Chapter One
1. Thermodynamics of Energy Transduction in Biological Membranes

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1-1. Introduction

Thermodynamics is a classical area of physics and chemistry for which there exists a large number of major treatises that form the background for this chapter. These include excellent treatises that emphasize the application of thermodynamics to problems in biochemistry and biophysics (Bloomfield, *Biophysics Textbook On-Line* ; Eisenberg and Crothers, 1979; Tinoco et al., 1978). Other sources are (Hill, 1966; Mahan, 1964; Chandrasekhar, 1957). The present treatment specifically emphasizes the application of thermodynamics to bioenergetics, or energy transduction in biological membranes. This energy transduction involves (i) the transduction of light and redox energy to "free energy" stored in a trans-membrane ionic electrochemical potential ($\Delta\tilde{\mu}$), and (ii) the use of this energy to synthesize ATP and to drive active transport across membranes and the accumulation of needed solutes and metabolites in the cell. This present discussion of a subset of the topics of thermodynamics in the context of biological membrane energy transduction is derived from that in (Cramer and Knaff, 1991).

The laws of thermodynamics, obeyed by physical and chemical reactions in living as well as inanimate systems, describe the energetic limitations on state changes of systems of molecules, the relative molecular populations of energetically accessible states, and predict the direction of the reactions that link these states. In the thermodynamic formalism, a "system" is described physically and mathematically by a small number of state properties or variables: (a) **intensive variables** such as the density, dielectric constant, molar free energy or chemical potential, pressure, specific heat, and temperature, which are **independent of the size of the system**; (b) **extensive variables**, such as the mass, energy, enthalpy, and entropy, and volume, which are **dependent on system size**. The state of a system is said to be defined when all of its properties can be specified. A defined state of a system is in **equilibrium** when (a) the values of its state variables are independent of time and (b) there is no flux of mass or energy across the boundaries of the system. If the system variables are constant, but there is a net flux of mass or energy moving across the system, then this system is not in equilibrium, but in a **steady-state**. Mathematical relationships between state variables are called **equations of state**. One example of such an equation, relating the pressure, p, volume, V, and temperature, T, is the ideal (dilute) gas equation,

$$pV = nRT \qquad (1\text{-}1)$$

where the constants of proportionality, n, R, are, respectively, the number of moles in the system and the gas constant, R (Table 1-1, Constants and Conversions). This equation of state applies to gases at a pressure and concentration small enough that molecular interactions can be neglected. The same equation of state, with the variable, concentration (c), replacing that of pressure, can be used as an equation of state for solutes in dilute solution, so that the dependence of the energy change of an ideal gas at constant temperature on its change of pressure, $dE = Vdp$, is the same as the dependence of the partial free energy of a solute in aqueous solution on its concentration, $dG = Vdc$ (cf., section 1-7 below.)

A summary of physical constants that are utilized in discussions of thermodynamics, their values in the Standard International (SI) and cgs (cm-gm-sec) systems of units, and the conversion factors between the two systems, is presented in Table 1-1.

Table 1-1. Constants and Conversions
A. Fundamental Constants

Constant	Symbol	SI Units	Value	cgs Units
Avogadro's number	N		6.02×10^{23} mol^{-1}	
Speed of light in vacuum	c	$3 \cdot 10^{8}$ m s^{-1}		$3 \cdot 10^{10}$ cm s^{-1}
Bottzmann's constant	k	$1.38 \cdot 10^{-23}$ J K^{-1}		$1.38 \cdot 10^{-16}$ erg K^{-1}
Gas constant (Nk)	R	8.31 J K^{-1} mol^{-1}		1.99 cal K^{-1} mol^{-1}
Elementary charge	e	$1.6 \cdot 10^{-19}$ coulomb		4.8×10^{-10} statcoulomb ($g^{1/2}$cm$^{3/2}$ s^{-1})
Faraday constant (Ne)	F	$9.65 \cdot 10^{4}$ C mol^{-1}		
Planck's constant	h	$6.63 \cdot 10^{-34}$ J s		$6.63 \cdot 10^{-27}$ erg s
	$\hbar = h/2\pi$			

B. Inter-Conversion of Units
1 Joule (SI) = 10^7 ergs (cgs) = 1 coulomb-volt
1 electron-volt (eV) = $1.6 \cdot 10^{-19}$ J = 23.06 kcal · mol^{-1} = 96.48 kJ mol^{-1}
1 calorie = 4.184 Joule
1 ampere = 1 coulomb · s^{-1} = $6.2 \cdot 10^{18}$ charges · s^{-1}
1 Siemen = 1 amp/volt
1 Einstein = 1 mole of photons

C. Useful Mixed Constants
2.3 RT = 1.36 kcal · mol^{-1} = 5.69 kJ mol^{-1} (25°C)
2.3 RT/F = 59.1 mV (25°C)

Thought Problems:

1. Consider two systems: (a) hemoglobin and O_2, each at a constant total concentration, in a sealed test tube at constant temperature (isothermal) and pressure (isobaric), where the binding of O_2 is described by Hb + O_2 $\leftrightarrows$ Hb-O_2; (b) a suspension of bacterial cells in a solution of nutrient ("chemostat"), amino acids, and salts in which the cells are diluted with fresh nutrient medium so they remain at a constant cell concentration. Which system, (a) or (b), is at equilibrium and which is at steady-state?

2. Consider the heat energy absorbed by a protein when it is heated at constant pressure in a scanning calorimeter from room temperature, 20° C, to its melting temperature, T_m = 60° C. The average specific heat, C_p, of the protein in this temperature range is 40 kJ mol^{-1}°K^{-1}. For what extensive variable is a value needed to calculate the total amount of absorbed heat energy ?

3. The hydrophobic interior of biological membranes is an energy barrier for transport across the membrane of charged ions and metabolites. The hydrophobicity of the membrane bilayer semi-continuum is described by the dielectric constant, ε, which describes the polarity of the medium, and has the value of 78 in water, and of approximately 3 in the center of a lipid bilayer membrane. The energy required to transfer an ion of radius, r, and valence, z, from water with dielectric constant, ε_w, to the center of the membrane with dielectric constant, ε_m, is the energy, $E_b = 695(z)^2/r\ [1/\varepsilon_m - 1/\varepsilon_w]$,

in kJ/mol, that forms a barrier for the translocation of the ion to the membrane interior. What is the physical constant (Table 1-1) that allows one to calculate the energy barrier *per ion*?

1-2. The First Law of Thermodynamics

A fundamental aspect of the First Law, which states that **the energy of an insulated system is conserved**, is that **heat and work are both considered as equivalent forms of energy**. The energy level of a system can be changed by (i) an increment or decrement of mechanical work (**dW**), exerted by a force (F) or pressure (P) acting, respectively, over a distance dx, or an element of volume, dV, or by (ii) heat (**dQ**) transmitted through a temperature gradient. The **First Law**, the **law of conservation of energy**, is well known from other branches of chemistry and physics and states that the total energy of a system insulated from its surroundings does not change. Thus, addition of amounts of heat (**dQ**) and work (**dW**) to a system that is insulated from exchange of energy with its surroundings must be reflected in a change, **dE**, of the energy of the system. That is,

$$\mathbf{dE = dQ + dW} \qquad (1\text{-}2)$$

1-2.1 Properties of the energy, E:

1. **E** is an extensive property, whose units in thermodynamic problems are calories or joules (**1 cal = 4.184 J** or **electron-volts** (1 **eV** = 1.6×10^{-19} **J**).

- For an ideal or dilute gas, the energy is a function only of the intensive variable, temperature.
- Energy is a **state function**. The value of the energy function is unique for a given state, and therefore its change, ΔE, between two states is independent of the pathway that links the states. Because of the latter two properties, the infinitesimal change of energy, **dE**, is a **perfect differential**, i.e.,

$$\Delta E = E(2) - E(1) = \int_1^2 dE \qquad (1\text{-}3)$$

for the change of energy, Δ**E,** between the value of E in the final state, "2," and the initial state, "1." This is the mathematical definition of a state function. In contrast, the integral of an arbitrary mathematical function is dependent on the pathway between initial and final states, and thus the change in its value from the initial to the final state is not unique.

1-2.2. Work & Heat.

Neither of the two components, work and heat, of the energy defined in eqn (1-2) is a state function because their changes are dependent on the pathway between states. This can be visualized by comparing **reversible** vs. **irreversible** transitions between initial and final states for various processes. The reversible transition is ideally characterized by a pathway consisting of small steps or changes in coordinates. The occurrence of each such step is infinitesimally more probable than its non-occurrence. In the irreversible transition, each state or sub-state states reached during the transition is far more probable than that which preceded it. The probability that the reaction can reverse itself in the latter case is then extremely small and the transition is considered irreversible. A mechanical example of a reversible and irreversible reaction is provided by a comparison of (i) the slow reversible, and (ii) sudden irreversible, expansion of a gas, maintained under isothermal conditions (Fig. 1-1). The reversible expansion may occur through many small consecutive decreases of pressure (solid arrow pathway in Fig. 1-1A), mediated by a piston (Figure 1-1B). The irreversible expansion is initiated by a sudden decrease of the external pressure (multi-arrow pathway, Fig. 1-1A). In the reversible expansion, the system is at equilibrium during the transition, and it is possible to specify the pressure on, and the volume under, the piston during each small step, and at any time, and thus to calculate the work done during the compression of the gas. In the rapid expansion, the pressure on the gas cannot be specified, as it will depend on several parameters including the magnitude of the pressure decrease and the velocity of the moving piston.

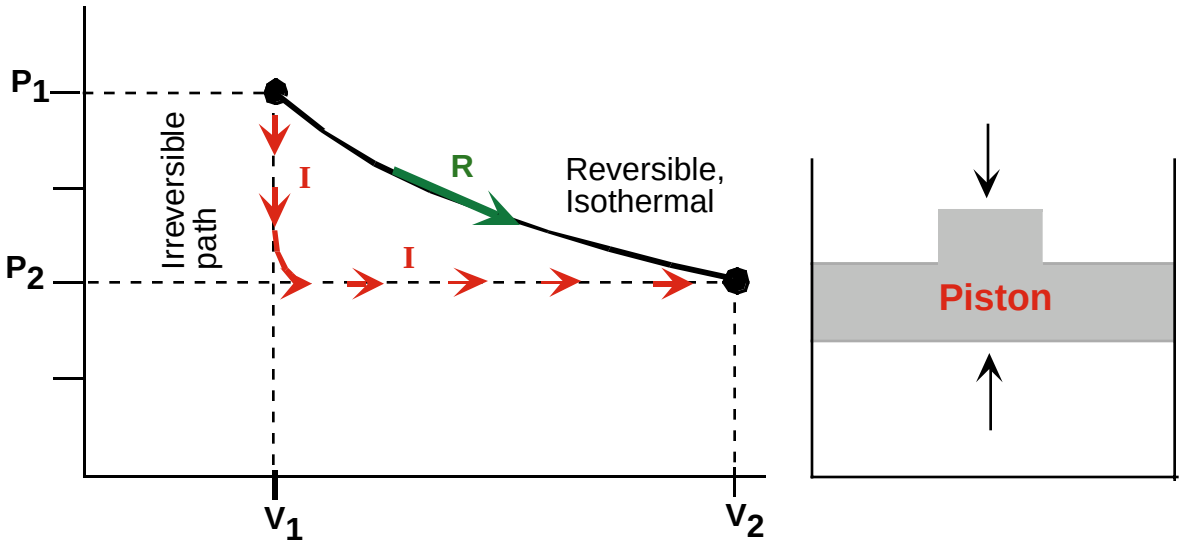


Figure 1-1. (A) Pathway of a reversible (R) and irreversible (I) isothermal (constant temperature) expansion of a gas by: (R) a gradual decrease of pressure from $\mathbf{p_1}$ to $\mathbf{p_2}$, and associated increase of volume from $\mathbf{V_1}$ to $\mathbf{V_2}$. For the I transition, a sudden decrease in the pressure on the piston would be followed by an increase in volume. (B) Closed chamber with piston mediating expansion and compression of an enclosed gas.

When the force of the piston is not in equilibrium with the reaction force from the gas molecules, its effective pressure (**p**) on the gas is always smaller than in the reversible transition ($\mathbf{p_I} < \mathbf{p_R}$). Thus, more work will always be done in the reversible transition, and more heat generated in that which is irreversible. Because the initial and final states of the reversible and irreversible transitions are the same, the sum of the work done and heat energy generated in the two transitions, i. e., the total energy change, is the same.

The pressure-volume work accomplished by expansion (or compression) of a gas at pressure, p, through a volume change, dV, as in the above example, is:

$$dW = -pdV \qquad (1\text{-}4)$$

Note that the convention for pressure-volume work is to carry the negative sign (dW = -pdV); the result of this sign is that when expansion work is done **by** the system so that $\Delta V > 0$ and the final volume is greater than that in the initial state, as in an expansion experiment using the piston of Fig. 1, ΔW and ΔE of the system are negative, and the energy of the system decreases**.** Conversely, when compression work is done **on** the system, $\Delta V < 0$, the final volume is less than the initial, the work term will be positive, and the energy level of the system the energy of the system is increased.

Then, from eqn (1-1), the First Law of Thermodynamics can be written in terms of the energy change due to work done on (or by) the system, and the change in its heat energy, as:

$$dE = dQ - pdV \qquad (1\text{-}5)$$

As noted above, for an ideal or dilute gas, the energy, E, is a function only of its temperature, i. e., E = E(T).

Returning to the isothermal (constant T) expansion experiment shown in Fig. 1-1, the change in energy, $\Delta\mathbf{E}$, between initial and final states = 0 because T is constant. Therefore, the magnitude of the work done and change in heat energy level are equal, i. e., dQ = pdV = - dW, or for non-infinitesimal changes, $\Delta\mathbf{Q} = -\Delta\mathbf{W}$.

Then, for the **reversible** pathway in Fig. 1, in which the pressure is decreased slowly and continuously as the volume increases:

$$\Delta W = -\int_{V_1}^{V_2} pdV = -RT\int_{V_1}^{V_2} \frac{dV}{V} = -RT1n\frac{V_2}{V_1}, \qquad (1\text{-}6)$$

where the equation of state (1) was used to substitute for the pressure, p. Because the work done by the system in the expansion of the gas is negative, the heat change is positive, which means that heat was added to the system to drive the expansion of the gas.

For the **irreversible** pathway in Fig. 1-1, again $\Delta\mathbf{E} = \mathbf{0}$ because the transition of the state function, energy, occurs between initial (p_1, V_1, T) and final (p_2, V_2, T) states that are at the same temperature. Therefore, again, $\Delta\mathbf{Q} = -\Delta\mathbf{W}$. However, unlike the change in the state function, $\Delta\mathbf{E}$, the values of $\Delta\mathbf{W}$ and $\Delta\mathbf{Q}$ are different in the irreversible, compared to the reversible**,** pathway shown in Fig. 1-1A.

Homework problem 1. (a) Show that the work done by the system in the irreversible transition of Fig. 1-1 is $\Delta W = - p_2 (V_2 - V_1)$. (b) By comparing the areas under the functions describing the reversible and irreversible transitions in Fig. 1-1, show that more work is done in the reversible transition.

Homework problem 2. Calculate the work done by a system such as that shown in Fig. 1-1 for (a) reversible and (b) irreversible transitions, for each of which $\Delta E = +20$ kJ, but for which $\Delta Q = +40$ kJ and +30 kJ, respectively.

1-3. Different Kinds of Work.

The different kinds of work, chemical, electrical, and mechanical, respectively, are each a product of an intensive and extensive variable, i.e., $\boldsymbol{\mu}\mathbf{dn}$, $\phi\mathbf{dq}$, and $\mathbf{pdV}$, etc., where μ, ϕ, and p, chemical potential, electrical potential, and pressure, are intrinsic variables. The extrinsic variables, n, q, and V, represent the number of moles of the chemical species and of the electrical charge, respectively, and V is the volume available for the reaction.

1-4. Enthalpy

The state function, **H**, called enthalpy, defined as

$$H \equiv E + pV, \qquad (1\text{-}7)$$

is useful because there is a simple expression for the relation between the enthalpy and heat changes of a reaction under conditions that are common experimentally: i. e., where the only work done is mechanical, p-V work (i.e., no chemical or electrical work), and the pressure is constant. Under these conditions, the change in enthalpy, **dH**, equals the absorption of heat at constant pressure, $\mathbf{dQ_p}$. That is,

$$dH = dQ_p \qquad (1\text{-}8)$$

Homework problem 3. Derive eqn. (1-8) for **dH**, using the expression for the First Law (eqn 1-5), substituting **dW = -pdV** for p-V work, rearranging terms to solve for **dQ**, and substituting for **dH.**

1-5. The Direction of Reactions and Events: The Need for the Second Law

The First Law of Thermodynamics expresses the impossibility of constructing a machine that can create energy. However, the First Law places no limitations on the possibility of transforming energy from one form to another, in particular on the conversion of heat energy to work. The **Second Law of Thermodynamics** exists because: (i) The First Law does not predict the direction of reactions arising from thermal motion, particularly the direction relative to the equilibrium state. (ii) Although heat and mechanical energy are equivalent in their fundamental nature as forms of energy, there are **limitations** on the ability to convert heat energy into work. It is, in fact, impossible to have a physical or chemical process whose only result is: (i) to transform heat extracted from a source at a single temperature into work, or (ii) to transfer heat from an object at a given temperature to one at a higher temperature without performing work and consuming work energy from an external source. The impossibility of the occurrence of these events relates to the everyday experimental fact that heat energy is transferred spontaneously only down a temperature gradient, from a higher to lower temperature. This transfer is in the direction toward the state of **thermal equilibrium**.

