1 – THE LAWS OF THERMODYNAMICS CONCEPT OF CHEMICAL EQUILIBRIUM

1.1. THE LAWS OF THERMODYNAMICS

Living organisms are centres of numerous transformations during which energy is converted from one form to another. Today, ***bioenergetic conversion*** constitutes an important chapter in Biochemistry. Energy can be transformed into chemical, mechanical or electrical work, or indeed radiated as heat. These bioenergetic conversions are accompanied by a loss of energy, which must be compensated for by an external supply or a transformation of reserve substances. Metabolism as a whole is under the control of thermodynamic laws. It is important to understand how living beings both extract energy from the world around them and how they use this energy. Without going too much into detail here, it is essential however to recall the principal thermodynamic laws. We will concentrate in particular on the practical aspects necessary for understanding those fundamental energetic principles that underlie the chemical reactions involved in metabolism.

Every phenomenon in the universe is governed by the energy content of a system (in thermodynamics, a "system" is made up of all matter within a well-defined space) and by the exchange of energy between this system and its surroundings.

- The **first law** of thermodynamics teaches us that the total energy of a system and its surroundings is constant. In other words, the total energy content of the universe is constant, which is the *law of energy conservation*.
- The **second law** of thermodynamics states that the entropy of the universe increases continually: the *law of energy loss* (degradation).
- The **third law** states that there is zero entropy at absolute zero (–273°C).

1.1.1. FIRST LAW

The first law describes a thermodynamic relationship, which is obeyed by a system being transformed from an initial to a final state:

$$\Delta E = E_A - E_B = Q - W$$

J. Yon-Kahn, G. Hervé, *Molecular and Cellular Enzymology*,
DOI 10.1007/978-3-642-01228-0_2, © Springer-Verlag Berlin Heidelberg 2010

E_A is the energy of the system in its initial state; E_B, the energy of the system in its final state. Q is the heat absorbed by the system and W, the work done by the system. By convention, Q is positive when heat is absorbed by the system; W is positive when work is carried out by the system on its surroundings.

From the first law, **the change in internal energy of the system depends solely on the initial and final states, but is independent of the path taken by the energy transformation.** The first law, the law of energy conservation, is a universal principle.

If W represents some mechanical work at constant pressure P, with the system changing from volume V_A to V_B, we have:

$$\Delta W = P\,\Delta V \qquad \text{where } \Delta V = V_A - V_B$$

and consequently: $$Q = (E_B + P\,V_B) - (E_A + P\,V_A)$$

giving: $$\Delta H = \Delta E + P\,\Delta V$$

The quantity E + PV defines a function describing the state of the system and is the heat content or **enthalpy**. The enthalpy change associated with a transformation is equivalent in magnitude to the heat of the reaction at constant pressure, but with the opposite sign.

If a transformation not only takes place at constant pressure but also in a constant volume without work done in the system, the previous relationship simplifies to:

$$\Delta H = \Delta E$$

The internal energetic change is equal to the change in heat content at constant pressure and volume. The enthalpy change of a chemical reaction is expressed in kilocalories . mol^{-1} (kcal . mol^{-1}) or now more commonly in kilojoules . mol^{-1} (kJ . mol^{-1}). Indeed, a mechanical equivalent of heat exists, where a calorific energy of 1 cal . g^{-1} corresponds to a mechanical energy of 4.185 J.

The enthalpy change of a chemical reaction at a given temperature T may result from two contributing factors: chemical and thermal.

$$\Delta H = \Delta H_0 \int_0^T \Delta C_p dT$$

ΔH_0 represents the chemical contribution, or in other words, the enthalpy change that would be observed were the reaction to take place at absolute zero. The thermal contribution depends on the difference between the heat capacities of the system in its initial and final states at constant pressure, ΔC_p. If $\Delta C_p = 0$, ΔH is constant.

1.1.2. SECOND LAW

The first law of thermodynamics does not enable us to predict if a reaction could arise spontaneously. Now, certain reactions do occur spontaneously even if ΔE is

positive. The thermodynamic parameter allowing such a prediction is known as **entropy**.

The second law states that **a process may occur spontaneously when the sum of the entropy of the system and the external entropy increases**. In a spontaneous process:

$$\Delta S_i + \Delta S_e > 0$$

In order to illustrate this notion of entropy, let us consider two metal blocks placed together in an isolated enclosure; initially each block has a different temperature (Fig. 1.1a). Over time the temperatures will adjust until they become equal. Similarly, we shall consider the chemical example in Fig. 1.1b, where a 1 M salt solution is placed in a chamber separated from a chamber of pure water by a semi-permeable membrane. The molecules of NaCl from the left compartment will diffuse into the right compartment until the salt concentrations are equal. Another example is provided by gas molecules diffusing from a region of high pressure to one of low pressure. These examples show that such processes have a direction, yet they cannot be predicted from the first law.

