + \underset{\text{(Fatty acyl-CoA)}}{\text{R.CO\~S–CoA}}$$

Thus, this activation reaction proceeds by a pyrophosphate cleavage of ATP, in contrast to the usual orthophosphate cleavage (refer page 362). This reaction, whose $\Delta G^{\circ\prime}$ value is + 0.2 kcal/mol, results in a loss of free energy as heat which ensures that the reaction will proceed to the right as written. Further, the hydrolytic splitting of PPi has also a large $\Delta G^{\circ\prime}$ valaue of –6.9 kcal/mol. This also aids the above reaction to go to the right.

$$\underset{\text{(Pyrophosphate)}}{\text{PPi}} + H_2O \xrightarrow{\text{Inorganic pyrophosphatase}} \underset{\text{(Phosphate)}}{2\text{Pi}}$$

It is noteworthy that activations via the pyrophosphate pathway result in the loss of 2 high energy phosphates (~P) rather than one, as happens when ADP and P*i* are formed. The various interrelationships are represented cyclically in Fig. 20–15.

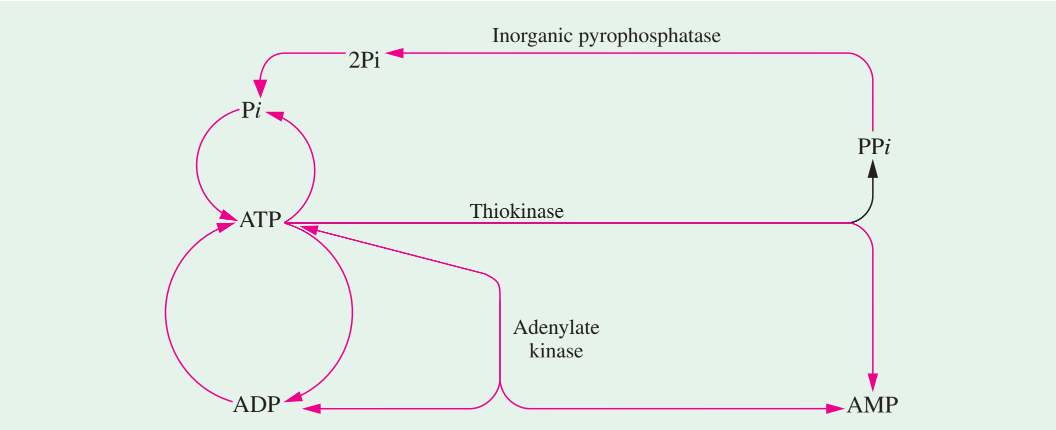


Fig. 20–15. Phosphate cycles and interconversion of adenine nucleotides

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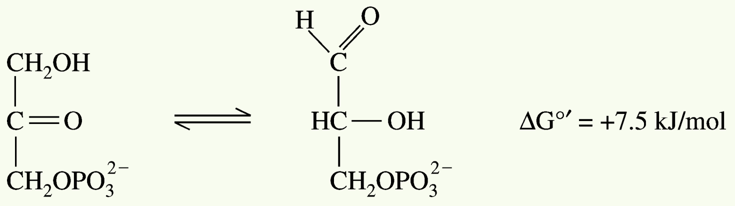
PROBLEMS