1-5.1. The approach to equilibrium.

At temperatures low enough so that heat and molecular motion can be neglected, the equilibrium state of a mechanical system is defined by the minimum in its potential energy, ϕ. ϕ will decrease ($d\phi < 0$) until it reaches a minimum ($d\phi = 0$). In all of these processes, energy is conserved according to the First Law.

However, the First Law of Thermodynamics and the criterion for equilibrium, that potential energy decreases to a minimum, does not explain many intuitively obvious phenomena that occur in the presence of heat and thermal motion. For example, it does not explain the ordinary observable process of evaporation. Considering only the First Law, why should a liquid in an open vessel evaporate, since the binding forces between liquid molecules are much stronger in the liquid than in the vapor state? The answer is that evaporation is a result of the statistical tendency of the molecules to fill all available space. One can calculate that it is extremely unlikely, to the point of an impossibility, that in the absence of constraining forces, a large number of molecules would remain confined to a small fraction of the volume available to them.

Thus, one can calculate that the probability that one molecule would be found in one half of a box (Fig. 1-2) with volume, V, is $(1/2V)/V = 1/2$. The probability that n molecules would all be found in that half is $(1/2)^n$. If n is about 10^{23} for a real box, this event will never be observed. The most likely state is, intuitively, that in which the particles move freely about the box, and at any given time are found to be spread evenly about the box, so that the number of distinct rearrangements of the particles in the box tends toward a maximum. This can be stated in terms of probability. The Statistical Statement of the **Second Law of Thermodynamics** is: (i) *a system of particles or molecules that undergoes an irreversible transition, in the absence of external forces, moves toward a state of* ***greater probability*** or greater ***disorder***; (ii) *In reversible processes,* the *system remains in, or very close to, a state of maximum probability*

1-6. Entropy and the Second Law of Thermodynamics

The **entropy (from Gr., EMBED Equation.3 change, turn) is a state function whose change in a reaction describes the direction of a reaction due to changes in heat input or output and the associated molecular rearrangements. There are two ways to describe the entropy function, S.**

1-6.1. In the **statistical formulation** due to Boltzmann,

$$S = k \ln W, \tag{1-9}$$

where **k** = Boltzmann's constant, which equals R, the gas constant (8.31 J $^{\circ}K^{-1}mol^{-1}$) divided by Avogadro's number, = 1.38 X 10^{-23} J/°K. That is, Boltzmann's constant is the value of the gas constant, R, per molecule or atom. In the Boltzmann formulation, **W** is a probability function associated with a macroscopic (large) state that consists of many molecules. The value of **W** in a given state is determined by the number of arrangements of the molecules of which it is composed, with each arrangement called a "microstate." The larger the number of arrangements or microstates associated with a macrostate, the greater the probability of the macrostate.

Because of the use of the logarithmic function in eqn 8, the product $\mathbf{W_1}$ x $\mathbf{W_2}$ that describes the joint occurrence of two different states characterized separately by independent probabilities, $\mathbf{W_1}$ and $\mathbf{W_2}$, is converted to a sum, $\ln(W_1 x W_2) = \ln W_1 + \ln W_2$. This property of **additivity**, possessed by state functions such as energy, confers the properties of a state function to the **entropy**, **S**, as defined by Boltzmann (eqn 1-9). Then, the change in the entropy, $\Delta\mathbf{S}$, between some final state, "2", and initial state, "1", is

$$\Delta S = S(2) - S(1) = k \ln \frac{W_2}{W_1} \qquad (1\text{-}9a)$$

This formulation of the second law in terms of an entropy function that is determined by the relative probability of molecular distributions emphasizes that irreversible (**I**) transitions are associated with increases of the entropy function, and reversilbe (R) transitions with no change of the entropy. That is,

$$dS_I > 0 \qquad (1\text{-}10a)$$
$$dS_R = 0 \qquad (1\text{-}10b)$$

1-6.1.1 Example of Calculation of Thermodynamic Probability, W:

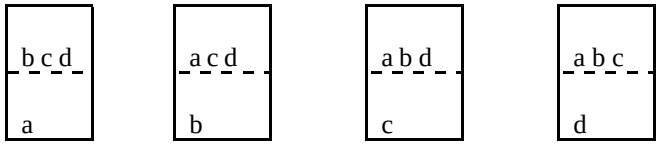
Calculate the number of combinations, $\mathbf{W_{j,k}}$ of four identical molecules, N = 4, contained in a box, on two distinguishable sides, j and k, of the box (Fig. 1-2). The number of independent arrangements $\mathbf{W_{j,k}}$ resulting from placement of $\mathbf{N_j}$ molecules on one side and $\mathbf{N_k}$ on the other, with the two sides of the box having the same volume, each subset with the same number of molecules weighted equally, and no preferred arrangement on each side of each subset, is:

$$W_{j,k} = \frac{N!}{N_j! N_k!} \qquad (1\text{-}11)$$

where **N!** = **N(N – 1)(N – 2)** ...(**1**).

For $N_j = 3$, $N_k = 1$, $\mathbf{W_{3,1}} = \frac{4!}{3!1!} = 4$, as seen by:

A.



The number of rearrangements with $N_j = N_k = 2$ is $\mathbf{W_{4,2}} = \frac{4!}{2!2!} = 6$, as shown below.

B.

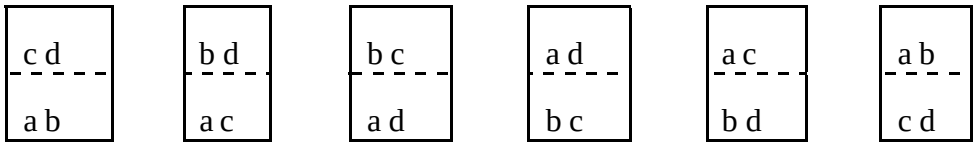


Figure 1-2. Description of possible arrangements of four identical molecules between two sides of a box, with (A) 3 molecules on one side and 1 on the other, and (B) 2 on each side. The third combination would have 4 on one side and none on the other, for which there is only one arrangement, $\mathbf{W_{4,0}} = \frac{4!}{4!} = 1$. The 4 identical molecules are labeled a, b, c, d for purposes of discussion.

Thus, the state or combination with the largest number of different arrangements is the one with equal numbers of molecules on each side of the box. The relative probability of the state $\mathbf{W_{N/2,N/2}}$ with $\mathbf{N_j = N_k}$ becomes increasingly greater with increasing N, and becomes overwhelmingly more probable compared to arrangements with $N_j >> N_k$ or $N_k >> N_j$ when the particle number is large or macroscopic, i.e., ca. 10^{23}.

1-6.2. Second Formulation of the Entropy Change; Relation between Heat and Entropy Change.

The 19th century physicist, **Clausius**, proposed that the differential entropy change, **dS**, is proportional to the heat absorbed,$\mathbf{dQ_{rev}}$, for a reversible process, with **1/T** [(absolute temperature)$^{-1}$] as the constant of proportionality. 1/T is an "integrating factor" for **dQ** that makes the function, **dQ/T,** a **perfect differential,** as required for a state function. [An integrating factor is a coefficient for a function that makes it a perfect (i. e., integrable) differential]. It should be emphasized that a theorem in the mathematics of the integral calculus states that if an integrating factor exists, then it is unique (Chandrasekhar, 1957).

With $\mathbf{dQ_{rev}}$ as the heat energy reversibly absorbed by the system, the entropy change, **dS**, from Clausius, is:

$$dS = \frac{dQ_{rev}}{T} \tag{1-12}$$

and

$$\Delta S = \int_1^2 \frac{dQ_{rev}}{T} = S(2) - S(1) \tag{1-12a}$$

1-6.2.2. Statement of the Second Law of Thermodynamics Using the Clausius Formulation of Entropy.

(i) There is a state function called entropy. (ii) The entropy of any isolated system increases when an irreversible process takes place in the system, $\mathbf{dS_I > 0}$ (eqn. 1-10). In an **isolated system**, when **dQ = 0**, the entropy remains constant (and maximum) in all reversible processes, where the probability of the system configuration remains the same. That is,

$$dS_I > 0, \quad (1\text{-}13a)$$
$$dS_R = 0 \quad (1\text{-}13b)$$

as stated above (Eqns. 1-10a,b) in the Boltzmann formulation.

For irreversible processes in a non-isolated system,

$$dS_I > dQ/T \quad (1\text{-}14)$$

1-6.2.3 Brief Mathematical Summary of Second Law of Thermodynamics

I. Isolated system ($dS \geq 0$)
   A. Reversible: $dS_R = 0$
   B. Irreversible: $dS_I > 0$

II. Non-Isolated System ($dS \geq \frac{dQ}{T}$)
   A. Reversible: $dS_R = \frac{dQ}{T}$
   B. Irreversible: $dS_I > \frac{dQ}{T}$

It can be seen from formulae (1-8) and (1-11 to 1-13) that the units of entropy are cal $^{\circ}K^{-1}$ or Joule $^{\circ}K^{-1}$.

1-6.3. Application of the Second Law to Heat Flow and Temperature Gradients.

Consider an isolated system that consists of two subsystems, "1" and "2." If an amount of heat, dQ, is transferred from subsystem 1 at temperature T_1 to subsystem 2 at temperature T_2, then "2" absorbs dQ and "1" loses dQ. If there are no other configuration changes in the system, and the subsystems are at equilibrium, then

$$dS_{total} = dS_1 + dS_2 = -dQ/T_1 + dQ/T_2 \quad (1\text{-}15a)$$

We know that heat transfer is only possible from system "1" to "2" if $T_1 > T_2$. Therefore, from (1-13) and the temperature inequality,

$$dS_{total} > 0, \quad (1\text{-}15b)$$

and heat transfer can only occur from a higher temperature to a lower temperature system, which is a spontaneous, irreversible process that is readily associated with a positive entropy change.

1-6.4. Relationship between Boltzmann (eqn 1-9) and Clausius (eqn 1-12)

Expressions for the **entropy** change can again be illustrated by the "molecules in the box" problem: Consider particles initially confined to one side of a box by a piston (Fig. 1-3A) or a sliding partition (Fig. 1-3B):

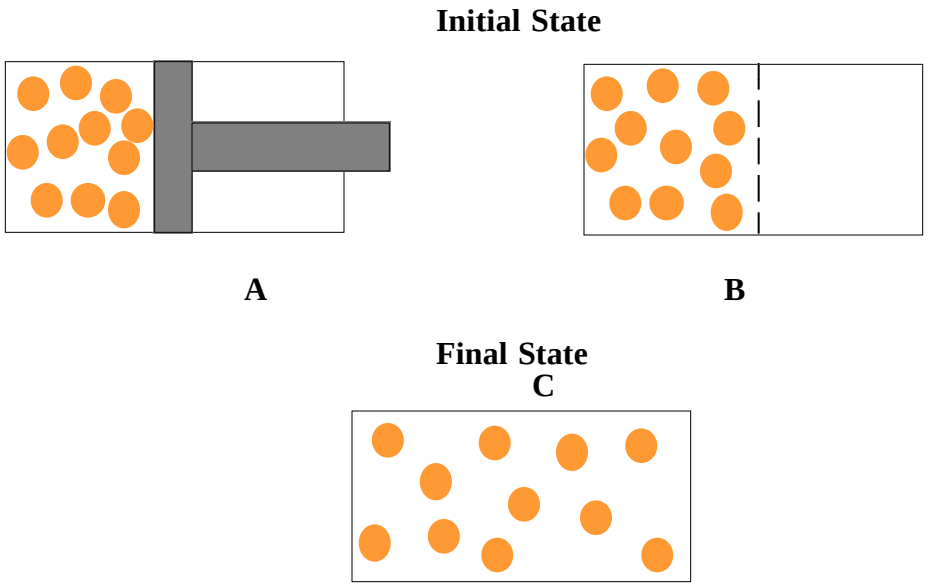


Figure 1-3. Two experiments on an isothermal (constant temperature, dE = 0) expansion of a dilute molecular population in an enclosed vessel. Initial state for (A), a **reversible** expansion through absorption of heat, **dQ**, that exerts a pressure on the piston, performing work, dW = -dQ ≠ 0 on the system, and $\mathbf{dS_{rev} = dQ/T}$; (B) initial state for **irreversible** expansion caused by removal of a partition dividing the chamber, with dW = dQ = dE = 0, and $\mathbf{dS_{irr} > 0;}$ (C) the identical final state that is reached in experiments (A) and (B), with the final volume = twice initial volume. Note: The expansion of the gas defined by (A, B) could be one phase of the complete cycle of a heat engine, sometimes called a Carnot cycle.

There are two paths to the final state, in which molecules fill the box, either by pushing the piston (Fig. 1-3A), or by removing the partition (Fig. 1-3B). The final state achieved through either path is shown in Fig. 1-3C. The entropy change will be shown below to be $\Delta\mathbf{S = Rln2}$ for both transitions, independent of path as is required for a state function.

1-6.4.1. In the first experiment (Figs.1-3A/C), the change in piston position and molecular distribution is a result of the absorption of heat. The temperature is held constant by placing the system in contact with a heat sink. The entropy change from the initial (Fig. 1-3A) to the final (Fig. 1-3C) state is:

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

for an incremental change of added heat. If the energy, **E**, is a function only of temperature, using the First Law, **dE = dQ + dW**, for constant temperature (dE(T) = 0), then dQ = -dW, and from eqn. (4),

$$dS = -\frac{dW}{T} = \frac{pdV}{T}$$

For 1 mole of a dilute molecular population, from eqn (1-1),

$$pV=RT$$

Then, as in the discussion of the reversible transition in Fig. 1-1,

$$dS = \frac{RT}{T}\frac{dV}{V} = R\frac{dV}{V},$$

and

$$\Delta S = R\int_1^2 \frac{dV}{V} = R\,ln\frac{V_2}{V_1} = R\,ln\,2 \qquad (1\text{-}16a)$$

since $V_2=2V_1$ (Fig. 1-3C).

Homework problem 4. Calculate the entropy change for a reversible expansion of 10 mol of an ideal gas from an initial volume of 0.1 L to a final volume of 100 L at 27 ° C.

1-6.4.2. In the second experiment, the partition is removed to allow a redistribution of particles into the entire volume of the box. For a large number of particles (e. g., 1 mole), this redistribution can never be reversed. If there are N identical molecules, and $\mathbf{N_j}$ and $\mathbf{N_k}$ in each compartment, then the relative probability of having a given number N_j and N_k on each side of the partition is proportional to the number of different ways, $\mathbf{W_{j,k}}$, in which this may occur, as written above in eqn (1-10).

$$W_{j,k} = \frac{N!}{N_j!\,N_k!}$$

For the problem in Fig. 1-3A, the initial state has all N particles on one side before removal of the partition. Therefore,

$$W_{j,k} = W_{N,0} = \frac{N!}{N!0!} = W_1 \equiv 1$$

(the symbol "≡" means, "equivalent by definition").

After the partition is removed, there are $N/2$ particles on each side. Then,

$$W_{j,k} = W_{N/2,N/2} = \frac{N!}{\left(\frac{N}{2}!\right)^2}$$

From the Boltzmann expression (eqn.1- 7) for **S**,

$$\Delta S = k\ln\frac{W_2}{W_1} = k\ln\frac{N!}{\left(\frac{N}{2}!\right)^2}$$

since $W_1 = 1$. One obtains a value of the argument of the logarithm by using "Stirling's approximation" for large values of *N*,

$$\ln N! = N\ln N - N$$

Then, substituting in the expression for ΔS,

$$\Delta S = k\left[N\ln N - N - 2\left(\frac{N}{2}\ln\frac{N}{2} - \frac{N}{2}\right)\right]$$
$$= kN\ln\frac{N}{\frac{N}{2}} = kN\ln 2$$

the gas constant $\mathbf{R \equiv kN}$ (i. e., Boltzmann's const x Avogadro's number, the number of molecules in a mole, 6.02 x 10^{23}; then for 1 mole,

$$\Delta S = R\ln 2 \qquad (1\text{-}16b)$$

Thus, the entropy change is the same when calculated from "particle in the box" experiments based on the view of Clausius (section 1-6.4.1; Figs. 1-3A,C) or (ii) that of Boltzmann (section 1-6.4.2, Figs. 1-3B,C). These experiments described:

(i) a reversible isothermal change involving the input of heat, the performance of work by the system to drive the piston, a net dE =0, and $\mathbf{dS_{rev} = dQ/T}$;

(ii) an irreversible isothermal change involving a redistribution of particles with dQ =dW =dE = 0, but $\mathbf{dS_{irr} > dQ/T}$. The conclusion from the above calculations, that ΔS for the two experiments (pathways) connecting the same initial and final states is the same, supports the concept that entropy is a state function.

Homework Problem 5. (a)What is the entropy change associated with ice (18 g) melting to water at 0° C, if the heat of melting is 5.98 kJ/mol. (b) What fractional volume change of this molar quantity of water vapor would yield the same entropy increase?

6.5. Second Law Implies < 100 % Efficiency of Heat Engines. The simplest heat engine in which heat can be converted to net work would involve a four step cycle of reversible changes of pressure, volume, and temperature that is described in the p-V plot (Fig. 1-4) similar to that described above in Fig. 1-1. Fig. 1-4 describes a cycle made of two isothermal (constant temperature) transitions, the A→B expansion at temperature T_2 and C→D compression at temperature T_1, with $T_2 > T_1$. The cycle is completed and the isotherms connected, by the two adiabatic (ΔQ = 0) transitions, the B→C expansion and the D→ A compression.