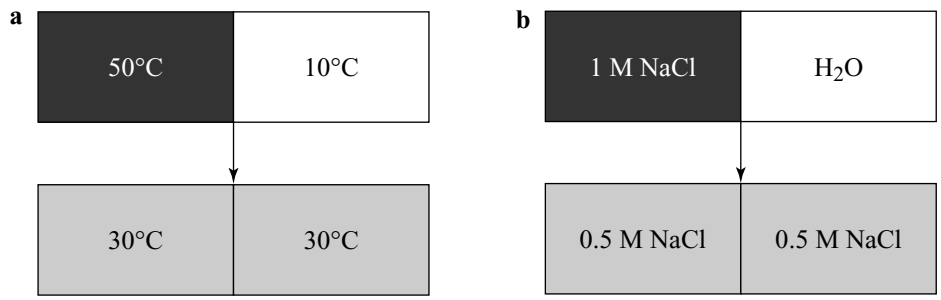


Fig. 1.1 (a) temperature equilibration of two identical metal blocks placed together (b) diffusion of NaCl across a semi-permeable membrane until the concentrations in the two compartments are equal

All systems tend towards a state of equilibrium, or rest, where pressure, temperature and all parameters of the state are uniform. This equilibrated state cannot be reversed by itself; systems at equilibrium do not spontaneously revert to a non-uniform state.

Entropy is a property of state. It represents the state of disorder of a system. When disorder increases:

$$\Delta S > 0$$

Thus, entropy is characteristic of a disordered state of energy incapable of carrying out work. The criterion for the spontaneity of a transformation is defined by the change in entropy associated with it.

If the transformation of a system is due to the reversible absorption of a quantity of heat, dQ, at temperature T the corresponding gain in entropy is:

$$dS = \frac{dQ}{T}$$

Entropy has dimensions of calories . mol^{-1} . K^{-1} or, in other words, dimensions of a **capacity factor**. Multiplying by T (an **intensity factor**) gives the energy. **Extensive variables**, like volume or the heat capacity of chemical bodies, are proportional to the quantity of matter in question; **intensive variables**, such as pressure, temperature or molal heat capacity, are independent of material quantity. At a given temperature, entropy values are relatively low for solids, intermediate for liquids and much higher for gases, as the gaseous state is the most disordered.

During a spontaneous process, the entropy of the system can decrease if the external entropy increases such that the sum of both is a positive value. Thus, it is thermodynamically possible for a highly ordered biological structure to form if the external entropy rises.

The second law establishes that the entropy of the universe continually grows with irreversible evolving processes. It indicates the impossibility to return to an ordered state without the external environment compensating. Theoretically, if the entropy of an evolving system remains constant, the transformation is reversible. In practice, real phenomena are nearly always irreversible due to friction (see Chap. 3).

1.1.3. THIRD LAW

The third law teaches us that the entropy of a substance having a perfect crystalline structure is nil at absolute zero. At absolute zero, thermal motion does not exist; all atoms possess perfect order. In absolute values, the entropy at a temperature T is essentially, therefore, the entropy of passing reversibly from absolute zero to T. The entropy increases with temperature because thermal motion becomes greater.

If, at absolute zero, a substance exists in only one conformation, the entropy at temperature T will be:

$$S_T = \int_0^T \frac{dQ}{T} = \int_0^T C_p \frac{dT}{T} = \int_0^T C_p d\ln T$$

If the material undergoes a sudden change of phase between absolute zero and T, the heat of this transition must be taken into account, and:

$$S_T - S_0 = \int_0^T C_p d\ln T + \sum_i \frac{\Delta H_i}{T_i}$$

ΔH_i is the enthalpy of the transition produced at temperature T_i.

Statistical thermodynamics gives a more concrete idea of entropy. Physical parameters and thermodynamic quantities are **macroscopic** quantities. Statistical thermodynamics relates entropy to microscopic states of the system.

$$S = k \log W$$

It expresses the evolution of the arrangement of these microscopic states towards the most probable arrangement. W is the number of possible arrangements of those states in which the system energy is distributed: the larger the number, the greater the entropy.

1.1.4. FREE ENERGY

All phenomena tend to reach a state of maximum entropy. This is the origin of all spontaneous transformations. Clearly, the entropy of both the system and its surroundings increases. The total entropy of the system with its surroundings is, in fact, difficult if not impossible to measure. Without a precisely determined value, the total entropy can only really provide a measure of whether or not a process could occur spontaneously. This limitation was overcome by the introduction of another thermodynamic quantity, namely **free energy**, or **G**, which was defined by Josiah Willard GIBBS in 1878 and combines the first and second laws of thermodynamics:

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

In this expression, **ΔG is the change in free energy** of the system that is transformed at constant pressure and at temperature T. ΔG and ΔS are, respectively, the change in enthalpy and entropy of the system. Indeed, if we consider the expression:

$$(\Delta S)\ \text{system} + (\Delta S)\ \text{surroundings} > 0$$

or:

$$\left(\frac{\Delta Q}{T}\right)\text{system} + \left(\frac{\Delta Q}{T}\right)\text{surroundings} > 0$$

and the first law:

$$\left[\frac{\Delta Q}{T}\right]\text{surroundings} = \left[\frac{\Delta(E+PV)}{T}\right]\text{system} = \left[\frac{-\Delta H}{T}\right]\text{constant pressure system}$$

and bearing in mind the previous relationship, we have:

$$\frac{\Delta Q}{T} - \frac{\Delta H}{T} \geq 0$$

giving:

$$\Delta H_{system} - T\Delta S_{system} \leq 0$$

This expression comprises only properties of the system and not the surroundings. Gibbs free energy can be considered to be the component of the total energy that can perform work under isothermal conditions.