1. What is the direction of each of the following reactions when the reactants are initially present in equimolar amounts ? Use the data given in Table 20-5.
   (*a*) ATP + creatine $\rightleftharpoons$ creatine phosphate + ADP
   (*b*) ATP + glycerol $\rightleftharpoons$ glycerol 3-phosphate + ADP
   (*c*) ATP + pyruate $\rightleftharpoons$ phosphoenolpyruate + ADP
   (*d*) ATP + glucose $\rightleftharpoons$ glucose 6-phosphate + ADP
2. What information do the $\Delta G^{o\prime}$ data given in Table 20-5 provide about the relative rates of hydrolysis of pyrophosphate and acetyl phosphate ?
3. Consider the following reactions :
   ATP + pyruate $\rightleftharpoons$ phosphoenolpyruate + ADP
   (*a*) Calculate $\Delta G^{o\prime}$ and K'_{eq} at 25ºC for this reaction, by using the data given in Table 20-5.
   (*b*) What is the equilibrium ratio of pyruate to phosphoenolpyruate if the ratio of ATP to ADP is 10 ?
4. The formation of acetyl CoA from acetate is an ATP-driven reaction :
   Acetate + ATP + CoA $\rightleftharpoons$ acetyl CoA + AMP + PP_i
   (*a*) Calculate $\Delta G^{o\prime}$ for this reaction by using data given in this chapter.
   (*b*) The PP_i formed in the preceding reaction is rapidly hydrolyzed *in vivo* because of the ubiquity of inorganic pyrophosphatase. The $\Delta G^{o\prime}$ for the hydrolysis of PPi is –4.6 kcal mol^{-1}. Calculate the $\Delta G^{o\prime}$ for the overall reaction. What effect does the hydrolysis of PP_i have on the formation of acetyl-CoA ?
5. The p*K* of an acid is a measure of its proton group-transfer potential.
   (*a*) Derive a relation between $\Delta G^{o\prime}$ and p*K*.
   (*b*) What is the $\Delta G^{o\prime}$ for the ionization of acetic acid, which has a p*K* of 4.8 ?
6. What is the structural feature common to ATP, FAD, NAD^+, and CoA ?
7. Glycolysis is a series of 10 linked reactions that convert one molecule of glucose into two molecules of pyruvate with the concomitant synthesis of two molecules of ATP. The $\Delta G^{o\prime}$ for this set of reactions is –8.5 kcal mol^{-1} (– 35.6 kJ mol^{-1}), where as ΔG is –18.3 kcal mol^{-1} (– 76.6 kJ mol^{-1}). Explain why the free-energy release is so much greater under intracellular conditions than under standard conditions.
8. The combustion of glucose to CO_2 and water is a major source of energy in aerobic organisms. It is a reaction favoured mainly by a large negative enthalpy change.
   $$C_6H_{12}O_6(s) + 6O_2(g) \longrightarrow 6CO_2(g) + 6H_2O(l)$$
   $$\Delta H^o = -2816 \text{kJ/mol} \qquad \Delta S^o = +181 \text{ J/K·mol}$$
   (*a*) At 37ºC, what is the value for $\Delta G^{o\prime}$?
   (*b*) In the overall reaction of aerobic metabolism of glucose, 38 moles of ATP are produced from ADP for every mole of glucose oxidized. Calculate the standard state free energy change for the *overall* reaction when glucose oxidation is coupled to the formation of ATP.
   (*c*) What is the *efficiency* of the process in terms of the percentage of the available free energy change captured in ATP ?

9. In another key reaction in glycolysis, dihydroxyacetone phosphate (DHAP) is isomerized into glyceraldehyde-3-phosphate (G3P) :



DHAP **G3P**

Because $\Delta G^{\circ\prime}$ is positive, equilibrium lies to the left.

(*a*) Calculate the equilibrium constant, and the equilibrium fraction of G3P from the above, at 37ºC.

(*b*) In the cell, depletion of G3P makes the reaction proceed. What will ΔG be if the concentration of G3P is always kept at $^1/_{100}$ of the concentration of DHAP ?

10. A protein molecule, in its folded native state, has *one* favoured conformation. But when it is denatured, it becomes a random coil, with *many* possible conformations.

(*a*) What must be the sign of ΔS for the change native ⟶ denatured ?

(*b*) Will the contribution of ΔS to the free energy change be + or – ?

What requirement does this impose on ΔH if proteins are to be stable structures ?

11. We give a value of the standard state free energy change for ATP hydrolysis to be –31 kJ/mol. Would you expect the same value for the reaction $ATP^{4-} + H_2O \longrightarrow ADP^{2-} + P_i^{2-}$? Explain.

12. For the reaction $A \rightleftharpoons B$, $\Delta G^{\circ\prime} = -7.1$ kcal mol^{-1}. At 37ºC, $-2.303\ RT = -1.42$ kcal mol^{-1}. What is the equilibrium ratio of B/A ?

13. Organophosphate compounds are irreversible inhibitors of acetylcholinesterase. What effect does an organophosphate inhibitor have on the transmission of nerve impulses ?

14. Calculate the standard free-energy changes of the following metabolically important enzyme-catalyzed reactions at 25ºC and pH 7.0 from the equilibrium constants given.

(*a*) $\text{Glutamate + oxaloacetate} \xrightleftharpoons{\text{aspartate aminotransferase}} \text{aspartate + }\alpha\text{-ketoglutarate} \qquad K'_{eq} = 6.8$

(*b*) $\text{Dihydroxyacetone phosphate} \xrightleftharpoons{\text{triose phosphate isomerase}} \text{glyceraldehyde-3-phosphate} \qquad K'_{eq} = 0.0475$

(*c*) $\text{Fructose-6-phosphate + ATP} \xrightleftharpoons{\text{phosphofructokinase}} \text{fructose-1,6-bisphosphate + ADP} \qquad K'_{eq} = 254$

15. Calculate the equilibrium constants K'_{eq} for each of the following reactions at pH 7.0 and 25ºC, using the $\Delta G^{\circ\prime}$ values of Table 20-3:

(*a*) $\text{Glucose-6-phosphate} + H_2O \xrightleftharpoons{\text{glucose-6-phosphatase}} \text{glucose} + P_i$

(*b*) $\text{Lactose} + H_2O \xrightleftharpoons{\beta\text{-galactosidase}} \text{glucose + galactose}$

(*c*) $\text{Malate} \xrightleftharpoons{\text{fumarase}} \text{fumarate} + H_2O$

16. Consider the following interconversion, which occurs in glycolysis :

$\text{Fructose-6-phosphate} \rightleftharpoons \text{glucose-6-phosphate} \qquad K'_{eq} = 1.97$

(*a*) What is $\Delta G^{\circ\prime}$ for the reaction (assuming that the temperature is 25ºC) ?