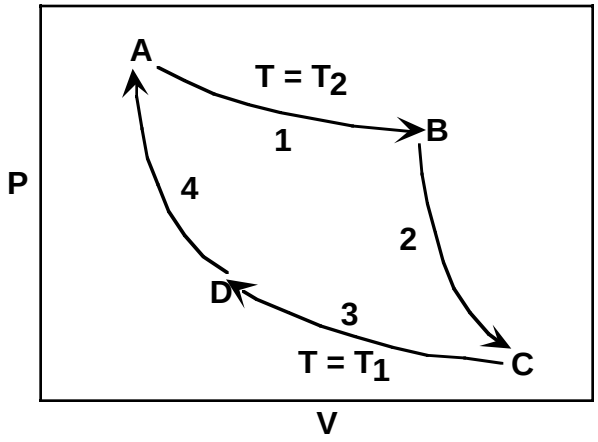


Figure 1-4. P-V-T plot of of a reversible (Carnot) engine. The direction of the engine cycle, as described in the text is along p-V coordinates, A → B → C → D. The A-B and C-D curves describe isotherms at constant temperatures T_2 and T_1, and the adiabatic (ΔQ = 0) transitions, B→ C and D →A, connect the two isotherms.

1-6.5.1. Work Done by System, (I) Isothermal Transitions

In the A→B transition at temperature T_2, from eqn. (1-6) the work done by the system

$$\Delta W_2 = -nRT_2 \ln \frac{V_B}{V_A} \tag{1-17a}$$

and, since $\Delta E = 0$ at constant T, an amount of heat, ΔQ_2, of equal magnitude but opposite sign (for purposes of energy book-keeping) is absorbed by it in this expansion. Similarly, in the compressive C→ D transition, work done on the system,

$$\Delta W_1 = -nRT_1 \ln \frac{V_D}{V_C} \tag{1-17b}$$

and an equal amount of heat, ΔQ_1, is released by it.

1-6.5.2. (II) Adiabatic Transitions, B→C and D→ A: The critical features of the two adiabatic transitions, B→C and D→A, are: (i) $\Delta Q = 0$, (ii) the work done in the B→C and D→A transitions is equal and opposite and cancels out because, since $\Delta Q = 0$, $\Delta E_{BC} = -\Delta W_{BC} = -\Delta E_{DA} = \Delta W_{DA}$; (iii) the ratios of the volumes linking the two adiabatic transitions (Fig. 1-4) are equal,

i. e., $V_C/V_B = V_D/V_A$, and $V_C/V_D = V_B/V_A$ (1-18)

Then, the net work done by the system,

$$\Delta W_{done} = \Delta W_2 + \Delta W_1 = -nRT_2 \ln \frac{V_B}{V_A} - nRT_1 \ln \frac{V_D}{V_C}$$

$$= -nRT_2 \ln \frac{V_B}{V_A} + nRT_1 \ln \frac{V_C}{V_D}, \text{ and from (1-18),}$$

$$= -nR \ln \frac{V_B}{V_A}(T_2 - T_1) \tag{1-19a}$$

Because $\Delta Q_2 = -\Delta W_2$ the heat absorbed is (1-17a):

$$\Delta Q_{abs} = \Delta Q_2 = nRT_2 \ln \frac{V_B}{V_A} \tag{1-19b}$$

The efficiency, ε, of this ideal "Carnot" heat engine is defined as the magnitude of the net work done, ΔW_{done} (formula 1-19a), divided by the heat absorbed, ΔQ_{abs} (formula 1-19b), and

$$\varepsilon = \left(1 - \frac{T_1}{T_2}\right); \tag{1-19c}$$

Note: because $T_2 > T_1$, $\varepsilon < 100$ %. This is a result of the work that has to be done on the system at the lower temperature, T_1, to return the engine to its original starting position.

1-6.5.3. The Entropy Change, ΔS, for the Carnot Heat Engine Cycle Equals Zero. The result (1-19c) for the efficiency < 100 % for the ideal (Carnot) engine could also have been derived from assuming that the entropy change for the whole cycle must be zero, $\Delta S_{cycle} = 0$, since it returns to its starting position and entropy is a state function. As an alternative approach, we will use the information given above for the heat changes in the steps of the cycle to prove that $\Delta S_{cycle} = 0$.

The entropy change for one complete cycle of the heat engine described in Fig. 1-4 is:

$$\Delta S_{cycle} = \Delta S_{AB} + \Delta S_{BC} + \Delta S_{CD} + \Delta S_{DA} \tag{1-20a}$$

Because $\Delta Q_{BC} = \Delta Q_{DA} = 0$ for the adiabatic transitions, $\Delta S_{BC} = \Delta S_{DA} = 0$, and

$$\Delta S_{cycle} = \Delta S_{AB} + \Delta S_{CD} = \Delta Q_{AB}/T_2 + \Delta Q_{CD}/T_1 \quad . \qquad (1\text{-}20b)$$

From the first law (eqn 1-2), and dE = 0 for constant T, the heat absorbed in the A→B isothermic transition at temperature T_2, $\Delta Q_{AB} = -\Delta W_2 = nRT_2 \ln(V_B/V_A)$, and the heat released in the C→ D isotherm at $T_1 = \Delta Q_{CD} = nRT_1 \ln(V_D/V_C) = -nRT_1 \ln(V_B/V_A)$. Therefore,

$$\Delta S_{cycle} = \Delta Q_{AB}/T_2 + \Delta Q_{CD}/T_1 = nR\ln(V_B/V_A) - nR\ln(V_B/V_A) = 0 \qquad (1\text{-}20c)$$

Thus, the heat engine problem demonstrates that the factor (1/T) is unique in its ability to make a state function out of a function proportional to $\Delta\mathbf{Q}$ or **dQ.**

1-7. Free Energy.

The entropy inequality $\mathbf{dS \geq dQ_{rev}/T}$ specifies the direction of reactions in which only the heat change and temperature are specified. Inequalities specifying the direction of chemical reactions in non-isolated systems are useful. From the first law, dE = dQ + dW, where dW can include chemical and electrical work as well as that arising from pressure–volume changes.

If $dW = dW_{pV} = -pdV$ for pressure–volume work, then,

$$dE = dQ - pdV$$
$$dQ = dE + pdV$$
$$dQ_p = d(E + pV)_p$$

at constant p, where $dQ_p = d(E + pV)_p \equiv dH$, the heat change at constant pressure (eqn. 1-8).

From the second law, $dS \geq dQ/T$**,** and by substitution,

$$TdS \geq dH,$$

the statement of the 2nd law for a non-isolated system. Rearranging terms,

$$dH - TdS \leq 0$$

Rewriting,

$$d(H - TS)_{p,T} \leq 0$$

or defining the Gibbs Free Energy, G, is defined as $\mathbf{G \equiv H - TS}$,

$$dG_{p,T} \equiv dH - TdS \qquad (1\text{-}21)$$

The second law of thermodynamics is then reformulated to describe reactions at constant temperature and pressure:

$$dG_{p,T} \leq 0. \qquad (1\text{-}22)$$

The reaction is at equilibrium and reversible if

$$dG_{p,T} = 0 \qquad (1\text{-}22a)$$

This is illustrated in a graph of the free energy, **G**, of an isolated spontaneously reacting system, e. g., reactant "A" ⇔ product "B", as a function of the quotient ($q = n_A/n_B$) of the number of moles of A, to those of B (Fig. 1-5). At equilibrium, the free energy, G, is at a minimum and $\frac{dG}{dq} = 0$, i. e., the slope of G as a function of q is zero at the position of the minimum of G, for

small displacements of G in either direction on the q-axis. At all other points on this graph away from equilibrium, the second law says that the tendency of G will be to decrease, i. e., $dG < 0$, to a minimum value.

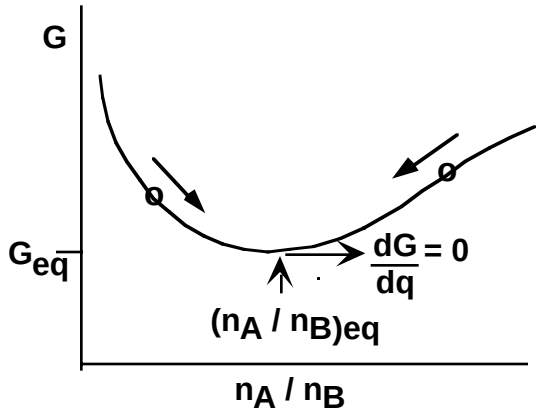


Fig. 1-5. Free energy, G, of the reaction "A" ⇔ "B", as a function of the quotient, q, of the molar quantity, n_A and n_B, of compounds A and B. Note that the equilibrium value of G for the system, $G_{eq} = G_{min}$. The directions toward equilibrium, marked by arrows, of reactions with $G > G_{min}$, at coordinates $(G, n_A/n_B)$ marked by open circles, are shown.

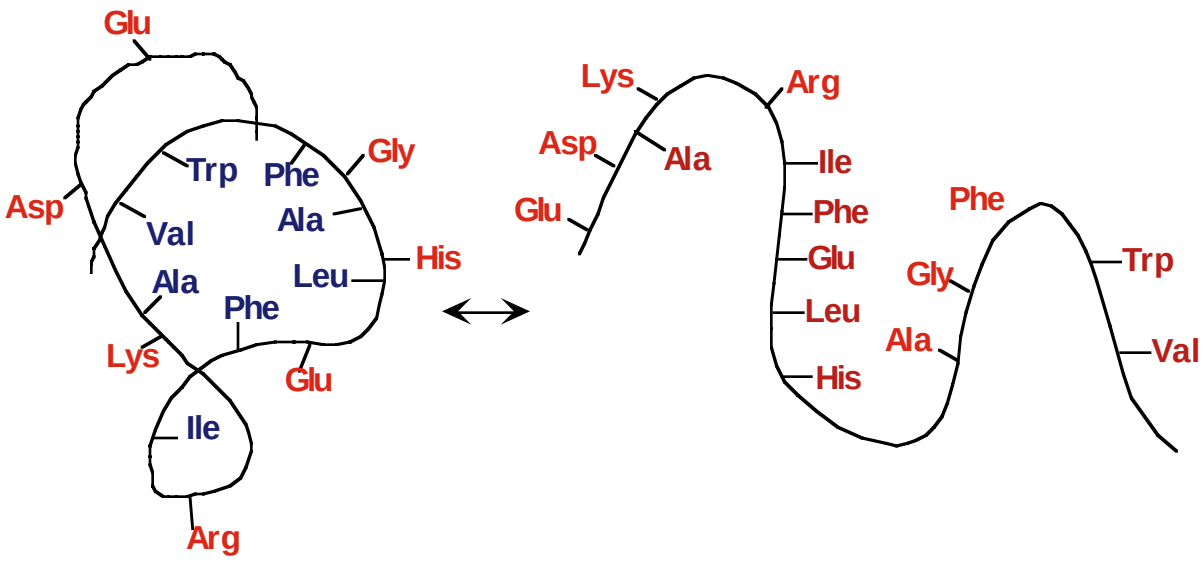
1-7.1. Separation of ΔG into ΔH and $T\Delta S$ terms. In biochemical and chemical systems that do not perform any mechanical work, it is useful to consider the nature of the contributions of the $\Delta \mathbf{H}$ and $\mathbf{T\Delta S}$ components to $\Delta \mathbf{G} = \Delta \mathbf{H} - \mathbf{T\Delta S}$ for reactions in which $\Delta \mathbf{G} \neq \mathbf{0}$. The ΔH term includes the components of the binding energy and intra- and inter-molecular interactions; ΔS includes effect of molecular rearrangement. At low temperatures, statistical effects in the contribution from ΔS will have relatively little weight and the ΔH term will be a major determinant of the $\Delta \mathbf{G}$. However, at high temperatures, the direction of the reaction is dominated by the $T\Delta S$ term and the effects of statistical rearrangements, so that the sign of ΔG will tend to be negative.

1-7.1.1. For example, protein denaturation in aqueous solution tends to occur at somewhat elevated temperatures (≥ 60°C) where the $T\Delta S$ term that expresses the increase in configurational space occupied by the protein in the denatured state carries more weight and offsets the large positive ΔH required to break the hydrogen bonds and favorable hydrophobic interactions that are responsible for favorable packing of the protein in the native state. Thus, at high temperatures, ΔG tends to assume more negative values, favoring a high population of the denatured state (Fig. 1-6A). The "melting" temperature, T_m, of the protein in a calorimetry experiment (Fig. 1-6B), will be defined by the temperature of the equilibrium, i. e., $\Delta \mathbf{G} = \mathbf{0}$, between the native and denatured states, where there can be a reversible transition between them. Therefore,

$$T_m = \frac{\Delta H}{\Delta S} \tag{1-23}$$

For a discussion of the determination of ΔH and ΔS from differential scanning calorimetry, see V. Bloomfield, Thermodynamics and Statistical Thermodynamics, section 1.7.

(A)



(B)

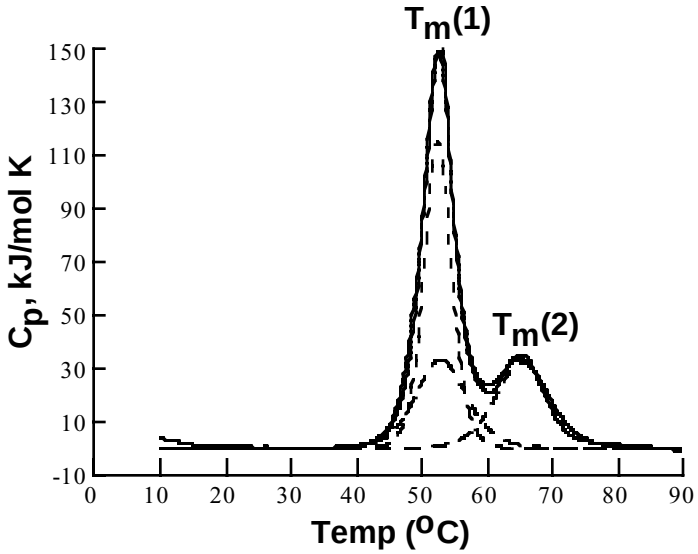


Fig.1-6. Protein denaturation. (A) Schematic diagram of the initial and final final states of a native → denatured transition; (B) Endothermic transitions associated with thermal denaturation of the 3 functional domains of colicin E1 modified from (Griko et al., 2000); deconvolution of the major endotherm into two melting transitions is shown. A representative value of ΔS^o for protein denaturation is ~100 cal/mol-˚K.

Homework Problem 6. If the free energy and enthalpy of denaturation of myoglobin are +13.6 kcal/mol and +42 kcal/mol at 25°C, (a) calculate the associated entropy change; (b) discuss briefly the reason(s) that the free energy for transfer from the protein core to the bulk aqueous

phase of the side chains of “hydrophobic” amino acids, for example, valine, leucine, and isoleucine is positive.

1-7.2. Concentration Dependence of the Free Energy.

An expression for the dependence of the free energy, G, on the concentrations of reactants and products in solution can be obtained by using the identity of the equations of state for ideal (non-interacting) gases and dilute (non-interacting) solutions. There is an identical dependence of (i) the free energy of an ideal gas at constant temperature on its partial pressure, $dG_i = Vdp_i$, and (ii) the partial free energy, d**G** or dμ, of a solute "i" in aqueous solution on its concentration, $dG = Vdc_i$.

Because G ≡ H - TS ≡ E + pV – TS, it is clear that G is a function of pressure. Then, from the differential expression for a reversible non-isolated transition (**dQ** = **TdS**) involving only p-V work:

$$\mathbf{dG = dE + pdV + Vdp - TdS - SdT},$$

and $dE = dQ - pdV$; cancelling terms,

$$dG = Vdp - SdT;\ \text{p, T not constant.}$$

showing that G is a function of p and T, i. e., G = f(p,T).

In an isothermal (dT = 0) transition, **dG = Vdp;** (1-24a)
and by analogy with dilute solutions

$$\mathbf{dG = Vdc\ \ (at\ constant\ p)} \qquad (1\text{-}24b)$$

Integration of expression (1-24b) between initial state 1 and final state 2 for n moles yields:

$$\Delta G = \int_1^2 dG = G(2) - G(1) = n\int_1^2 Vdc = nRT\int_1^2 \frac{dc}{c} = nRT\,ln\frac{c_2}{c_1} \qquad (1\text{-}25)$$

Then, from (1-25), with G(1) = G^o and G(2) = G,

$$G - G^o = nRT\,ln\frac{\{c\}}{\{c^o\}}, \text{ and}$$

$$G = G^o + nRT\ln\frac{\{c\}}{\{c^o\}} = G^o + RT\ln\{c\}^n \qquad \text{when } C^o = 1M \qquad (1\text{-}26)$$

where G(2) = G, and G(1) = is the standard free energy, c^o is the concentration under standard conditions (c^o = 1M), and the brackets refer to partial activities in the event that the concentration, *c*, is not dilute.

1-7.3. Dependence of ΔG on concentrations of reactants and products.