The transformation of the system is spontaneous when $\Delta G < 0$, termed **exergonic**. If $\Delta G > 0$, the reaction is **endergonic**. This is only possible when coupled to another reaction that is sufficiently exergonic that the sum of their ΔG values is negative. In the case of a reaction at equilibrium, $\Delta G = 0$, and the change in entropy, ΔS, of the system is given by:

$$\Delta S = \frac{\Delta H}{T}$$

During irreversible transformations the free energy decreases, whereas the entropy increases. **Free energy, G, and enthalpy, H, as well as entropy, S, are all functions of the system's state.**

A reaction may be accompanied by a large, positive enthalpy change (a strongly endergonic reaction) and, however, be spontaneous if the gain in entropy is sufficient to compensate for ΔH in such a way that ΔG is negative. A great many reactions exist for which large changes in enthalpy are compensated by a significant increase in entropy. This will be later reemphasised with the help of a few examples (see Part VI).

Thus, the free-energy change, ΔG, is a criterion that enables us to predict if a reaction will occur spontaneously. To summarise:

- a reaction can only occur spontaneously if $\Delta G < 0$;
- a system is at equilibrium and will not undergo a transformation if $\Delta G = 0$;
- a reaction cannot occur spontaneously if $\Delta G > 0$; it must be coupled to another reaction sufficiently exergonic to give a combined $\Delta G < 0$.

The free-energy change is independent of the reaction pathway and depends only on the initial and final states.

1.2. CONCEPT OF EQUILIBRIUM – STANDARD FREE ENERGY

Let us consider the following reaction:

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

The change in free energy of the reaction is given by the following expression:

$$\Delta G = \Delta G_0 + RT \ln \frac{[C][D]}{[A][B]}$$

where ΔG_0 is the change in standard free energy. [A], [B], [C] and [D] are the activities of the reaction components. ΔG is a function that changes continuously until equilibrium is attained and represents the affinity, having a positive value (see the definition of this affinity pp. 69). In a very dilute solution, the activities of the reaction components are interchangeable with their molar concentrations.

The relationship between concentration, c, and activity, a, is given by the equation:

$$a = \gamma c$$

where γ, the activity coefficient, is a function of the ionic strength of the solution and is described by the DEBYE equation:

$$\log\gamma = \frac{-Az^2\sqrt{\mu}}{1+Br\sqrt{\mu}}$$

The coefficients A and B depend on the temperature and the dielectric constant of the medium; r is the radius of the molecule and μ, the ionic strength of the medium. In water at 25°C, this equation becomes:

$$\log\gamma = \frac{-0.505z^2\sqrt{\mu}}{1+0.347\times10^8\sqrt{\mu}}$$

In dilute solutions, the above formula simplifies to:

$$\log\gamma = -0.505z^2\sqrt{\mu}$$

The change in standard free energy is the free energy of the reaction under standard conditions i.e. when the concentrations of both reactants and products are 1 M.

At equilibrium, $\Delta G = 0$ and the free energy equation becomes:

$$\Delta G_0 = -RT\ln\frac{(C)_e(D)_e}{(A)_e(B)_e}$$

Since the equilibrium constant, K_{eq}, is defined as follows:

$$K_{eq} = \frac{(C)_e(D)_e}{(A)_e(B)_e}$$

the free energy equation may be written:

$$\Delta G_0 = -RT\ln K_{eq}$$

or alternatively:

$$K_{eq} = e^{-\Delta G_0/RT}$$

Converting to base-10 logarithms, we obtain the common expression for calculating the standard free-energy change:

$$\Delta G_0 = -2.3\log K_{eq}$$

Thus, the standard free-energy change is related in a simple way to the equilibrium constant of a reaction. This is the most frequently used parameter in bioenergetics.

◤ It is more rigorous to demonstrate this relationship in terms of chemical potentials. The **chemical potential is defined by the partial molar free energy:**

$$\mu = \frac{\partial G}{\partial n}$$

n being the number of reacting molecules and μ is related to the activity of a component by the equation:

$$\mu = \mu_0 + RT \ln a$$

The reference state is that in which the activity equals 1. If a component switches from an activity a to an activity a′, the chemical potential is:

$$\mu' = \mu'_0 + RT \ln a'$$

and the change in chemical potential:

$$\Delta\mu = -RT \ln \frac{a}{a'}$$

or in molality:

$$\Delta G_0 = -RT \ln \frac{n'_A a'}{n_A a}$$

Let us consider the following relationship:

$$n_A A + n_B B = n_C C + n_D D$$

At equilibrium, we can write in terms of chemical potentials:

$$n_A \mu_A + n_B \mu_B = n_C \mu_C + n_D \mu_D$$

giving:

$$\Delta G_0 = n_C \mu_C + n_D \mu_D - n_A \mu_A - n_B \mu_B$$

and thus:

$$\Delta G_0 = -RT \ln \frac{(C)_e^n (D)_e^n}{(A)_e^n (B)_e^n} = -RT \ln K_{eq}$$ ◢

1.3. EXPERIMENTAL DETERMINATION OF THERMODYNAMIC PARAMETERS

Knowledge of the thermodynamic parameters of the major biochemical reactions forms the basis of all bioenergetics and for understanding the mechanisms by which metabolic reactions take place.

1.3.1. ENTHALPY CHANGE

The change in enthalpy, or the heat of a reaction at constant pressure, can be measured directly by microcalorimetry. From this technique we obtain ΔH from which ΔH_0 can be deduced. In the case of reversible reactions, it is typical to study the change in equilibrium constant with temperature.