(*b*) If the concentration of fructose-6-phosphate is adjusted to 1.5 M and that of glucose-6-phosphate is adjusted to 0.5 M, what is ΔG ?

(*c*) Why are $\Delta G^{\circ\prime}$ and ΔG different ?

17. The free energy released by the hydrolysis of ATP under standard conditions at pH 7.0 is –30.5 kJ/mol. If ATP is hydrolyzed under standard conditions but at pH 5.0, is more or less free energy released ? Why ?

18. Glucose-1-phosphate is converted into fructose-6-phosphate in two successive reactions:

$$\text{Glucose-1-phosphate} \longrightarrow \text{glucose-6-phosphate}$$
$$\text{Glucose-6-phosphate} \longrightarrow \text{fructose-6-phosphate}$$

Using the $\Delta G^{\circ\prime}$ values in Table 20–3, calculate the equilibrium constant, K'_{eq}, for the sum of the two reactions at 25°C :

$$\text{Glucose-1-phosphate} \longrightarrow \text{fructose-6-phosphate}$$

19. Calculate the physiological ΔG (not $\Delta G^{\circ\prime}$) for the reaction,

$$\text{Phosphocreatine + ADP} \longrightarrow \text{creatine + ATP}$$

at 25°C as it occurs in the cytosol of neurons, in which phosphocreatine is present at 4.7 mM, creatine at 1.0 mM, ADP at 0.20 mM, and ATP at 2.6 mM.

20. The ATP concentration in muscle tissue (approximately 70% water) is about 8.0 mM. During strenuous activity, each gram of muscle tissue uses ATP at the rate of 300 µmol/min for contraction.

(*a*) How long would the reserve of ATP last during a 100 meter dash ?

(*b*) The phosphocreatine level in muscle is about 40.0 mM. How does this help extend the reserve of muscle ATP ?

(*c*) Given the size of the reserve ATP pool, how can a person run a marathon ?

21. The synthesis of the activated form of acetate (acetyl-CoA) is carried out in an ATP-dependent process :

$$\text{Acetate + CoA + ATP} \longrightarrow \text{acetyl-CoA + AMP + PP}_i$$

(*a*) The $\Delta G^{\circ\prime}$ for the hydrolysis of acetyl-CoA to acetate and CoA is –32.2 kJ/mol and that for hydrolysis of ATP to AMP and PP_i is –30.5 kJ/mol. Calculate $\Delta G^{\circ\prime}$ for the ATP-dependent synthesis of acetyl-CoA.

(*b*) Almost all cells contain the enzyme inorganic pyrophosphatase, which catalyzes the hydrolysis of PP_i to P_i. What effect does the presence of this enzyme have on the synthesis of acetyl-CoA ? Explain.

22. Electron transfer in the mitochondrial respiratory chain may be represented by the net reaction equation

$$\text{NADH} + \text{H}^+ + \tfrac{1}{2}\text{O}_2 \rightleftharpoons \text{H}_2\text{O} + \text{NAD}^+$$

(*a*) Calculate the value of $\Delta E'_0$ for the net reaction of mitochondrial electron transfer.

(*b*) Calculate $\Delta G^{\circ\prime}$ for the this reaction.

(*c*) How many ATP molecule can theoretically be generated by this reaction if the standard free energy of ATP synthesis is 30.5 kJ/mol ?

23. List the following substances in order of increasing tendency to accept electrons :

(*a*) α-ketoglutarate + CO_2 (yielding isocitrate), (*b*) oxaloacetate, (*c*) O_2, (*d*) $NADP^+$

24. Distinguish between anabolic and catabolic reactions.

25. How is the energy generated during metabolic processes usually stored for later use ?

26. DNA, RNA and ATP are all composed of :

(*a*) nucleotides (*b*) purines

(*c*) nucleic acids (*d*) pentose sugars

27. The role played by ATP in biochemical reactions is that of :

(*a*) a reducing agent (*b*) an energy-donor substance

(*c*) a coenzyme (*d*) an energy-donor substance or a coenzyme

28. ATP synthesis occurs in :

(*a*) chloroplasts

(*b*) mitochondria

(*c*) chloroplasts and mitochondria both

(*d*) all cell organelles