Considering the reaction,

$$n_A A + n_B B \rightarrow n_C C + n_D D,$$

where the coefficients, n_A, n_B, etc., refer to the number of moles of compounds “A, B, C, and D” that participate in the reaction, the free energy, G_A, of reactant “A” at concentration, c_A, is:

$$\Delta G^o + RT\ln\{c_A\}^{n_A};\ \ c_A^o = 1\,M$$

and combining the free energy expressions for each compound, yields the total free energy change:

$$\Delta G = \Delta G^o + RT \ln \frac{\Pi(products)}{\Pi(reactants)} \quad (1\text{-}27a)$$

$$\Delta G = \Delta G^o + RT \ln \frac{\{c_C\}^{n_C}\{c_D\}^{n_D}}{\{c_A\}^{n_A}\{c_B\}^{n_B}} \quad (1\text{-}27b)$$

where $\Delta G = (G_C + G_D) - (G_A + G_B)$, **and** $\Delta G^o = (G_C^o + G_D^o) - (G_A^o + G_B^o)$, and the quantities, c_A, c_B, etc., in curly brackets are the concentrations or activities of compounds A, B, C, and D. At equilibrium, $\Delta G = 0$, and therefore

$$\Delta G^o = -RT \ln \left[\frac{\{c_C\}^{n_C}\{c_D\}^{n_D}}{\{c_A\}^{n_A}\{c_B\}^{n_B}} \right]_{eq},$$

where the subscript, "*eq*," refers to evaluation of the concentrations or activities at equilibrium. The quotient of products to reactants evaluated at equilibrium is defined as the equilibrium constant K_{eq} Then,

$$\Delta G^o = -RT \ln K_{eq} = -2.3RT \log K_{eq}; \; \Delta G^o < 0 \text{ when } K_{eq} > 1 \quad (1\text{-}28)$$

and $\Delta G^o > 0$ when $K_{eq} < 1$.

1-7.4. Using ΔG^o to predict reaction direction near its mid-point

ΔG^o is the free energy change for conversion of a standard amount of reactant [1 molar for solutes, 1 atmosphere of pressure [1.013 x 10^5 Pascal (Pa)] for gases, at 25°C] to the same standard amount of product. The complete conversion of a standard amount of reactant to product may be regarded as involving (i) a conversion of the reactant to an equilibrium mixture (with negative ΔG because the reaction goes toward equilibrium), and then (ii) conversion of the equilibrium mixture to products (with a + ΔG since the reaction is going away from equilibrium). If $K_{eq} > 1$, then the negative ΔG step outweighs that with positive ΔG.

In addition, the sign of ΔG^o can indicate the direction of the reaction at or near its midpoint, where the concentration of products, "p", equals that of the reactants, "r" [i.e., $[\pi_i(p_i)/ [\pi_i(r_i)] = 1$, $1n [\pi_i(p_i)/ [\pi_i(r_i)] = 1n\ 1 = 0$, and $\Delta G = \Delta G^o$ in Eq. (1-20)]. However, when the **activities of reactants and products** are not equal, it is the **sign of ΔG, and not that of ΔG^o that determines** the **direction of the reaction**.

Homework problem 7. (a) Applying the relation, $\Delta G^0 = -2.3RT \log_{10} K_{eq}$, to acid-base reactions, $AH \rightarrow A^- + H^+$, for which the $pK = -\log_{10} K_{eq}$, and $\Delta G^o = 2.3\ RT \bullet (pK)$. What is the difference in the ΔG^o at 25°C for an acid with a pK of (i) 4 and (ii) 7? (b) What is the change in ΔG^0 of a reaction that accompanies a 100-fold increase in K_{eq} at 25°C? (c) What is the effect on the K_{eq} of a decrease of 1.36 kcal/mol in the ΔG^0 at the same temperature?

Homework problem 8. Given the reaction, r(reactant) ↔ p(product) proceeding at 25°C with a ΔG^o = -1.36 kcal/mol for the direction r → p, in what direction does the reaction tend to proceed when it operates between concentrations or activities of {r} and {p} that are: (a) 0. 91 and 9.1 μM; (b) 5 and 5 μM; (c) 9.1 and 0.91 μM; (d) 9.91 and 0.09 μM?

Homework problem 9. The hydrophobic nature of amino acids has been determined from the following kind of experiment: The ΔH^o and ΔG^o for the transfer of pure benzene to H_2O at 18°C is 0 and 19.4 kJ/mol. What is the ΔS^o for this reaction? Explain the sign of the ΔS^o.

1-7.5 Additivity or Lack of Additivity of ΔG, ΔH, and ΔS.

Thermodynamic additivity of changes in free energy, enthalpy, and entropy is implicit in the nature of these quantities as state functions. However, a requirement of additivity is that the processes for which ΔG, ΔH, or ΔS are being added must be independent. Thus, if two components, A and B, contribute to a net process, the free energy change, $\Delta G = \Delta G_A + \Delta G_B$, is a valid expression only if components A and B are independent (Dill, 1997). It is, in fact, experimentally difficult to establish this requirement. One example cited by (Dill, 1997) is the transfer free energy, ΔG_g, of glycine from oil to water. It has been found that ΔG_g = 1.270 or 0.895 kcal/mol if ΔG_g is calculated, respectively, as the difference in the transfer ΔG of (i) the tripeptide minus the dipeptide, or (ii) the dipeptide minus glycine. The explanation of this difference is that the environment around the different Gly peptides is variable. Such experimental variability, in homogeneity, sometimes arising from conformational change, implies that additivity of thermodynamic state function parameters cannot be taken for granted.

1-8. Other Kinds of Work

The **free energy** change, **dG**, in a reversible transition at constant (p,T) is equal to the work done exclusive of p-V work. Other kinds of work commonly encountered in biochemistry and biophysics are chemical, electrical, and electrochemical, denoted by dW_{ce}. It can be shown by substituting (dW –pdV) into the expression for the First Law,

$$dE = dQ + dW = dQ + (dW_{ce} - pdV)$$

into the expression for

$dG = d(H\text{-}TS)]_{p,T} = [dE + pdV + Vdp - TdS - SdT]_{p,T} = dQ + (dW_{c,e} - pdV) + pdV - TdS$, that

$$dG_{p,T} = dW_{ce}. \qquad (1\text{-}29)$$

When it is specified that this work is done in a reversible transition, the work done is the maximum that can be done under the conditions of this transition. As discussed above, when dW is negative and positive, respectively, work is done *by* or *on* the system and the free energy of the system will correspondingly decrease or increase.

A logical extension of the equality/inequality relationships discussed above (Eqns. 1-22, 1-22a) is that $\Delta G = \Delta W_{ce} = 0$ at equilibrium for dW_{ce} work. The physical-chemical formalism for dW_{ce} also requires that $dG < 0$ for irreversible or spontaneous reactions.

1-8.1. Electrical, Chemical Work

Electrical work is carried out by moving a charge (units, coulombs, "C") across a potential, **ΔE or** $\Delta\psi$, in volts). The charge is equal to the Faraday constant (molar value of the electronic charge (9.6487×10^4 coulombs/mole, or 23.06 kcal mol^{-1} V^{-1})), multiplied by the valence of the charge, *z*, the sign of the charge, *e* (±1), and the number, *n*, of moles transferred. Then,

$$\Delta G_{elec} = \Delta W_{elec} = n_i zeF \bullet \Delta E \qquad (1\text{-}30)$$

where *ze* is the charge on the ionic species, and $\Delta\mathbf{E}$ is the electrical, or oxidation-reduction potential difference, in volts. The other common mode of electrical energy storage is in the trans–membrane electrical potential difference, $\Delta\psi$, which is typically approximately 100 mV. Then, for electrical work, the free energy level, G_{elec}, corresponding to a potential **E** or ψ, is,

$$G_{elec} = n_i zeF \bullet E \tag{1-31a}$$

and,
$$G_{elec} = n_i zeF \bullet \Psi \tag{1-31b}$$

The differential free energy change corresponding to a small change, $\mathbf{dn_i}$, in n_i at an electrical potential of **E** or ψ, is:

$$dG_{elec} = dn_i zeF \bullet E \tag{1-32a}$$

and,
$$dG_{elec} = dn_i zeF \bullet \Psi \tag{1-32b}$$

1-8.2. Chemical Work and Chemical Potential

The chemical potential, μ_i, of compound *i* is the free energy per mole,

$$\left[\frac{\partial G}{\partial n_i}\right]_{T,p,n_j \neq n_i} \equiv \mu_i \tag{1-33}$$

The symbol ∂ stands for partial derivative, the derivative with respect to n_i with *T, p,* with the other $n_j \neq n_i$ held constant.

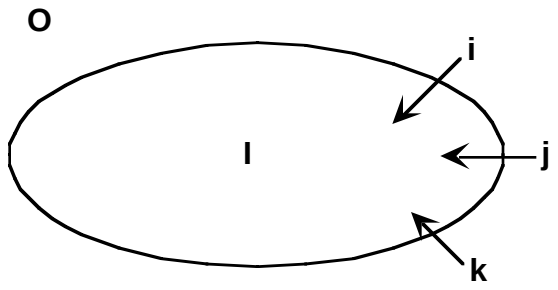


Figure 1-7. Transport of solutes *i, j, k* from the outside compartment **O** to the inside, **I**.

Problems concerning the movement of solutes, dn_i, across membrane boundaries define the field of **transport**. When the solute is accumulated against its concentration gradient, one speaks of **active transport** because this process requires energy. The chemical potential, μ, is an important parameter for transport problems because it represents the $\Delta\mathbf{G}$ per mole of component transported in or out of the membrane-enclosed system.

Consider a two-component system [outside, **O**; inside (e.g., cytoplasm), **I**]. Then, the free energy, $G = G^I + G^O$. If $\mathbf{dn_i}$, $\mathbf{dn_j}$, $\mathbf{dn_k}$ moles of components **i, j, and k** are transferred from the solution outside, **O**, to the inside of the cell, **I** (Fig. 1-7), then

$dn^{\mathrm{I}} = dn_i$ = moles of *i* gained by phase **I**, (1-34a)

$dn_j^{\mathrm{I}} = dn_j$ = moles of *j* gained by phase **I,** etc.

$dn_i^{\mathrm{O}} = -dn_i$ = moles of *i* lost by phase **O**, (1-34b)

$dn_j^{\mathrm{O}} = -dn_j$ = moles of *j* lost by phase **O**, etc.

The change in free energy of the system, $dG_{i,chem}$ for the chemical work done on it through movement of only dn_i is:

$$dG_{i,chem} = dG^{\mathrm{O}} + dG^{\mathrm{I}} \tag{1-35}$$

$$= \left(\frac{\partial G}{\partial n_{\mathrm{i}}}\right)^{\mathrm{O}} \cdot dn_{\mathrm{i}}{}^{\mathrm{O}} + \left(\frac{\partial G}{\partial n_i}\right)^{\mathrm{I}} \cdot dn_i{}^{\mathrm{I}}$$

$$= \left[\left(\frac{\partial G}{\partial n_{\mathrm{i}}}\right)^{\mathrm{I}} - \left(\frac{\partial G}{\partial n_i}\right)^{\mathrm{O}}\right] dn_{\mathrm{i}}, \tag{1-36a}$$

From Eqns. 1-33a, b above,

$$dG_i = (\mu_{\mathrm{i}}{}^{\mathrm{I}} - \mu_i{}^{\mathrm{O}}) \cdot dn_i,$$

from the definition given above for chemical potential. Thus,

$$dG_{\mathrm{i}(chem)} = \sum \mu_i \cdot dn_i, \tag{1-36b}$$

where the chemical potential of component *i* is summed $\left(\sum\ \right)$ over the compartments **O** and **I.**

For net transport, $\mu_i^{\mathrm{O}} \neq \mu_i^{\mathrm{I}}$. The reaction proceeds then in the "forward" (inward) direction if $\mu_i^{\mathrm{O}} > \mu_i^{\mathrm{I}}$. This corresponds to the concentration of component "I" being greater in the outside compared to the inside component. Therefore, this spontaneous flow of solute from higher to lower concentration, like that of heat from higher to lower temperature is characterized by dG < 0.

At equilibrium, $\mu_i^{\mathrm{O}} = \mu_i^{\mathrm{I}}$ and $dG_i = 0$.

In general, the free energy change arising from movement of dn_i at chemical potential μ_i is:

$$dG_i = \mu_i dn_i \tag{1-36c}$$

The combined free energy change for electrical (Eqns. 1-32a,b) and chemical work (Eqn. 1-36c) arising from movement of dn_i from chemical potential μ_i and electrical potential, E (or Ψ) is

$$dG_i = dn_i zeF \bullet E + \mu_i dn_i \tag{1-37}$$

Dropping the subscript *i*, the sum of the electrical (*e*) and chemical (*c*) terms in the free energy is the electro-chemical (*ec*) free energy dG_{ec}.

$$dG_{ec} = \mu \bullet dn + zeFE \bullet dn = \tilde{\mu} \bullet dn \tag{1-38}$$

where the electrochemical potential, $\tilde{\mu} = \mu + zeF \bullet E = dG_{ec}/dn$.

The significance of dG_{ec} is that the accumulation or escaping tendency of an ion is determined by both chemical and electrical potential gradients.

1-8.3 Electrochemical Work; The Electrochemical Potential.

The dependence of the chemical potential, μ, on concentration has the same form as does the free energy, G (Eqn. 1-26):

$$\mu = \mu^o + RT\ln\frac{\{c\}}{\{c^o\}} = \mu^o + 2.3RT log_{10}\frac{\{c\}}{\{c^o\}} \quad (1\text{-}39)$$

where μ^o, c^o are the chemical potential and concentration (1M) under standard conditions.

Then, from (1-37), the complete expression for the electrochemical potential of a charged solute is

$$\tilde{\mu} = \mu^o + RT\ln\frac{\{c\}}{\{c^o\}} + zeF \bullet \left(E - E^o\right), \quad (1\text{-}40)$$

where E^o is the electrical potential in the standard state.

As the difference in chemical potential is a measure of the escaping tendency of uncharged molecules due to a concentration gradient, the electrochemical potential describes this tendency of charged molecules due to both a concentration gradient and an electrical potential. When applied to H^+ or Na^+ gradients across energy transducing membranes, Eqn 1-38 underlies the **chemiosmotic** framework for energy coupling (Mitchell, 1966)

1-8.4. Thermodynamics of Ion Gradients

Consider the application of the electrochemical potential (eqn 1-40) to proton (H^+) translocation driven by a membrane potential , with the protonic charge, ze = +1,

$$\tilde{\mu} = \mu^o + RT \bullet \ln\{H^+\} + F \bullet \Psi \quad (1\text{-}41)$$

omitting the standard concentration, c_o, and standard or reference potential then,

$$\tilde{\mu} = \mu^o + 2.3RT \bullet \log_{10}\{H^+\} + F \bullet \Psi \quad (1\text{-}42)$$

$$\tilde{\mu} = \mu^o - 2.3RT \bullet pH + F \bullet \Psi \quad (1\text{-}42a)$$

since pH ≡ - log $\{H^+\}$.

The gradient of the electrochemical potential for protons, $\Delta\tilde{\mu}_{H^+}$, from one side of a membrane, for example, initial state, "outside" ≡ "O" to the final, "inside," ≡ "I", is:

$$\Delta\tilde{\mu}_{H^+}[(\text{Final} - \text{Initial})] = RT \bullet \ln\frac{H_I^+}{H_O^+} + F(\Psi_I - \Psi_O), \text{ in kJ/mol, or} \quad (1\text{-}43)$$

$$\Delta\tilde{\mu}_{H^+} = 2.3RT \bullet \log_{10}\frac{H_I^+}{H_O^+} + F(\Psi_I - \Psi_O) \quad (1\text{-}43a)$$

$$= 5.69 \bullet (pH_0 - pH_I) + F(\Psi_I - \Psi_O) \text{ at 25 °C,} \quad (1\text{-}43b)$$

where 2.3RT = 5.69 kJ/mol at 25°C, and (-log H^+) = pH. Then,

$$\Delta\tilde{\mu}_{H^+} = F \cdot \Delta\psi - 2.3RT \cdot \Delta pH \quad (1\text{-}44)$$

where $\Delta\Psi = \Psi_I - \Psi_O$ and $\Delta pH = pH_I - pH_O$.

Eqn (1-44) is the fundamental formula that describes energy transduction in energy-transducing proton-translocating membrane proteins in the 'chemiosmotic" framework (Mitchell,

1966). In this framework, energy is stored in (i) the pH gradient that is generated by electron transport or ATP hydrolysis, coupled to the pumping of protons across the membrane, and (ii) the trans-membrane electrical potential arising from electrically uncompensated transfer of electrons or protons across the membrane. It is assumed in Eqn (1-44) that the membrane is impermeable to the movement of protons except through pathways mediated by the energy transducing membrane proteins (i. e., electron transport, ATP synthase/ATPase, transport proteins).

Some organisms may alternatively utilize sodium to generate the trans-membrane electrochemical potential. Thus, for an analogous electrochemical gradient involving the translocation of Na^+ from its initial, "I", to its final state, "F,"

$$\Delta\tilde{\mu}_{Na^+} = F \cdot \Delta\psi + 2.3RT \cdot \log_{10} \frac{(Na^+)_F}{(Na^+)_I}, \qquad (1\text{-}45)$$

with $\Delta\psi = (\Psi_F - \psi_I)$.

1-8.4.1. Proton-Motive Force.