The VAN T'HOFF equation: $$\frac{-d \ln K}{dT} = \frac{\Delta H_0}{RT^2}$$

also written: $$\frac{d \ln K}{d(1/T)} = \frac{-\Delta H_0}{R}$$

enables determination of ΔH_0 from knowledge of the equilibrium constant, K, at several temperatures. The graphical representation of VAN T'HOFF gives a linear relationship between ln K and 1/T; the slope of the line is equal to $-\Delta H/R$ (Fig. 1.2). In this expression R, the gas constant, is equal to 1.98×10^{-3} kcal . mol^{-1} . K^{-1}.

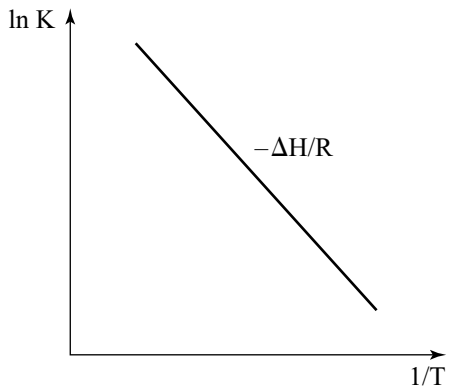


Fig. 1.2 VAN T'HOFF representation

This relationship shows that, when the temperature rises, K increases in endergonic reactions but decreases in exergonic reactions. Table 1.1 gives values of ΔH_0 for some important biochemical reactions.

Table 1.1 Values for the enthalpy of combustion of some biological molecules

Molecule	*ΔH_0 (kcal . mol^{-1})*
Glycine	–234
Lactic acid	–326
D-glucose	–673
Palmitic acid	–2 380

1.3.2. FREE-ENERGY CHANGE

ΔG_0 values for chemical reactions can be obtained in three different ways: from thermochemical data; by analysing the concentrations of reaction components at equilibrium; or by measuring the work done by a system.

1.3.2.1. THERMOCHEMICAL ANALYSIS

ΔG_0 can be obtained from the basic equation:

$$\Delta G_0 = \Delta H_0 - T\Delta S_0$$

when thermochemical data are available that allow the determination of ΔH_0 and ΔS_0. These values can be determined by calorimetric measurement when certain conditions are met. Thus, PRIVALOV and collaborators applied these methods to the study of the thermal denaturation of proteins. They showed that, for most proteins studied, the enthalpy change varies linearly with temperature, i.e. ΔC_p is constant. Under these conditions:

$$\Delta H_D = \Delta H_m + \Delta C_p(T - T_m)$$

ΔH_D being the enthalpy of denaturation, and ΔH_m its value at the melting temperature, T_m. The change in entropy is given by:

$$\Delta S_D = \Delta S_m + \Delta C_p \ln T/T_m$$

1.3.2.2. EQUILIBRIUM STUDY

Table 1.2 Standard free-energy change of some chemical reactions at pH 7.0 and 25°C

Reaction	*$\Delta G_0'$ (kcal . mol^{-1})*
Hydrolysis	
Acid anhydrides	
Acetic anhydride + H_2O ⟶ 2 acetates	–21.8
Pyrophosphate + H_2O ⟶ 2 phosphates	–8.0
Esters	
Ethyl acetate + H_2O ⟶ ethanol + acetate	–4.7
Glucose-6-phosphate ⟶ glucose + phosphate	–3.3
Amides	
Glutamine + H_2O ⟶ glutamate + NH_4^+	–3.4
Glycylglycine + H_2O ⟶ 2 glycines	–2.2
Glycosides	
Saccharose + H_2O ⟶ glucose + fructose	–7.0
Maltose + H_2O ⟶ 2 glucoses	–4.0
Rearrangement	
Glucose-1-phosphate ⟶ glucose-6-phosphate	–1.7
Fructose-6-phosphate ⟶ glucose-6-phosphate	–0.4
Elimination	
Malate ⟶ fumarate + H_2O	+0.75
Oxidation	
Glucose + $6O_2$ ⟶ $6CO_2 + 6H_2O$	–686.00
Palmitic acid + $23O_2$ ⟶ $16CO_2 + 16H_2O$	–2338.00

This method involves calculating ΔG_0 from the equation:

$$\Delta G_0 = -RT \ln K_{eq}$$

and is without a doubt the most often used in biochemistry. The technique requires a precise estimation of the activities or concentrations at equilibrium of each reaction component. Depending on the properties of each, various methods of measurement are employed.

The majority of biochemical reactions occur near to neutral pH and so standard free-energy change, designated by $\Delta G_0'$, generally refers to reactions at pH 7 and at 25°C. Table 1.2 opposite gives values of $\Delta G_0'$ for a few biochemical reactions and Table 1.3, $\Delta G_0'$ values for the formation of several biological molecules.

Table 1.3 Standard free-energy change for the formation of some components
(the values shown refer to a 1 M aqueous solution at pH 7.0 and 25°C)

Component	*$\Delta G_0'$ (kcal . mol^{-1})*
H^+ (standard reference)	0.00
NH_4^+	–19.00
OH^-	–37.60
Ethanol	–43.39
Water	–56.69
L-alanine	–88.75
$Acetate^-$	–88.99
Carbon dioxide (gaseous)	–94.45
$Pyruvate^-$	–113.44
Glycerol	–116.76
Lactate	–123.76
HCO_3^-	–140.33
$Fumarate^{2-}$	–144.41
$Succinate^{2-}$	–164.97
L-$aspartate^-$	–166.99
$Oxaloacetate^{2-}$	–190.53
α-$ketoglutarate^{2-}$	–190.62
L-$malate^{2-}$	–201.98
α-D-glucose	–219.22
cis-$aconitate^{3-}$	–220.51

1.3.2.3. DIRECT MEASUREMENT OF WORK SUPPLIED BY THE SYSTEM

For biochemical reactions involving an oxidation-reduction, the free-energy change for the reaction can be evaluated by measuring the potential difference between two electrodes. Indeed, it is possible to set up cells where the electrical work of the reaction is practically equal to the maximum work.