Using the same convention and notation for defining $\Delta\psi$ and ΔpH that form the basis for eqns (1-44, 1-45), the term "proton-motive force", Δp, the "pmf", is defined as

$$\Delta p = \frac{\Delta\tilde{\mu}_{H^+}}{F} = \Delta\Psi - \frac{2.3RT}{F} \cdot \Delta p\text{H}, \qquad (1\text{-}46)$$

with units of electrical potential, in volts or millivolts; substituting 59 mV for 2.3 RT/F evaluated at 25°C.

$$\Delta p = \Delta\Psi - 59 \cdot \Delta p\text{H} \text{ in millivolts} \qquad (1\text{-}46a)$$

The utility of formulation (1-46) for chemiosmotic energy transduction is that it makes clear that every change of one pH unit in the ΔpH is equivalent to a 59 mV change in the membrane potential, $\Delta\Psi$. On the other hand, it should be noted that the term "pmf" is a bioenergetics jargon incorrectly describes the dimensionality of expressions (1-44, 1-45).

1-8.5. Formation of $\Delta\tilde{\mu}_{H^+}$ Across the Inner Membranes of Bacteria and Mitochondria, and Chloroplast Thylakoid Membranes

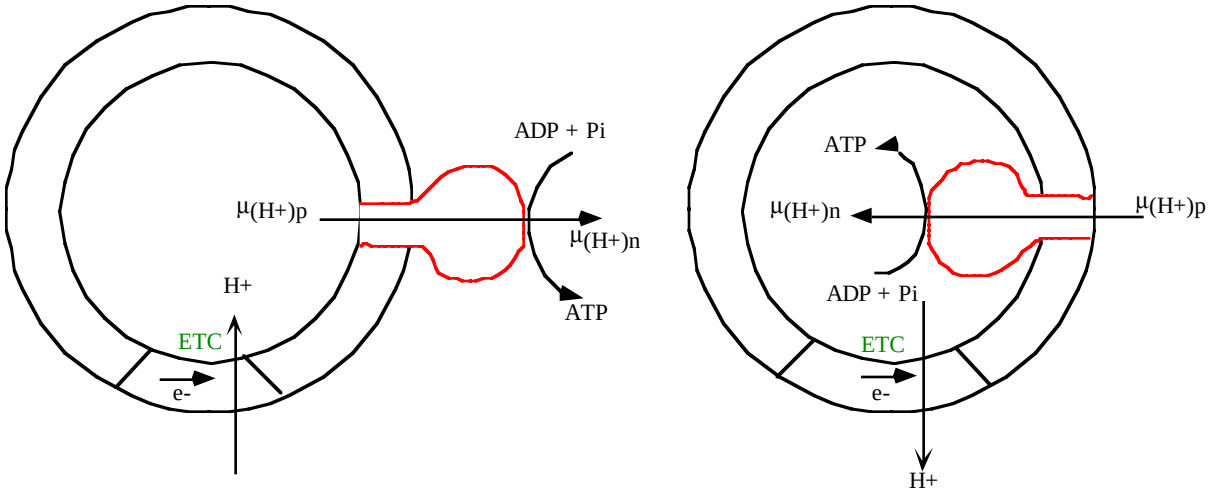
1. **Chloroplasts** (Fig. 1-8A). The $\Delta\mu_{H+}$ formed by H^+ movement driven by photosynthetic electron transport from the chloroplast stroma on the outside of the membrane (initial state) to the internal compartment of the lumen (final state), so that the designation of initial and final states for the formation of the $\Delta\tilde{\mu}_{H^+}$ is:

$$\Delta\tilde{\mu}_{H^+} = F\Delta\psi - 2.3RT \cdot \Delta p\text{H} \qquad (1\text{-}47)$$

$$\Delta\psi = \psi_{in} - \psi_{out},$$

$$\Delta p\text{H} = p\text{H}_{in} - p\text{H}_{out},$$

and $$\Delta\tilde{\mu}_{H^+} = (\tilde{\mu}_{H^+})_{in} - (\tilde{\mu}_{H^+})_{out}$$



(A) (B)

Figure 1-8. Vectorial proton pumping by the electron transport chain ("out" → "in") in (A) chloroplast thylakoid, sub-mitochondrial, and chromatophore (sub- photosynthetic bacterial) membranes; (B)"in" → "out" proton transfer in intact mitochondria and bacterial cells. H^+ flow through ATP synthase is also shown. ETC, electron transfer chain

2. Bacterial Cells and Mitochondria. In contrast to chloroplast thylakoid membranes, H^+ movement occurs from "in" to "out" in the energization of the inner mitochondrial or bacterial cytoplasmic membrane by electron transport or ATP hydrolysis. Then, it is important to keep in mind that the $\Delta\tilde{\mu}_{H^+}$ associated with this energization, referenced to the difference between final and initial states, "out" minus "in" has a sign opposite to those in eqns (1-44,47). That is, as in (1-47),

$$\begin{aligned} &\Delta\tilde{\mu}_{H^+} = F\Delta\psi - 2.3RT\cdot\Delta p\mathrm{H}\,;\ \text{however,}\\ &\Delta\psi = \psi_{out} - \psi_{in},\\ &\Delta p\mathrm{H} = p\mathrm{H}_{\mathrm{out}} - p\mathrm{H}_{\mathrm{in}}, \end{aligned} \qquad (1\text{-}48)$$

and $\Delta\tilde{\mu}_{\mathrm{H}^+} = (\mu_{\mathrm{H}^+})_{out} - (\mu_{\mathrm{H}^+})_{in}$, for the differences between initial and final states.

1-8.5.3. Comments on the Impermeability of Biological Membranes to Ions; the Born Energy.

It is implicit in figures 8A,B that the only pathways for protons to cross the membrane (a) to generate the trans-membrane potential or pH gradient, and (b) to utilize the electrochemical gradient for the synthesis of ATP, or the accumulation of solutes in active transport (see below, Fig. 9), are through specific integral membrane proteins. As a consequence of the apolar nature of the lipid acyl chain core of membranes, the lipid part of the membrane is essentially impermeable to the

penetration and translocation of charged ions. The apolar nature of membranes can also be described in terms of the low value of the **dielectric constant** ($\varepsilon = 2$) of the acyl chains. The dielectric constant, denoted by ε, of a medium is a measure of its electrical polarizability. As a result of the low dielectric constant, an ion that is inserted into the bilayer will find no counter charges to balance it. In the absence of counter charges, the ion will thus generate a high electric field intensity and electric field energy density inside the apolar medium. Thus, the difficulty in inserting an ion into a "greasy" medium of low dielectric constant is explained by the relative absence of counter charges or of the barrier created by the necessary generation of a high internal electric field energy. The barrier is called the "Born energy", after the physicist, Max Born, who described it. The energy barrier or Born energy, E_b, the difference in the energy of the ion electric field in the membrane and in water, is:

$$E_b = 695\frac{z^2}{r}\left(\frac{1}{\varepsilon_m} - \frac{1}{\varepsilon_{H_2O}}\right), \text{ in kJ/mol,}$$

where *z* and *r* are the valence and radius (Å) of the ion. For the fully charged group of the Arg, Asp, and Glu ion, z^2 and r are 1 and 2.5 Å, respectively (Krishtalik and Cramer, 1995). ε_m and ε_{H2O} are the dielectric constants of water and the lipid bilayer, which are 78 and approximately 2, respectively. The energy barrier can be calculated from the above formula for the Born energy to be 135 kJ/mol = 32 kcal/mol. This barrier can be thought of as an activation energy, $\Delta G^{\ddagger}$, for translocation of a single charge. From reaction rate theory, the effect of this $\Delta G^{\ddagger}$ on the rate of proton translocation is then $e^{\frac{-\Delta G^{\ddagger}}{RT}} = e^{\frac{-135}{2.48}} = e^{-55} = 10^{-24}$. This implies that the rate at which a single charge will penetrate a lipid bilayer membrane is very small, and the lipid bilayer membrane can be thought of as impermeable to single charges including protons.

8.5.4 How is the Membrane Potential Generated?

The generation of the membrane potential and pH gradient by the movement of the positively charged proton across the membrane is illustrated in Figs. 8A,B. If we know the relation

between electrical charge moved across the membrane per unit area, and the resulting electrical potential, we can know the number of protons, ions, or electrons that must move across this area to generate a physiologically meaningful membrane potential.

The physical relation between charge (Q) moved and the potential $\Delta\Psi$ is a constant of proportionality called the capacitance. Thus, Q = C x $\Delta\Psi$, or $\Delta\Psi$= Q/C. Thus, the larger the capacitance, the more charge has to be moved across the membrane in order to generate a given value of $\Delta\Psi$. For a planar electrical capacitor that can be viewed as a small segment of a biological membrane (Figure 8C), the specific capacitance, i. e., the capacitance per unit area, is proportional to the dielectric constant, ε, and inversely proportional to the distance, d, between the two sides of the capacitor. That is, C per unit area = ε/d. Because the distance between the two sides of the biological membrane is small, approximately 35 Å, the specific capacitance of such membranes is large, ca. 1 μFarad per cm^2, even though the dielectric constant is small.

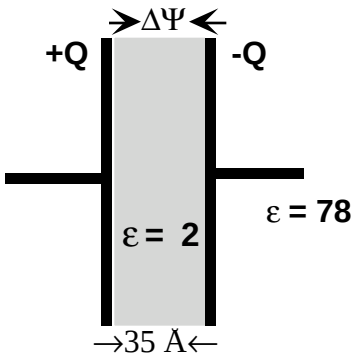


Figure 1-8C. A simple planar capacitor as a model for a local segment of a biological lipid bilayer membrane.

Relation between $\Delta\Psi$ and charge transfer across a membrane. The ability of membranes to sustain an energetically significant $\Delta\Psi$ as a result of the movement of a small number of electrons or ions across the membrane is a consequence of the large value of the specific membrane capacitance. The difference in the charge, Q, translocated to one side from the other results in a potential ($\Delta\Psi$) across the dielectric. With

$$Q = C \times \Delta\Psi$$

and C = 1 μFarad/cm^2, if the potential across the membrane is $\Delta\Psi$ = 100 mV, the number of transferred charges is approximately 1 per 10^4 Å^2, i. e., approximately (a) 1 charge per membrane protein complex subtending an area (100 Å x 100 Å) of this size, or (b) per 160 lipid molecules in the membrane monolayer if the area subtended per lipid molecule is 60 Å^2.

1-9. Thermodynamics of $\Delta\tilde{\mu}_{H^+}$-Linked Active Processes

1-9.1. Active transport; Symport

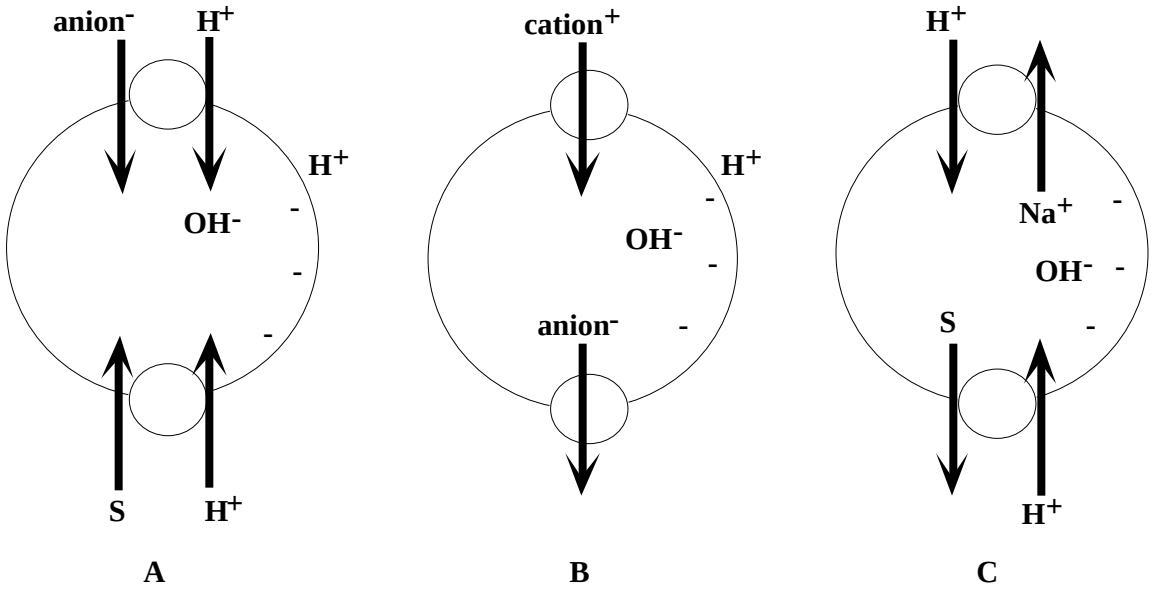


Fig. 1-9. Diagramatic representation of a neutral or charged solute into a cell by symport (A) or antiport (C) mechanisms, and of a charged solute S^+, e. g., K^+, by a uniport mechanism (B). Modified from (Cramer and Knaff, 1991).

The uptake of solute S^{+z} (charge z) from an initial state, "out," in the extracellular milieu outside the cell to a final state, "in," when the solute has been transported into the cytoplasm, is accompanied by the uptake **(symport)** of **n** protons (Fig. 1-9A), that is driven by a proton flux down the gradient of the proton electrochemical potential, $\Delta\tilde{\mu}_{H^+}$ from the side of the positive to that of negative $\tilde{\mu}_{H^+}$. The uptake results in a $\Delta\tilde{\mu}_{S^{+z}}$ for substrate accumulation. If all of the $\Delta\tilde{\mu}_{H^+}$ generated by electron transport and ATP hydrolysis is utilized by proton uptake into the cell associated in the active transport process, then this free energy change, $n\Delta\tilde{\mu}_{H^+}$, equals the $\Delta\tilde{\mu}_{S^{+z}}$ per mole of solute, S^{+z}, that is accumulated, from an initial extracellular state to a final cytoplasmic compartment:

$$\Delta G_{S^{z+}} = n\Delta\tilde{\mu}_{H^+} + \Delta\tilde{\mu}_S = 0, \quad (1\text{-}49)$$

where n is the number of moles of H^+ that would have to move down the $\Delta\tilde{\mu}_{H^+}$ gradient from outside to inside to generate the accumulation. Note that $\Delta\tilde{\mu}_S$ is positive for active transport for which the solute accumulation ratio is > 1, and $\Delta\tilde{\mu}_{H^+}$ for the utilization of the electrochemical potential initially generated by electron transport and ATP hydrolysis must then be negative in eqn (1-49). Then, for the utilization of the $\Delta\tilde{\mu}_{H^+}$ as described in Fig. 1-9A,

$$\Delta\tilde{\mu}_{H^+} = F\Delta\Psi - 2.3RT\Delta pH \quad (1\text{-}50)$$

where $\Delta pH = pH_I - pH_O$ and $\Delta\Psi = \Psi_I - \Psi_O$.

By analogy with eqns (1-43a,1-44),

$$\Delta\tilde{\mu}_S = 2.3RT \bullet \log_{10}\frac{\{S_I{}^{+z}\}}{\{S_O{}^{+z}\}} + zF \bullet \Delta\psi \quad (1\text{-}51)$$

Substituting (1-50) and (1-51) into (1-49),

$$2.3RT \bullet \log_{10}\frac{\{S_I{}^{+z}\}}{\{S_O{}^{+z}\}} + zF \bullet \Delta\psi + \text{n}[\, F\Delta\Psi - 2.3RT \bullet \Delta pH\,] = 0,$$

dividing by 2.3RT, and solving for the accumulation ratio, $\log_{10}\frac{\{S_I{}^{+z}\}}{\{S_O{}^{+z}\}}$,

$$\log_{10}\frac{S_i{}^{+z}}{S_0{}^{+z}} = n\Delta pH - (n+z)\cdot\frac{\Delta\psi}{Z} \quad (1\text{-}52)$$

with *2.3RT/F* = 59 mV ≡ Z at 25°C, expressed in millivolts. This is the expression for accumulation of solute *S* with charge z by a symport mechanism. The solute uses *n* protons $(n \cdot H^+)$ per solute molecule, with the transport driven by a trans-membrane pH gradient (ΔpH) and membrane potential ($\Delta\psi$).

Lactose is an example of a solute whose accumulation is driven by a symport mechanism (Ramos and Kaback, 1977) for which n = 1 (West and Mitchell, 1972) for transport by the lactose permease.

1-9.2 Special Case; Uniport.

For n = 0, The mechanism is called uniport (Fig. 1-9B), is driven only by Δψ because the proton flux does not participate in the transport mechanism. Setting n= 0 in eqn (1-52) leads to:

$$\log_{10}\frac{S_I{}^{+z}}{S_O{}^{+z}} = -z\cdot\frac{\Delta\psi}{Z} \quad (1\text{-}53)$$

This is the formula that describes accumulation of solute through active transport by a **Uniport** mechanism. To achieve an accumulation ratio of 100 for a singly charged species, a $\Delta\Psi = \Psi_I - \Psi_O$ of -118 mV would be required at 25°C. Note that negative sign for $\Psi_I - \Psi_O$ implies that the potential is negative on the inside of the membrane relative to the outside or external side. This is the sign of the trans-membrane electrical potential in almost all biological membranes, including the bacterial cytoplasmic membrane and the mitochondrial inner membrane, which are discussed below.

One major exception is the chloroplast thylakoid membrane (also, see below).

1-9.3. Antiport mechanism

For an antiport transport (e. g., Na^+ - H^+ exchange) as described in Fig.1-9C, in contrast to symport and uniport (Figs. 1-9A,B) the initial and final states for solute movement are, respectively, the inside, "I" and outside "O", of the membrane. The initial and final states for proton transfer and utilization of the energy stored in the proton electrochemical gradient are independent of the transport mechanism and are the same for the antiport as for the symport and uniport mechanisms. Then,

$$\Delta\tilde{\mu}_{S^{+z}} = 2.3RT \cdot \log_{10} \frac{S_o{}^{+z}}{S_i{}^{+z}} - zF \cdot \Delta\psi, \qquad (1\text{-}54)$$

with signs reversed from Eqn. (1-51) because of reversal of initial and final states for solute transport, and $\Delta\psi = \psi_{in} - \psi_{out}$.

Using $\Delta\tilde{\mu}_{H^+}$ as in the symport case above, with 'n'protons utilized per solute molecule transported, combining $\Delta\tilde{\mu}_{H^+}$ and $\Delta\tilde{\mu}_{S^{+z}}$, and solving for $\log_{10} \frac{S_i{}^{+z}}{S_o{}^{+z}}$,

$$\log_{10} \frac{S_i{}^{+z}}{S_o{}^{+z}} = (n - z) \cdot \frac{\Delta\psi}{Z} - n \cdot \Delta\text{pH}. \qquad (1\text{-}55)$$

This is the formula that describes the accumulation of solute through an **antiport** mechanism. If *n* = z, then the charge movement would be neutral, $\Delta\psi$ has no effect, and

$$\log_{10} \frac{S_i{}^{+z}}{S_o{}^{+z}} = -n \cdot \Delta\text{pH}. \qquad (1\text{-}56)$$

1-9.4. A mechanistic problem arises when the mechanism of proton translocation through the transport protein that is embedded in the membrane bilayer is considered. Carboxylate residues have been shown to be intraprotein and intramembrane H^+ carriers. Whereas the pK of Asp in solution is nominally about 4, the pK in the membrane environment is commonly 7-9. This reflects the energetic tendency of the residue to retain the neutral protonated, as opposed to the charged unprotonated, form in the low dielectric membrane. The pK of 7-9 facilitates H^+ transfer reactions in the membrane. However, to achieve such altered pK values, energy would be required in the assembly and biogenesis of the membrane protein.

Homework problem 10. Calculate the ΔG at 25 C for the protonation and neutralization at pH 7 of half the amount of aspartic acid (A^- + H^+ →AH) in a membrane protein, if the effective pK of this residue in the membrane is (a) 4.0, and (b) 8.0. It may be helpful to derive the formula ΔG = -1.36 (pK-pH).

Homework problem 11. (a) What is the accumulation ratio of a monovalent anionic species transported by *E. coli* through a proton symport mechanism, if ΔΨ = -118 mV and ΔpH = +1? (b) What is the ratio of accumulation under the same conditions of an uncharged sugar, e. g., lactose? The number of protons, n, translocated in (a) and (b) = 1. (c) Consider transport of Cl- into inside-out membranes, ΔΨ positive inside. (i) Derive an expression for the log of the accumulation ratio

according to a uniport mechanism. By how much does the ratio change when $\Delta\Psi$ is increased from + 59 to + 118 mV?

1-10. Thermodynamics of $\Delta\tilde{\mu}_{H^+}$-Linked ATP Synthesis

The synthesis of ATP coupled to membrane energy storage is also linked to the utilization of the proton electrochemical gradient across the membrane, again from the side of positive (p) $\tilde{\mu}_{H^+}$, to the side that has a $\tilde{\mu}_{H^+}$ that is more negative (n). The "p" side is in the inside or lumen side of the chloroplast thylakoid membrane (Fig. 1-8A), and the outside of the mitochondrial inner membrane or bacterial cytoplasmic membrane (Fig 1-8B). Thus, synthesis of ATP from ADP and orthophosphate that is coupled to a proton flux through the $\Delta\tilde{\mu}_{H^+}$, with n protons required per mole of ATP synthesized, can be written:

$$ADP + P_i + nH_p{}^+ \rightarrow ATP + H_2O + nH_n{}^+, \tag{1-57}$$

for which the free energy for ATP synthesis is:

$$\Delta G_{ATP} = \Delta G^o_{ATP} + 2.3RT \log_{10} \frac{\{ATP\}}{\{ADP\}\{P_i\}}, \tag{1-57a}$$

with ΔG^o_{ATP} for synthesis of ATP is ≈ 8 kcal/mol = 33.5 kJ/mol (pH 8, Mg^{+2}).
If all of the free energy stored in the proton electrochemical potential is utilized ($\Delta\tilde{\mu}_{H^+} < 0$) for ATP synthesis, the total free energy change would be zero. Thus,

$$\Delta G_{tot} = n \cdot \Delta\tilde{\mu}_{H^+} + \Delta G_{ATP} = 0 \tag{1-58}$$

substituting for ΔG_{ATP} and using (1-57a),

$$-n \cdot \Delta\tilde{\mu}_{H^+} = \Delta G^\circ + 2.3RT \cdot \log_{10} \frac{\{ATP\}}{\{ADP\}\{P_i\}}; \tag{1-59}$$

then,

$$\log_{10} \frac{\{ATP\}}{\{ADP\}\{P_i\}} = \frac{-1}{2.3RT}(\Delta G^\circ + n \cdot \Delta\tilde{\mu}_{H^+}) \tag{1-60}$$

1-10.1 ATP Synthesis in Chloroplast Thylakoid, Sub-Mitochondrial and Chromatophore Membranes.

A positive $\left(\Delta\tilde{\mu}_{H^+}\right)_p$ is generated in the chloroplast thylakoid lumen from protons pumped into the internal space by the photosynthetic electron transport chain or ATP hydrolysis. The discharge of this positive potential from the "inside" (initial state) to the "outside" (final state) is coupled to the synthesis of ATP by the ATP synthase (mushroom-shaped structure in Fig 1-8).

These membranes have a topology, with respect to the direction of proton pumping and the orientation of the ATP synthase that is the opposite of right side-out bacterial and mitochondrial membranes. Considering the energy balance, as in (1-58) and with $\Delta\psi = \psi_O - \psi_I; \Delta pH = pH_O - pH_I$, one obtains the expression,

$$\log_{10} \frac{\{\mathrm{ATP}\}}{\{\mathrm{ADP}\}\{\mathrm{P_i}\}} = \frac{-1}{2.3RT}(\Delta G^\circ + n \cdot \Delta\tilde{\mu}_{H^+}) \tag{1-61}$$

with $\Delta\mu_{H^+} = F \cdot \Delta\psi - 2.3RT \cdot \Delta pH$, from Eqn (1-44).

Using the parameters that are typical for the energized thylakoid membrane, one may estimate the poise (ratio) of ATP/ADP: $\Delta\psi$ = 0 mV, $\Delta pH = pH_{final} - pH_{initial} = pH_{out} - pH_{in}$ = + 2.5, and from the latest data on the proton:ATP stoichiometry in ATP synthesis, n = 3-4. If ΔG^o_{ATP} is 8 kcal/mol and the orthophosphate concentration (P_i) is 10 mM, and n = 4,

$$log_{10}\frac{\{\text{ATP}\}}{\{\text{ADP}\}\{\text{P}_i\}} = \frac{-1}{2.3RT}(\Delta G^\circ + n \cdot \Delta\tilde{\mu}_{H^+}) = \frac{-1}{2.3RT}[+8 + n(F \cdot \Delta\Psi - 2.3RT \cdot \Delta pH)]$$

$$\log_{10}\frac{\{ATP\}}{\{ADP\}\{P_i\}} = -\left(\frac{8}{1.36} - n \cdot \Delta pH\right) = -(5.9 - 10) = +4.1$$

Substituting {Pi} = 0.01 M,

$\log_{10}\frac{\{ATP\}}{\{ADP\}} = 2.1$, the ratio of concentration of ATP to ADP (ATP:ADP) = 126.

1-10.2 ATP Synthesis in Mitochondria and Bacteria.

A positive $(\tilde{\mu}_{H^+})_p$ in the periplasmic (bacteria) or inter-membrane (mitochondria) space is generated by H^+ pumped out of the membrane by the electron transfer chain or the ATPase. This also generates a relatively negative $(\tilde{\mu}_{H^+})_n$ in the cytoplasm or mitochondrial matrix (internal) space. Energy requiring synthesis of ATP from ADP and orthophosphate again results from the downhill discharge of the $\Delta\tilde{\mu}_{H^+}$ through the ATP synthase enzyme in which the proton flux is utilized enzymatically. In this case, for ATP synthesis, the initial and final states are "outside" (O) and "inside" (I), respectively (Fig 1-8B).

Example: Calculate the {ATP}:{ADP}{Pi} poise with ΔG^o_{ATP} = +8 kcal/mol (pH 8), for physiological values of $\Delta\Psi$ and ΔpH, e.g., $\Delta\psi$ = -118 mV = $\psi_{in} - \psi_{out}$; ΔpH = + 0.5 = $pH_{in} - pH_{out}$; assume that the number of H^+ that pass through the ATP synthase per molecule of ATP synthesized is 4 (n =4). These values for $\Delta\Psi$ and ΔpH are typical for bacterial and mitochondrial membranes. From (1-60),

$$log_{10}\frac{\{ATP\}}{\{ADP\}\{Pi\}} = \frac{-1}{2.3RT}\left(\Delta G^o + n \cdot \Delta\tilde{\mu}_{H^+}\right) = \frac{-1}{2.3RT}[+8 + n(F \cdot \Delta\Psi - 2.3RT \cdot \Delta pH)]$$

$$= -\left[\frac{8}{1.36} + n(\frac{\Delta\Psi}{Z}) - n \cdot \Delta pH\right] = -\left[5.9 + \frac{-118}{59}n - 0.5n\right], \quad where\ Z = 2.3RT/F$$

$= -[5.9 - 2(4) - (0.5)4]$ = - (5.9 - 10) = + 4.1

If {Pi} = 10 mM, then {ATP}:{ADP} = 126. The identical results obtained in the calculation of Section (1-10.1) for ΔpH = 2.5, and in (10.2) for $\Delta\Psi$ = -118 mV and ΔpH = +0.5 illustrate that these two sets of conditions generate the same $\Delta\tilde{\mu}_{H^+}$ (Eqn. 1-44) or proton-motive force (Eqn. 1-46). That is, the ΔpH and $\Delta\Psi$ are thermodynamically exchangeable in the energy transduction of biological membranes.

Homework problem 12. (a) Solve problem 11a for z = 1, n = 1, $\Delta\Psi$ = -177 mV, ΔpH = 0.

(b) Solve problem 11b for z = 0, n = 1, $\Delta\Psi$ = -59mV, ΔpH = 2.

Homework problem 13. What is the concentration of Na^+ ion in the *E. coli* cytoplasm if it is transported from the cytoplasm via a Na^+ - H^+ (n = 1) antiport exchange mechanism, the Na^+ concentration in the periplasm = 100 mM, and ΔpH = + 1?

Homework problem 14. Calculate the value of the membrane potential, $\Delta\psi$, needed to drive ATP synthesis to an effective equilibrium (ΔG = 0) if the required ΔG_{ATP} for ATP synthesis, ΔG_{ATP} = +12.25 kcal/mol in (i) chloroplasts, (ii) mitochondria, if the ΔpH associated with ATP synthesis is +3 and +1, respectively. Assume that the number of protons required for synthesis of one ATP molecule by the ATP synthase = 4.

Homework problem 15. (a) What value of the ΔpH is needed in chloroplast thylakoids to sustain an {ATP}/{ADP}{Pi} poise of 1000 if $\Delta\Psi$ = 0, and n = 4?; (b) what value of the $\Delta\Psi$ in mitochondria if the ΔpH = 0? ΔG^o_{ATP} = +8 kcal/mol for (a) and (b).

Homework problem 16. Calculate the ATP/ADP ratio that can be obtained from the $\Delta\tilde{\mu}_{H+}$ = + 4 kcal/mol that has been generated by an electron transport chain and whose complete discharge will release –4 kcal/mol for coupling to ATP synthesis. n(ATP) = 4 (a) for the mitochondrial or chloroplast ATP synthase, and (b) n(ATP) = 1 for the vacuolar ATPase of the mold, *Neurospora crassa*. Assume ΔG^o_{ATP} = + 8 kcal/mol, and $\{P_i\}$ = 10 mM

1-10.3. Summary of Tenets of the Chemiosmotic Model for Energy Coupling in Biological Membranes. (Mitchell, 1966)

1. Energy transducing membranes are sealed and impermeable to coupling ions except for the pathways involved in redox- and protein dependent ion translocation. The ions that have been documented to couple energy transduction are H^+ in most studies, and also Na^+.

2. The high energy intermediate in membranes that can be used to sustain active transport or ATP synthesis is the ion electrochemical potential, which for protons, is written as $\Delta\tilde{\mu}_{H^+}$. $\Delta\tilde{\mu}_{H^+}$ is determined by the trans-membrane pH gradient and membrane potential, which are energetically equivalent forms of energy storage. The dependence is written quantitatively as $\Delta\tilde{\mu}_{H+} = F \cdot \Delta\psi - 2.3RT \cdot \Delta pH$ with the actual sign of the $\Delta\Psi$ and ΔpH dependent on the initial and final states for the ion movement.

3. The ATP synthase/ATPase activity, along with the transmembrane H+ flux, is reversible: $ADP + P_i + nH_p^+ \rightarrow ATP + H_2O + nH_n^+$, (i) with H^+ flux from the p-side to the n-side of the membrane coupled to ATP synthesis, and (ii) H^+ flux from the n- to the p-side coupled to ATP hydrolysis.
4. Non-protein uncouplers of membrane energy transduction that de-energize the membrane are lipophilic (membrane-soluble) weak acids or bases that catalyze the equilibration of H^+ or OH^- across the membrane.

Homework problem 17. How many protons must be translocated across (a) a small spherical liposome (diam. = 300 Å)-neglect membrane thickness, and (b) a spherical *E. coli* cell of 1 µm diam., in order to generate a trans-membrane potential of 100 mV? Assume both membranes have the same specific membrane capacitance, 1µF/cm^2.

1-10.4 Experimental Tests of the Chemiosmotic Hypothesis;

1-10.4.1 Proton Movement

H^+ or Na^+ movement driven by electron transport or ATP hydrolysis across a closed and non-leaky organelle or bacterial membrane is always associated with formation of a $\Delta\tilde{\mu}_{H^+}$ across that membrane. Proton efflux (as in Fig. 1-8B) driven by an O_2 pulse delivered to anaerobic *E. coli* cells is shown (Fig. 1-10).

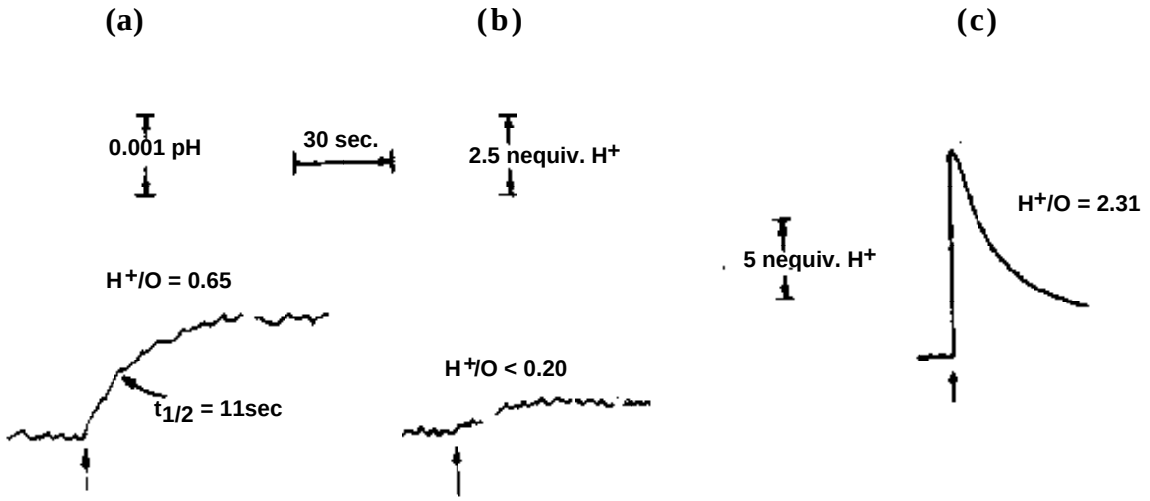


Figure 1-10. H^+ efflux induced by an O_2 pulse (upward arrows) of air-saturated buffer in *E. coli* cells, (a) untreated, (b) in the presence of uncoupler to short-circuit the H^+ flow, and (c) in the presence of thiocyanate (SCN^-) as a lipid-soluble counter anion to prevent polarization of the membrane (Gould and Cramer, 1977). A common pH scale, determined by adding known equivalents of HCl to the cell suspension, is shown for (a, b). The slow irreversible responses in (a, b) are slow"artificial background" responses that are not associated with redox-linked H^+ pumping.

1-10.4.2 ATP Synthesis by an Artificial $\Delta\tilde{\mu}_{H^+}$

A. The energetic ability of a trans-membrane ΔpH to support ATP synthesis was demonstrated by incubation of chloroplast thylakoid membranes in the dark at pH 4 in the presence of a permeant acid, followed by transfer to a solution containing ADP and P_i at pH 7. The membranes catalyzed the synthesis of 140 nmol ATP/mg chlorophyll ATP in the dark acid-base transition compared to 5 – 10 nmol ATP/mg Chl in controls without ADP, phosphate, or (Jagendorf and Uribe, 1966). Synthesis of ca. 150 nmol ATP/mg Chl corresponds to ~ 1 ATP per 6-7 chlorophyll molecules, approximately 100 times the content of the chloroplast electron transport chain components (stoichiometry approximately 1:600 Chl). Neither the presence of electron transport inhibitors, nor the redox state of the chloroplasts, affected the amount of ATP synthesized during the dark acid-base transition.