In the reaction: $\mathbf{A_{red} \rightleftharpoons A_{ox} + n\ e}$

n is the number of electrons, e.

The equilibrium constant is: $$K = \frac{(A)_{ox}(e)^n}{(A)_{red}}$$

If in the reaction an electron acceptor exists such that:

$$\mathbf{B_{ox} + ne \rightleftharpoons B_{red}}$$

then the oxidation-reduction equation is as follows:

$$\mathbf{A_{red} + B_{ox} \rightleftharpoons A_{ox} + B_{red}}$$

These two reactions are coupled (see below); one cannot take place without the other. The total free-energy change is equal to the sum of the free-energy changes of each individual reaction. The free-energy change can be measured based on the oxidation-reduction (also called redox) potential, Eh. This value can be determined from an oxidation-reduction cell as illustrated in Fig. 1.3. The cell comprises two separate compartments linked by conducting material that allows the flow of electrons between the two. A_{red} and B_{ox} are isolated in each of the compartments, which contain platinum electrodes. The potential difference is measured across the terminals under conditions in which the current is nearly zero i.e. in conditions close to reversibility.

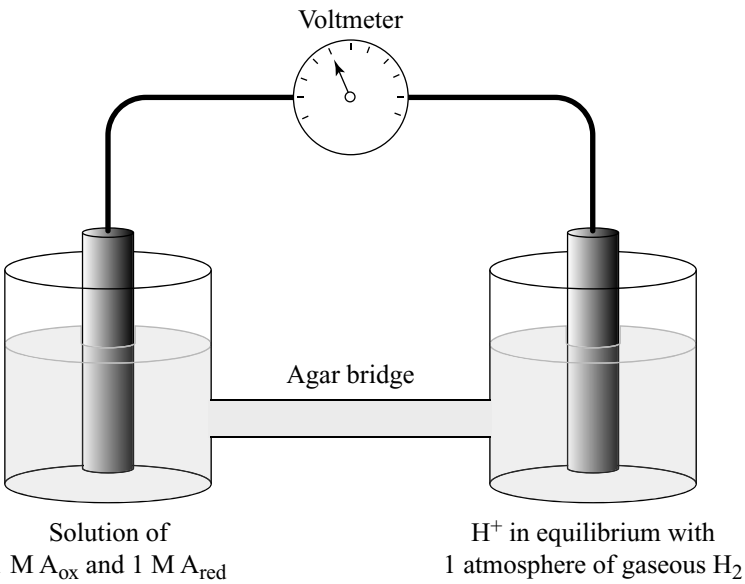


Fig. 1.3 Schematic representation of an oxidation-reduction cell

In one of the compartments the following reaction takes place:

$$H^+ + e \rightleftharpoons \tfrac{1}{2} H_2 \text{ (1 atmosphere)}$$

Therefore the potential difference between the electrodes, or:

$$Pt : A_ox, A_{red} : : H^+(a = 1), H_2(1 \text{ atm.}) : Pt$$

is the redox potential of the system. The change in free energy is related to this potential difference by the formula:

$$\Delta G + nRT \ln \frac{1}{e_a} = nFE_h$$

F is the Faraday equal to 96 500 coulombs, or 23.062 kcal . V^{-1}. The redox potential of the system is therefore:

$$E_h = \frac{RT}{nF} \ln K + \frac{RT}{nF} \ln \frac{A_{ox}}{A_{red}}$$

By definition:

$$E_0 = \frac{RT}{nF} \ln K$$

where E_0 is the normal potential obtained when $A_{ox} = A_{red}$. Thus, at 25°C we have:

$$E_h = E_0 + \frac{0.0586}{n} \log \frac{A_{ox}}{A_{red}}$$

Table 1.4 below lists the redox potentials for half-reactions at pH 7 of several biological systems. NAD^+ ($NAD^+ \rightleftharpoons$ NADH) and FAD (FAD $\rightleftharpoons$ $FADH_2$) are essential electron transporters during electron transfer within living organisms. NADPH is the principal electron donor in reductive biosynthesis.

1.4. COUPLED REACTIONS

1.4.1. DEFINITION OF ENERGETIC COUPLING

A thermodynamically unfavourable (endergonic) reaction can take place when coupled to a thermodynamically favourable reaction if in total the result is exergonic. For example, let us consider these reactions:

$$A + B \rightleftharpoons C + D \qquad \Delta G_1 = +5 \text{ kcal}$$

$$D + E \rightleftharpoons F + G \qquad \Delta G_2 = -7 \text{ kcal}$$

The first is endergonic and so would be impossible if not coupled to the second to give an exergonic reaction, overall:

$$\Delta G_{total} = \Delta G_1 + \Delta G_2 = -2 \text{ kcal}$$

The additivity of free-energy relationships stems directly from the law of mass action. Indeed, the equilibrium constants of these reactions are, respectively:

$$K_1 = (C)(D)/(A)(B) \quad \text{and} \quad K_2 = (F)(G)/(D)(E),$$

The global equilibrium constant is thus:

$$K = (C)(D)(F)(G)/(A)(B)(D)(E) = K_1 K_2$$

The two reactions are coupled via a **common intermediate**, D. The principle of the common intermediate forms the basis of all biological energy transfers. ATP acts as a common intermediate in consecutive reactions of this type.