B. Artificial ATP Synthesis in Submitochondrial Particles (SMP)

SMP subjected to a $\Delta\tilde{\mu}_{H^+}$ generated by an acid-base transition and a potassium diffusion potential in the presence of ADP and $^{32}P_i$, can generate 2-3 nmol ATP/mg protein compared to 10% of this amount in the control. The $\Delta\tilde{\mu}_{H^+}$ including the diffusion potential is generated by diluting SMP at pH 5.0 without potassium into a medium at pH 7.5 containing 100 mM K^+ in the presence of the lipophilic K^+–specific ionophore, valinomycin, MW = 1,110.0; cyclic peptide made of repeating units of (L-lactate)-(L-valine)-(D-hydroxyvalerate)-(D-valine) (Fig. 1-11A). The facilitated inward flow of K^+ provides a $\Delta\psi$ to drive the H^+ efflux (Thayer and Hinkle, 1975).

C. Reconstitution of Bacteriorhodopsin in Membrane Vesicles that Catalyze H^+ Uptake and ATP Synthesis

Reconstitution of the purified bacteriorhodopsin pigment-protein from halophilic bacteria into artificial membrane vesicles catalyzes H^+ uptake (Racker and Stoeckenius, 1974). Simultaneous incorporation of the mitochondrial ATP synthase into the vesicles (20 nmol ATP:mol bR) catalyzed light-dependent ATP synthesis (15 mol ATP: mol bR), compared to 0.5 mol ATP:mol bR in the control. The light-dependent ATP synthesis was sensitive to uncouplers (see below) of membrane energy transduction.

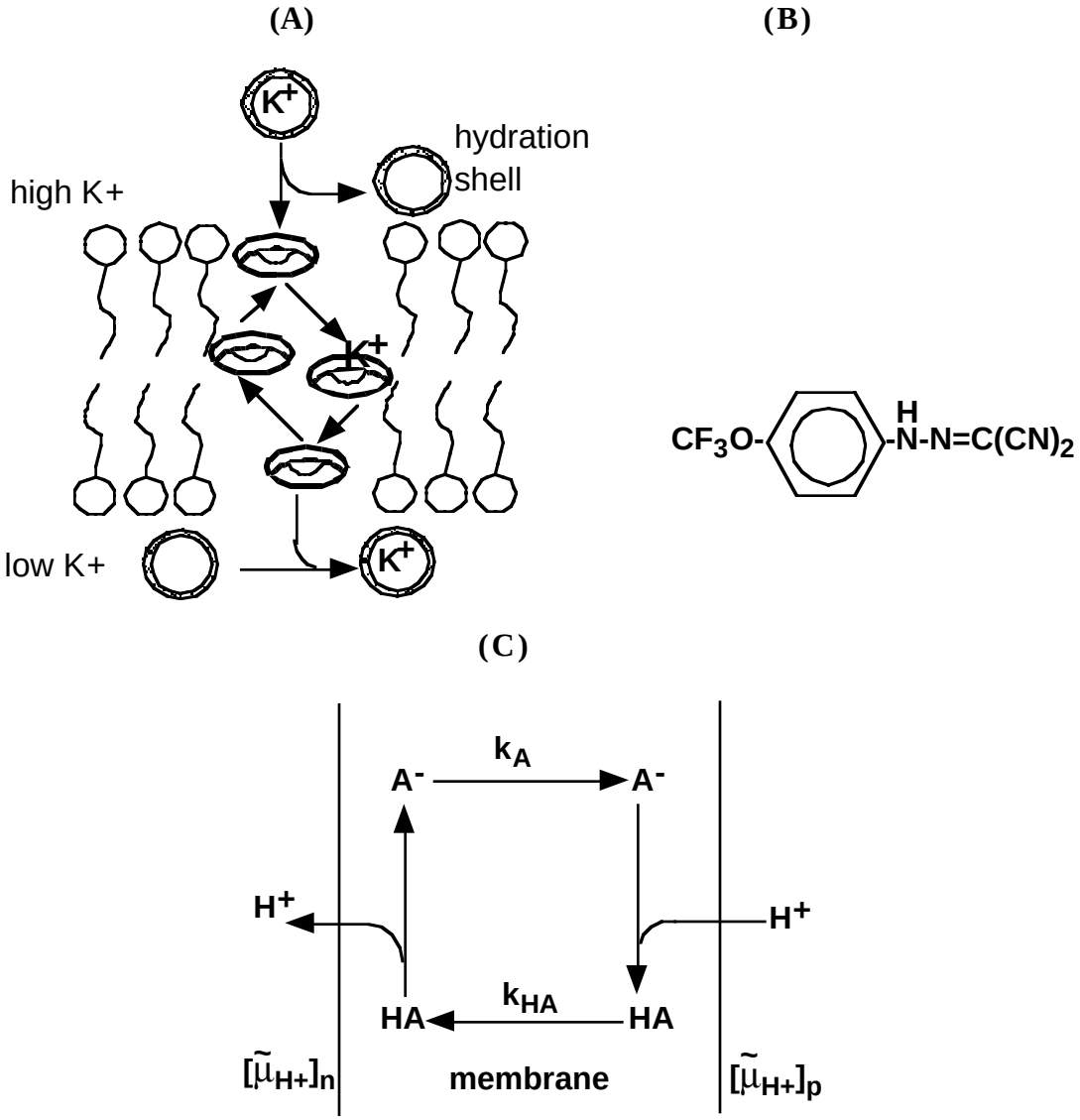


Fig. 1-11. (A) Schematic view of the ionophore, valinomycin (MW = 1,110, three repeating units of [(L-lactate)-(L-valine)-(D-hydroxy isovalerate)-(D-valine)], as a mobile carrier for a dehydrated monovalent cation. The valinomycin dodecamer can be thought of as a "doughnut," hydrophobic outside with a central polar niche into which the cation can fit. The ion selectivity is $Rb^+ > K^+ > Cs^+ > Ag^+ > NH_4^+ > Li^+$. (B) Structure of the protonated form of the weak acid lipophilic uncoupler, FCCP; (C) model for uncoupling action of weak acid anionic uncouplers that catalyze H^+ flow in the membrane from the side of positive $\tilde{\mu}_{H^+}$ to negative $\tilde{\mu}_{H^+}$ [modified from (Benz and McLaughlin, 1983)].

D. Mechanism of Action of Uncouplers of Phosphorylation

It was proposed in the chemiosmotic hypothesis (Section 1-10.3) that weak acid lipid-soluble uncouplers of ATP synthesis (e.g., carbonyl cyanide p-trifluoromethoxy-phenylhydrazone, FCCP; pK_a, 6.2 in 10% ethanol; Fig. 1-11A) act by increasing the conductance or permeability of the membrane to protons.

Uncouplers increase the proton conductance of planar bilayer membranes by 2 – 3 orders of magnitude, with the maximum increase occurring near the uncoupler pK_a suggesting that the

unprotonated form of the uncoupler (e.g., FCCP) is translocated across the membrane in a shuttle-like mechanism and thereby dissipates the $\Delta\tilde{\mu}_{H^+}$ (Fig. 1-11B). Uncouplers stimulate the rate of respiratory and photosynthetic electron transport that is coupled to ATP synthesis by a factor of 2 – 10, depending on ambient pH and the tightness of coupling of the membranes. This phenomenon is called "respiratory control" in mitochondria. The absence of mitochondrial respiratory control and the presence of high respiratory electron transport rates are a frequent manifestation of human mitochondrial myopathies (Luft et al., 1962).

Homework problem 18. ΔpH = 0 at pH = 7.5 in isolated right side-out *E. coli* membrane vesicles. At this pH, the source of all of the free energy available for active transport and ATP synthesis from a positive electrochemical potential gradient ($\Delta\tilde{\mu}_{H+}$ = + 4 kcal/mol) generated by respiration resides in the $\Delta\Psi$. However, the ΔpH can be solely responsible ($\Delta\Psi = 0$) for transport of some solutes at lower pH, e. g., pH 5.5. This is one indication that the ΔpH and $\Delta\Psi$ are thermodynamically and enzymatically interchangeable. Calculate the value of the $\Delta\Psi$ and the ΔpH that can drive active processes at pH 7.5 and 5.5, respectively. T = 25 °C.

D. Mechanism of Action of Uncouplers (continued)

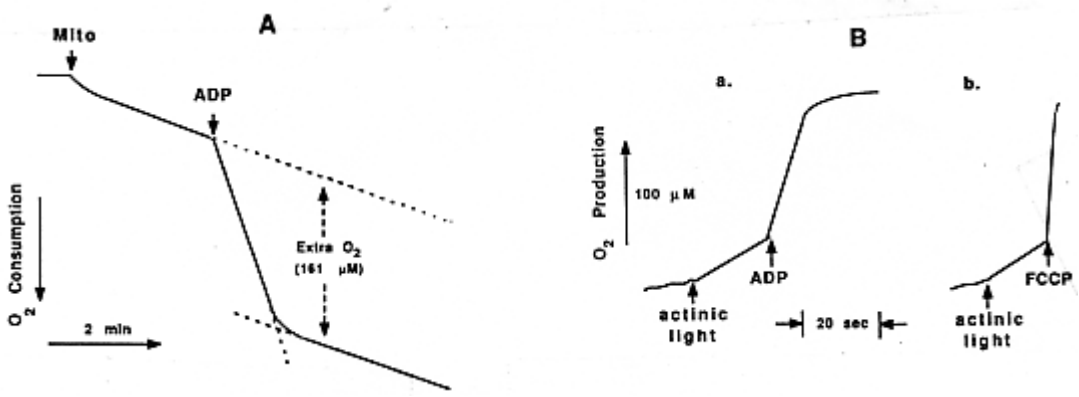


Fig. 1-12. Effect of ADP and uncouplers on rates of (A) respiratory electron transport and oxygen consumption in mitochondria, and (B) photosynthetic electron transport and oxygen evolution in chloroplasts.

1-11. ATP and "High-Energy" Bonds

The definition of a "high-energy" bond in biochemistry is that it is unstable and readily hydrolyzed with a ΔG^{o} at pH 7 ($\equiv \Delta G^{o'}$) more negative than that of the majority of simple phosphate esters, i.e., $\Delta G^{o'} \leq -7$ kcal/mol (Table 1-2). Most high-energy compounds involve phosphate or sulfur anhydrides. High-energy compounds other than the phosphate anhydride compounds shown in Table 1-2 include thiol acyl esters, sulfonium compounds, acyl imidazole, and acyl amino acids. ATP occupies a central position in the hierarchy of high energy phosphoryl compounds. Thus, the ADP/ATP couple can mediate phosphate flow from phosphorylated compounds with very negative $\Delta G^{o\prime}$ (i.e., phosphoenolpyruvate, 1,3-diphosphoglycerate, creatine phosphate) and can act as a phosphoryl donor or acceptor.

CTP, GTP, and UTP, which have free energies of hydrolysis very similar to that of ATP, are preferentially used as the energy source for different biosynthetic pathways. They are the general precursors, respectively, for biosynthesis of lipid, protein and cellulose, and polysaccharides.

The origins of the high-energy nature of ATP are: (i) the negative ΔH^{o} from electrostatic repulsion caused by the negative charges on the terminal β–γ phosphate groups, and (ii) the positive

ΔS^{o} arising from the increased number of substates or arrangements available in the phosphate product due to resonance, and a decrease in the amount of H_2O solvated (and ordered) in the products. The net charge is affected by the pK for $HATP^{3-} \rightarrow H^{+} + ATP^{4-}$, which is approximately 6.8.

Table 1-2. $\Delta G^{o\prime}$ for hydrolysis of phosphate anhydride and ester compounds

Compound	ΔG^{o} (kcal/mol)[a]
Phosphoenolpyruvate	–14.8
1,3-Diphosphoglyceric acid	–11.8
Creatine phosphate	–10.0
Acetyl phosphate	–10.0
Phosphoarginine (pH 8.0)	–8.0
$ATP \rightarrow ADP + P_i(+Mg^{++})$	–7.7
$ATP \rightarrow ADP$ (pH 8.0)	–8.4
$ATP \rightarrow ADP$ (pH 9.5)	–10.4
Glucose-1-phosphate	–5.0
Pyrophosphate	–4.0
Fructose-6-phosphate	–3.8
Glucose-6-phosphate	–3.3
Glycerol-1-phosphate	–2.2

[a]pH = 7.0, except where noted.

The contribution of entropy or resonance stabilization to the large ΔG^{o} of hydrolysis is a consequence of the atomic properties of phosphorous, i.e., large bond lengths with weaker binding energies. This leads to a partial double bond character of the orthophosphate P–O bonds. The average P–O bond length in orthophosphate is 1.54 Å compared to 1.73 Å expected for a single P-O bond. The greater electron delocalization is associated with resonance stabilization and a positive contribution to the ΔS^{o} of hydrolysis (Table 1-3). Another major contribution to the large positive ΔS^{o} and negative ΔG^{o} of hydrolysis of phosphate anhydride compounds arises from differential solvation by H_2O of products and reactants. The solvation energies of $H_2PO_4^{-}$, HPO_4^{2-}, and PO_4^{3-} are 76, 299, and 637 kcal/mole (25°C). This implies that the large ΔS^{o} of ATP hydrolysis arises from a smaller amount of solvated or ordered water in the products because they carry a smaller average charge than the reactants.

Table 1-3. Factors contributing to large negative ΔG^o of hydrolysis of phosphate anhydrides such as adenosine 5'-triphosphate (ATP)

Factor	$-\Delta H^o$ or $+T\Delta S^o$
1. Electrostatic charge repulsion	$-\Delta H^o$
2. Large number of resonant forms of orthophosphate	$+T\Delta S^o$
3. Smaller amount of solvated or ordered water in less highly charged products	$+T\Delta S^o$

1-11.1 Experimental determination of the ΔG^o for ATP Hydrolysis

At pH 8, in the presence of 10^{-2} M Mg^{2+}, $\Delta G^o = -9$ kcal/mol, and $K_{eq} > 10^6$, which is too large to be measured accurately through only the ATP hydrolysis reaction, ATP + $H_2O \rightarrow$ ADP + P_i (See problem 19). Therefore, the K_{eq} for ATP hydrolysis must be measured in two or more coupled reactions such as the glutamine synthetase and glutamine hydrolysis reactions [Eqns (i) and (ii)] below (Rosing and Slater, 1972). Thus,

(i) Glutamic acid + NH_4^+ + ATP $\xrightarrow{K_I}$ Glutamine + ADP + P_I; $\mathbf{K_I} = 700$

(ii) Glutamine + H_2O $\xrightarrow{K_{II}}$ Glutamic acid + NH_4^+; $\mathbf{K_{II}} = 230$

The sum of reactions (i) and (ii) is:

ATP + $H_2O \rightarrow$ ADP + P_i; with $K_\Sigma = K_I K_{II}$; $K_\Sigma = 1.6 \times 10^5$. Then,

$\Delta G_\Sigma^o = \Delta G_{ATP}^o = \Delta G_I^o + \Delta G_{II}^o = -RT1nK_\Sigma = 2.3RT \cdot \log K_\Sigma = -30.5$ kJ/mol.

Homework problem 19. (a) If 1 mM ATP is added to a solution at pH 8 in which it can be hydrolyzed to equilibrium, calculate the concentration of ATP at equilibrium if it is assumed that $K_{eq} = 10^6$. (b) Briefly describe a method that could be used to determine the K_{eq} for ATP hydrolysis more accurately.

1-12. Oxidation-Reduction Reactions

1-12.1. Direction of redox reactions

As discussed in section 1-8.1, a free energy change, ΔG, results from movement of electrical charge, nzF, through an electrical potential gradient. Thus,

$$\Delta G = +nzF\,\Delta E = -nF\,\Delta E, \qquad (1\text{-}62)$$

for the free energy change in a reversible reaction associated with electrical work driven by a change in oxidation-reduction potential, ΔE , with $z = -1$ for electrons, and

$$\Delta G^o = -nF\,\Delta E^o \qquad (1\text{-}62a)$$

for the changes in standard free energy and standard potential. Then, $\Delta G_{final} - \Delta G_{initial} =$

$$\Delta G^o = -RT1nK_{eq}, \quad \text{and} \qquad (1\text{-}63)$$

$$E_F^o - E_I^o = \Delta E^\circ = \frac{RT}{nF} \bullet 1n K_{eq} = \frac{2.3RT}{F} \log_{10} K_{eq} = 59 \log_{10} K_{eq} \qquad (1\text{-}64)$$

(i.e., $\Delta E^\circ = 59 \log K_{eq}$)in mV when n = 1; 2.3RT/F = 59 mV at 25˚C.

Using the criteria that reactions at their midpoint go forward if $\Delta G^o < 0$ and away from the midpoint when $\Delta G < 0$ with $\Delta G = -nF\Delta E$, the criterion for the forward or spontaneous direction of an oxidation-reduction reaction is, respectively,

$$\Delta E > 0, \text{ or } \Delta E^o > 0 \qquad (1\text{-}65)$$

The criteria $K_{eq} > 1$, $\Delta E^o > 0$, $\Delta E > 0$ are used to predict the direction of a redox reaction.

1-12.2 Properties of Oxidation-Reduction Potentials

A. Electron transfer tends to proceed in the direction of more positive *E* and E^o.

B. A strong reductant results from a redox couple with a negative oxidation-reduction potential, and a relatively strong oxidant from a redox couple with a positive potential.