Table 1.4 Half-reaction redox potentials, E_{m7}, at pH 7 of various systems at around 30°C

System	*E_{m7} (volts)*
Flavins and flavoproteins	
Riboflavin	–0.208
Riboflavin 5′ phosphate (FMN)	–0.219
Flavin adenine dinucleotide (FAD)	–0.219
Xanthine oxidase	–0.350
D-lacticodehydrogenase (anaerobic yeast)	–0.178
Vitamin B2	–0.123
D-amino acid oxidase	–0.110
Glucose dehydrogenase	+0.080
Haems and haemoproteins	
Horseradish peroxidase	–0.271
Haem	–0.120
Cytochrome b_6 (chloroplast)	–0.060
Metmyoglobin	+0.046
Cytochrome b (heart)	+0.068
Methaemoglobin	+0.144
Cytochrome c (heart)	+0.250
Cytochrome oxidase	+0.285
Cytochrome a (heart)	+0.290
Metabolites and others	
Cysteine-cystine	–0.400
Thioglycolic acid	–0.340
NADH-NAD^+	–0.320
NADPH-$NADP^+$	–0.316
Propanol-acetone	–0.286
L-β-hydroxybutyrate-acetoacetate	–0.284
Glutathione	–0.230
Ethanol-acetaldehyde	–0.200
D-lactate-pyruvate	–0.190
L-malate-oxaloacetate	–0.157
Glycerate-hydroxypyruvate	–0.150
Succinate-fumarate	+0.024
Ascorbic acid-dehydroascorbic acid	+0.058

1.4.2. ROLE OF ATP

The main supplier of energy in living organisms is adenosine triphosphate or ATP. From 1905, HARDEN and YOUNG noticed that alcoholic fermentation could only occur in the presence of phosphate. A certain number of phosphate esters were identified. In 1930, MEYERHOFF and LIPMANN showed that, through phosphate ester intermediates, the cell is able to harness part of the energy of chemical bonds contained in nutrients. Isolated for the first time around 1930 from muscle tissue, ATP was considered for a long while to be a specific component of muscle. Later it was discovered that ATP exists in cells from all species of animal, plant and microbe. Figure 1.4 displays the chemical structure of an ATP molecule. It is strongly charged: at pH 7.0 there are four negative charges due to the complete ionisation of each of the three phosphate groups.

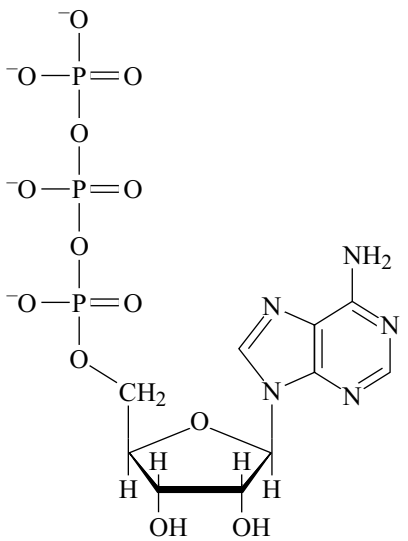


Fig. 1.4 The ATP molecule

The energetic role of ATP was discovered in 1941 by LIPMANN and KALCKAR. ATP hydrolysis can take place in two different ways:

$$ATP^{4-} + H_2O \rightleftharpoons ADP^{3-} + P_i + H^+$$

$$ATP^{4-} + H_2O \rightleftharpoons AMP^{2-} + PP_i + H^+$$

Each of these reactions is accompanied by a free-energy change of $\Delta G_0' = -7.3\ \text{kcal} \cdot \text{mol}^{-1}$ under standard conditions. Compared to simple esters, glycosides and amides, this is significantly more exergonic. For this reason, ATP is classed as an "energy-rich substance". Its specific bonds, whose hydrolysis leads to large, negative ΔG values, are also described as being "rich in energy". In fact, this notion is incorrect and ambiguous. **The so-called energy of the phosphate bond does not apply to the energy of the covalent bond, but to the difference in the energy content of the molecule and its hydrolysis products**.

ATP hydrolysis is utilised to facilitate processes that require energy. Thus, chemical work such as biosynthesis and active transport across membranes and mechanical work, for instance, muscle contraction, all make use of the energy liberated by ATP hydrolysis. Some examples of energetic coupling will be examined later.

ATP is reconstituted in the course of the oxidation of combustible molecules during metabolism. However, the hydrolysis energy of the phosphodiester bond is much less than the quantity of energy released by the catabolism of a single glucose molecule (686 kcal . mol^{-1}). Were this to occur in a single metabolic step, there would be insufficient energy to form several "energy-rich bonds" and hence glucose catabolism requires several steps. In this way, each metabolic step releases an amount of energy equivalent to the free energy of hydrolysis of a single "energy-rich bond".

Coupling a chemical reaction to ATP hydrolysis shifts the equilibrium of the coupled reaction by a factor of the order of 10^8. So let us consider the following reaction:

$$A \rightleftharpoons B$$

with an equilibrium constant $K_{eq} = 10^{-3}$, and $\Delta G_0' = 4$ kcal . mol^{-1}. In the presence of ATP:

$$A + ATP \rightleftharpoons B + P_i + ADP + H^+$$

the value of $\Delta G_0' = -3.3$ kcal . mol^{-1}.

So
$$K'_{eq} = \frac{(B)_{eq}}{(A)_{eq}} \frac{(ADP)_{eq}(P_i)_{eq}}{(ATP)_{eq}} = 10^{3.3/1.36}$$

$$K'_{eq} = 2.67 \times 10^2$$

In cells, the ATP-regenerating system maintains the $(ATP)/(P_i)$ ratio near to 500. Under these conditions:

$$K_{eq} = \frac{(B)_{eq}}{(A)_{eq}} = 2.67 \times 10^2 \times 500 = 1.35 \times 10^5$$

which is about 10^8 times greater than the equilibrium constant of the uncoupled reaction.

1.4.3. FREE ENERGY OF HYDROLYSIS OF SOME PHOSPHORYLATED COMPOUNDS

The most important "energy-rich" substance is ATP, however other molecules also store energy. Some are directly formed during catabolic reactions, others are synthesised as a result of the energy produced from ATP hydrolysis. Table 1.5 opposite shows the free-energy changes of the principal phosphorylated molecules

found in living organisms; phosphates are transferred from molecules at the top of the table towards those at the bottom.

Table 1.5 Change in free energy of hydrolysis of some major phosphorylated compounds

Compound	$\Delta G_0'$ *(kcal . mol^{-1})*
Phosphoenol pyruvate	–14.8
1,3-diphosphoglycerate	–11.8
Phosphocreatine	–10.3
Acetyl phosphate	–10.1
Phosphoarginine	–7.7
ATP	**–7.3**
Glucose-1-phosphate	–5.0
Fructose-6-phosphate	–3.8
Glucose-6-phosphate	–3.3
Glycerol-1-phosphate	–2.2

The substances listed at the top of the scale have a propensity to lose their phosphate groups; conversely, those lower down tend to retain their phosphate. It is important to note that ATP possesses by no means the greatest free energy of hydrolysis amongst phosphate esters. It has an intermediate value and may be considered to be in the middle of a thermodynamic scale. **ATP occupies an intermediate position on a thermodynamic scale of phosphorylated biomolecules.** The ATP-ADP system acts as a bridge between phosphorylated compounds that have a high phosphoryl transfer potential and those whose transfer potential is low. Furthermore, its particular role is explained by the fact that the many ATP-dependent processes take place with the assistance of enzymes that harbour a binding site for ATP and ADP. For instance, the reaction:

$$\text{ATP} + \text{AMP} \rightleftharpoons 2\ \text{ADP}$$

is catalysed by adenylate kinase. ATP and ADP behave like a shuttle for phosphate groups, always in the general direction away from "energy-rich" substances towards "energy-poor" substances. In cells, the terminal phosphate group of ATP is reformed very quickly, estimated to be within a fraction of a second.

Many metabolic reactions are controlled by the energetic state of the cell. The concept of **energy charge** is commonly used (ATKINSON). The energy charge is proportional to the molar fraction of ATP plus a half-molar fraction of ADP, since ATP contains two anhydride bonds whereas ADP has only one; it is expressed by the following relationship:

$$\text{energy charge} = \frac{(\text{ATP}) + \tfrac{1}{2}(\text{ADP})}{(\text{ATP}) + (\text{ADP}) + (\text{AMP})}$$

The phosphorylation potential is defined thus:

$$\frac{(ATP)}{(ADP)(P_i)}$$

The metabolic pathways that produce ATP are inhibited by a high energy load. Conversely, those pathways that consume ATP are activated by a high energy load. As such, this mechanism provides an "energetic buffering effect".

1.4.4. SOME EXAMPLES OF ENERGETIC COUPLING

1.4.4.1. FORMATION OF ATP FROM THE OXIDATION ENERGY OF NUTRIENTS

The first step in the energy conversion of nutrients is the transformation of an energy-rich substance able to transfer its energy to ADP in order to produce ATP. The formation of a carboxylic acid from free aldehyde releases energy:

$$R—CHO + H_2O \rightleftharpoons 2\,H + R—COO^- + H^+$$

where $\Delta G_0' = -7$ kcal . mol^{-1}.

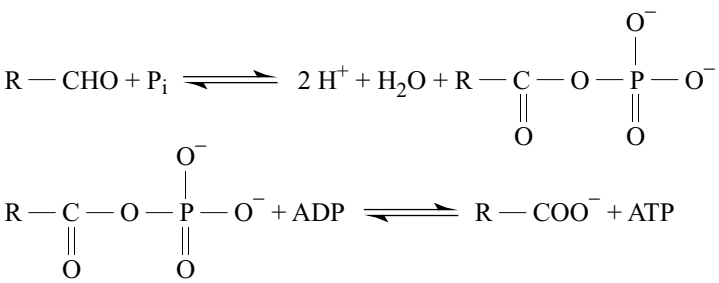
In the cell, this reaction is catalysed by an enzyme in such a way that the energy is not dispersed but stored in the form of phosphate:

$$\text{3-phosphoglyceraldehyde} \rightleftharpoons \text{3-phosphoglycerate}$$

This reaction is linked to the formation of ATP from ADP:

$$R—CHO + P_i + ADP \rightleftharpoons 2\,H^+ + RCOO^- + ATP$$

and the overall energy change, $\Delta G = 0$ kcal . mol^{-1}. The drop in free energy resulting from the aldehyde oxidation (–7 kcal . mol^{-1}) is exactly compensated by the formation of ATP from ADP (+7 kcal . mol^{-1}) and made possible only by coupling the reactions. In fact, the reaction comprises two distinct steps catalysed by two different enzymes, yet involving a common intermediate: 1,3-diphosphoglycerate. To restate, the conservation of oxidative energy from ATP is only possible because the oxidative reaction and the phosphorylating reaction share this common intermediate.



1.4.4.2. USE OF THE ENERGY FROM ATP FOR CHEMICAL WORK

The biosynthesis of complex macromolecules within cells absolutely requires energy. Indeed, the synthesis of a single protein molecule involves assembling correctly a sequence of hundreds of amino acids linked by peptide bonds. Peptide-bond formation is an endergonic process. Similarly, for the construction of polysaccharides such as cellulose, starch and glycogen, hundreds of glucose molecules must be linked by glycosidic bonds, which is also endergonic. The same holds true for the formation of nucleic acids. The general equation for biosynthesis may thus by written:

$$\text{building blocks} \rightleftharpoons \text{macromolecules} + H_2O$$

which represents a highly endergonic reaction. Table 1.6 displays the energies of formation of the major biological macromolecules.

Table 1.6 Chemical work during biosynthesis

Macromolecule	*Element*	*Type of bond*	*$\Delta G_0'$ (kcal . mol^{-1})*	*Number of bonds*
Protein	Amino acid	Peptide	+4	1.2×10^2 to 10^4
Nucleic acid	Mononucleotide	Phosphodiester	+5	3×10^3 to 10^6
Polysaccharide	Monosaccharide	Glycosidic	+4	2×10^3 to 10^4

Each time that a large, complex structure is formed from simple but disordered elements, the system entropy decreases. Given that all complex, organised systems have a natural tendency to return to a state of disorder, it is vital to supply energy to the system in order to counteract this effect.

The reactions are mostly coupled to the hydrolysis of ATP. For instance, the formation of saccharose, a disaccharide, from its building blocks, glucose and fructose, is coupled to ATP hydrolysis by the following sequential reactions:

$$\text{ATP} + \text{glucose} \longrightarrow \text{ADP} + \text{glucose-1-phosphate}$$

$$\text{glucose-1-phosphate} + \text{fructose} \longrightarrow \text{saccharose} + P_i$$

The first reaction is endergonic ($\Delta G_0' = +5.5$ kcal . mol^{-1}) and alone, therefore, is highly unlikely. When coupled to ATP hydrolysis, however, the overall $\Delta G_0'$ is -1.5 kcal . mol^{-1} and hence is exergonic, the common intermediate being glucose-1-phosphate.

Similarly, during protein synthesis, we find the following reaction sequence:

$$\text{amino acid} + \text{ATP} \longrightarrow \text{AA—AMP} + PP_i$$

The formation of an activated amino acid or an adenylated amino acid is then followed by a transfer reaction to the corresponding tRNA:

$$\text{AA—AMP} + \text{ARNt} \longrightarrow \text{AA—ARNt} + \text{AMP}$$

Then: AA—ARNt + polypeptide chain of n amino acids

$\longrightarrow$ ARNt + polypeptide chain of (n + 1) amino acids

These diverse reactions, which always involve a common intermediate, are catalysed by specific enzymes: amino-acyl tRNA synthetases.

1.4.4.3. OSMOTIC WORK

All cells are able to transport and store certain essential substances from the external environment. This results in their much higher concentration within the cell. On the contrary, cells expel or secrete unwanted or degraded substances, even if their internal concentrations are much lower than outside of the cell.

Now, the movement of molecules against a concentration gradient is not spontaneous. This process is assisted by the energy supplied, in general, from ATP hydrolysis and is referred to as active transport. The work of transport pumps is critical in enabling the cell to maintain constantly an appropriate, internal composition, within an environment that may be extremely different or even life-threatening. Besides, thanks to these pump mechanisms, cells are able to extract from the environment the required molecules even if they are present in only very dilute quantities.

The electrical work carried out by certain cells is a form of osmotic work. When certain charged ions, Na^+ or K^+, are transported across the membrane, an unequal distribution of charge, or potential difference, develops. The potential difference is essential in nerve and muscle cells, for example, to create an active state by excitation and for transmitting the signal.

1.4.4.4. MECHANICAL WORK

Mechanical work is a type of biological work, the most striking example of which in higher animals results from muscle contraction. However, muscle contraction is simply the visible manifestation of a property that is possessed by all cells, but which has been perfected. In other cells, this property has the more general effect of enabling the exertion of intracellular traction forces by means of contractile filaments. During cell division, contractile filaments are responsible for the separation of cellular components.

Structures possessing movement such as cilia and flagella also carry out mechanical work, i.e. propulsion. It is worth noting that the mechanical work done in cells is directly sustained by chemical energy. Systems designed by humans to supply mechanical work are generally powered by thermal or electrical energy, whereas in living organisms chemical energy is directly used. These diverse processes are, of course, linked to ATP hydrolysis.

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