C. Absolute values of the oxidation-reduction potential, like the absolute value of the free energy, have no meaning in nature. The absolute values are set by the reference or

standard that is chosen, which in this case is the potential of hydrogen half-cell reaction: $2H^+ + 2e^- \rightarrow H_2(g)$; $E^o = 0.0$ V; pH = 0 (1 M H^+).

D. Oxidation potentials, *E* and E^o, are not state functions. A consequence is that these functions are not necessarily additive. That is, for a series of sequential redox reactions A→B→C→D,

$$\Delta E_{AD} \neq \Delta E_{AB} + \Delta E_{BC} + \Delta E_{CD}, \qquad (1\text{-}66a)$$

although the free energy, as a state function, is additive,

$$\Delta G_{AD} = \Delta G_{AB} + G_{BC} + \Delta G_{CD}. \qquad (1\text{-}66b)$$

E. The oxidation-reduction reaction of a single redox-compound is written as a "half-cell" reaction:

$$[A(ox) + e^- \rightarrow A(red); \text{ or for a pH-dependent reaction,} \qquad (1\text{-}67a)$$

$$A(ox) + 2\ e^- + 2H^+ \rightarrow AH_2] \qquad (1\text{-}67b)$$

The free energy changes associated with half-cell reactions are:

$$\Delta G^o = -nFE^o, \qquad (1\text{-}68a)$$

and

$$\Delta G = -nFE \qquad (1\text{-}68b)$$

1-12.3. The hydrogen half-cell. One can see from the hydrogen half-cell reaction, $\mathbf{2H^+ + 2e^- \leftrightarrow H_L(g);\ E^o = 0.0V}$, that its equilibrium constant and E^o are both pH dependent. The E_o' standard potential (in mV) at pH = 7, $E_o' = E_o - 7(59.1)$ = is −414 mV at 25° C (cf., pH dependence of midpoint potential, section 1-12.9 below). Using the hydrogen electrode as the standard, a short list of half-cell redox reactions of interest to membrane energy transduction is shown in Table 1-4.

Table 1-4. Oxidation-reduction potentials of some redox couples or half-cell reactions important in bioenergetics

Half-reaction	Midpoint potentials (mV) E^{o} (pH = 0)	$E^{o'}$ (E_{m7})
Ferredoxin (ox) + $e^- \rightarrow$ Fd (red) [2Fe–2s]	___	−432
$2H^+ + 2e^- \rightarrow H_2(g)$ [Reference]	0.0	−414
$NAD^+ + H^+ + 2e^- \rightarrow$ NADH	−113	−324
FMN(ox) + $2e^- + 2H^+ \rightarrow FMNH_2$	+209	−205
$O_2(1) + e^- \rightarrow O_2^{\bullet-}$	___	−160
$UQ + e^- \rightarrow UQ^{\bullet-}$	___	−150
cytochrome *b* + $e^- \rightarrow$ cytochrome *b* (red)	___	−150 - +50
ubiquinone + $2e^- + 2H^+ \rightarrow$ ubiquinol	___	+ 60
plastoquinone + $2e^- + 2H^+ \rightarrow$ plastoquinol	___	+ 90
cytochrome c_1 + $e^- \rightarrow$ cytochrome c_1,(red)	___	+220
cytochrome *c* + $e^- \rightarrow$ cytochrome *c*(red)	___	+250
cytochrome *f* + $e^- \rightarrow$ cytochrome *f*(red)	___	+370
plastocyanin + $e^- \rightarrow$ plastocyanin(red)	___	+370
Rieske [2Fe-2S](ox)+ $e^- \rightarrow$ Rieske [2Fe-2S](red)	___	+390
cytochrome *a* + $e^- \rightarrow$ cytochrome *a*(red)	___	+250–+400
Photosystem I-Chl (ox) + $e^- \rightarrow$ Chl(red)	___	+500[1]
$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$	+1,230	+815
Photosystem II-Chl (ox) + $e^- \rightarrow$ Chl(red)	___	+1,200[2]

[1]Photosystem I, [2]photosystem II reaction center Chl in oxygenic photosynthesis.

1-12.4 Oxidation-Reduction Potential as a Group-Transfer Potential; Comparison of Standard Potentials and p*K* Values

The standard potential is a measure of the tendency to donate or accept electrons. The more negative the potential, the greater the tendency of the reduced compound in a half-cell reaction (1-65a) to donate (reducing ability), and the more positive, the greater the affinity for the electron of the oxidized compound (oxidizing ability). Thus in Table 1-4, reduced ferredoxin and NAD(P)H are strong reductants, and molecular O_2 is a strong oxidant. Strong reductants and oxidants are good

electron donors and acceptors, respectively, just as strong and weak acids are good proton donors and acceptors. The standard potential, E^o, of an oxidation-reduction reaction is analogous to the pK of an acid-base reaction (Table 1-5). As the pK is the pH at which an acid is half-protonated, the E^o is the redox potential under standard conditions at which an electron donor-acceptor is 50% reduced. For this reason, the notation E_m is often used instead of E^o to designate the midpoint potential. The E_m becomes E^o under standard conditions.

Table 1-5. The oxidation-reduction potential as a group transfer potential.

	Proton-transfer potential (acid-base)	Electron-transfer potential (redox potential)
Reaction	$A^- + H^+ \rightarrow HA$	$A^+ + e^- \rightarrow A$
Measure of transfer potential	$pKa = \frac{\Delta G^0}{2.3RT}$	$E^0 = -\frac{\Delta G^0}{nF}$

1-12.5 Dependence of ΔE on Reaction Pathway: Reduction of O_2 to H_2O

ΔE , unlike ΔG, is not a state function and depends on the path of the reaction. As an example, consider the four different pathways for reduction of O_2 to H_2O involving the intermediates, superoxide ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2), A→B→C→D (Fig 1-12). One path would be the four-electron reduction:

$O_2 + 4e^- + 4H^+ \rightarrow 2H_2O$, with the midpoint potential at pH 7, E_{m7} = 0.82V. The relationships between the E_{m7} values for the different reactions can be determined from Eqns (1-66a) and (1-68a). The value for one of them [e.g. $E_{AB} = E_{m7}(O_2/O_2^{\cdot-})$] can be solved from the E_{m7} and n values for the O_2/H_2O (E_{AD} = 0.82 V, *n* = 4) and $O_2^{\cdot-}/H_2O$ (E_{BD} = 1.20 V, *n* = 3) couples. E_{AB} ($O_2 \rightarrow O_2^{\cdot-}$) can be obtained from E_{AD} ($O_2 \rightarrow H_2O$) and E_{BD} ($O_2^{\cdot-} \rightarrow H_2O$) because $\Delta G_{AD} = \Delta G_{AB} + \Delta G_{BD}$. One can write the half cell reactions as:

$O_2 + e^- \rightarrow O_2^{\cdot-}$	$E_{AB} = ?$	(1e⁻ reaction)
$O_2^{\cdot-} + 3e^- + 4H^+ \rightarrow 2H_2O$	$E_{BD} = +1.20\,V$	(3e⁻ reaction)
$O_2 + 4e^- + 4H^+ \rightarrow 2H_2O$	$E_{AD} = +0.82\,V$	(4e⁻ reaction)

From

$$\Delta G^o_{AD} = \Delta G^o_{AB} + \Delta G^o_{BD}, \text{ substituting for } \Delta G$$

$$-4F \cdot E^o_{AD} = -(1)F \cdot E^o_{AB} - (3)F \cdot E^o_{BD}; \text{solving for } E^o_{AB}$$

$$E^o_{AB} = \frac{(1+3)(0.82) - (3)(1.2)}{1} = -0.32\ V$$

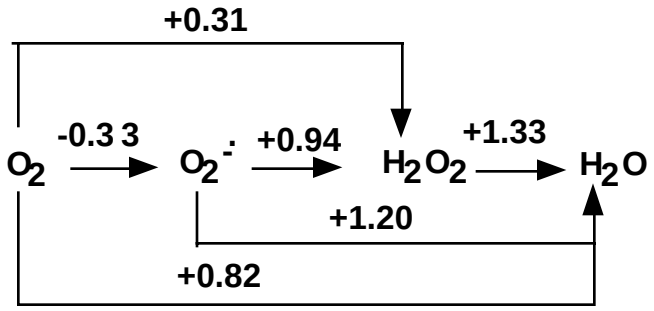


Figure 1-12. Standard reduction-oxidation potentials for the intermediates involved in the reduction of O_2 to H_2O at 25 C and pH 7.0. The pathway, $O_2 \rightarrow O_2^{\cdot-} \rightarrow H_2O_2 \rightarrow H_2O$ is designated A $\rightarrow$ B $\rightarrow$ C $\rightarrow$ D in the text.

1-12.6 Reduction of compound B by A

$$A(ox) + n_1 e^- \rightarrow A(red), \qquad E^o = E_A{}^o; \qquad G^o = G_A{}^o \tag{1-69a}$$

$$B(ox) + n_2 e^- \rightarrow B(red), \qquad E^o = E_B{}^o; \qquad G^o = G_B{}^o \tag{1-69b}$$

with $n_2 \neq n_1$ the charge balanced reaction is,

$$\left(\frac{n_2}{n_1}\right)A(red) + B(ox) \rightarrow \left(\frac{n_2}{n_1}\right)A(ox) + B(red),$$

for transfer of n_2 electrons from A to B. The standard free energy change, $\Delta G_{AB}{}^o$, for transfer of the n_2 electrons from A to compound B that requires 2 electrons for reduction is:

$$\Delta G^o_{AB} = G^o_B - \left(\frac{n_2}{n_1}\right)G^o_A . \tag{1-70}$$

Using Eqn 1-60, and substituting into eqn. (1-70),

$$-n_2 F \cdot \Delta E^o_{AB} = -n_2 F \cdot E^o_B - \frac{n_2}{n_1}\left(-n_1 F \cdot E^o_A\right),$$

it follows that:

$$\Delta E^o_{AB} = E^o_B - E^o_A, \tag{1-71a}$$

which is the standard potential of electron acceptor minus that of the donor. Similarly,

$$\Delta E_{AB} = E_B - E_A, \tag{1-71b}$$

so that the change of standard potential in a complete redox reaction is the difference of the standard potentials for the two half-cell reactions, as discussed in section 1-12.1. For the reaction to proceed in the forward direction, $\Delta E > 0$ and $E_B{}^o > E_A{}^o$, i.e., in the direction of more positive E^o and higher electron affinity.

1-12.7 Concentration dependence of the oxidation-reduction potential

From $G = G^o + RT\,ln\left(\frac{\{c\}}{\{c^o\}}\right)$,

$\Delta G = \Delta G^o + RT \ln \frac{\Pi\{products\}}{\Pi\{reac\tan ts\}}$, where the brackets indicate activities; then, because $\Delta G = -nF \cdot \Delta E$, and

$$\Delta G^o = -nF \cdot \Delta E^o$$

for the reaction, $[ox] + ne^- \rightarrow [red]$, involving the transfer of *n* electrons, it follows:

$$\Delta E = -\frac{\Delta G^o}{nF} - \frac{RT}{nF} \ln \frac{\Pi\{prod\}}{\Pi\{react\}}$$

$$= \Delta E^o - \frac{RT}{nF} \ln \frac{\Pi\{prod\}}{\Pi\{react\}}$$

$$= \Delta E^o - 2.3 \frac{RT}{nF} \log_{10} \frac{\Pi\{prod\}}{\Pi\{react\}}. \quad (1\text{-}72)$$

For a half-cell reaction, one would write:

$$E = E^o - 2.3 \frac{RT}{nF} \log_{10} \frac{\Pi\{prod\}}{\Pi\{react\}} \quad (1\text{-}73)$$

From (1-72), since 2.3RT/F = 59.1 mV at 25 C,

$$\Delta E(mV) = \Delta E^o - \frac{59}{n} \log_{10} \frac{\Pi\{prod\}}{\Pi\{react\}} \quad (1\text{-}74)$$

and for a half-cell reaction,

$$E = E^o - \frac{59}{n} \log_{10} \frac{\{red\}}{\{ox\}} \quad (1\text{-}75)$$

The fact that the E^o values can be considered midpoint potentials when there is no pH or ligand dependence of the E_m, and at dilute concentrations of reactants and products, is illustrated in Eqns. (1-73) and (1-74). It can be seen that $E = E^o$ and $\Delta E = \Delta E^o$ when the activities of the products or reduced species equals those of the reactant or oxidized component of the half-cell. Thus,

$$\Delta E = \Delta E_m - \frac{59}{n} \log_{10} \frac{\Pi\{prod\}}{\Pi\{react\}} \quad (1\text{-}74a)$$

$$E = E_m - \frac{59}{n} \log_{10} \frac{\{red\}}{\{ox\}} \quad (1\text{-}75a)$$

As discussed above (Section 1-7.4) for ΔG, the direction of an oxidation-reduction reaction is determined by the concentrations of products and reactants as well as by the ΔE_m, i.e., by the ΔE.

Note: In the context of the group transfer potential (section 1-12.4), compare (1-75a) to the Henderson-Hasselbach equation for H^+ buffering: $pH = pK + \log \frac{\{A^-\}}{\{AH\}}$.

1-12.7.1 Concentration dependence in donor-acceptor reactions

For the reaction A(red) + B(ox) →A(ox) + B(red); 1 e- reaction (n = 1),

$$\Delta E = \Delta E_m - 59 \log \frac{A(ox)}{A(red)} \cdot \frac{B(red)}{B(ox)}, \qquad (1\text{-}75b)$$

for every 10-fold increase in the ratio of A(red)/A(ox), or B(ox)/B(red), the ΔE will increase by +59 mV, and the ΔG for the reaction will be more negative by –5.69 kJ/mole.

1-12.8 Graph of titrations of the oxidation-reduction reaction

For a half-cell reaction involving transfer of one e^- (n = 1), e.g. cytochrome *c*,

$$cyt\ c(ox) + e^- \rightarrow cyt\ c(red)$$

$$E = E_m - 59 \log \frac{\{c_r\}}{\{c_o\}}.$$

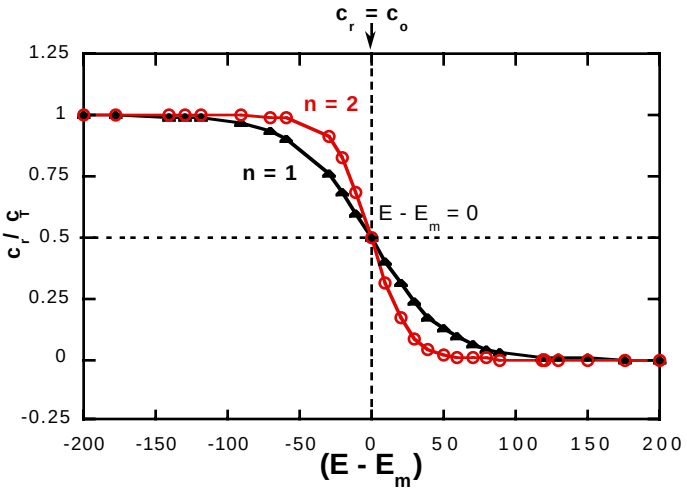
Note that for every 10-fold increase in c(red)/c(ox), E decreases by 59 mV. (Note the analogy with acid-base problems). Then,

$$E = E_m - 59\, log \frac{1}{\left(\frac{c_r}{c_T}\right)^{-1} - 1};\ \{c_r\} + \{c_o\} = \{c_T\}, \text{ the total concentration} \qquad (1\text{-}76)$$

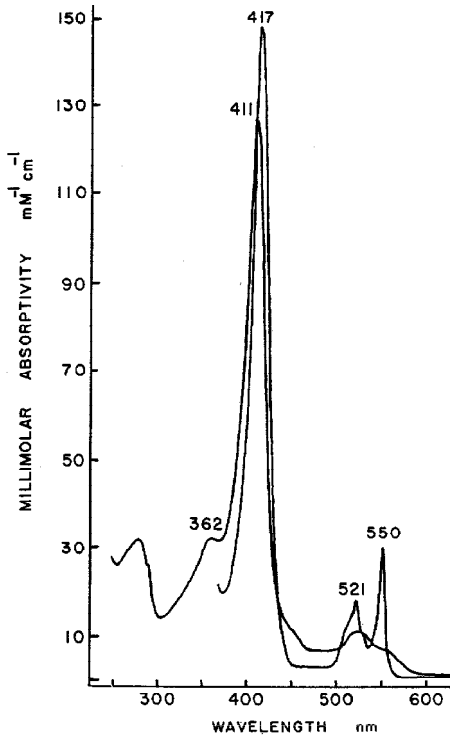
for n = 1 at 25 C, in mV; c_T = total cytochrome concentration (reduced + oxidized), c_r/c_T = fraction cytochrome reduced.

Two aids in graphing the titration are: (i) $c_r = c_o$ and $c_r/c_T = 0.5$ when E = E_m, (ii) c_r/c_o changes by a factor of 10 for every change of 59 mV in the potential. Thus, $c_r/c_T = 0.5$ and $c_o/c_r = 1$, when E = E_m. $c_r/c_T = 0.91$ and $c_r/c_o = 10$ when E = E_m – 59 mV; $c_r/c_T = 0.09$ and $c_r/c_o = 1/10$ when E = E_m + 59 mV (Fig 1-13A). The actual titration involves measuring changes in absorbance at a convenient wavelength (e.g. 550 nm) of the cytochrome as it is progressively reduced and oxidized. The spectrum of the reduced protein, with sharp peaks at 417 and 550 nm, and that for the oxidized cytochrome, relatively flat in the region of 550 nm, of horse heart cytochrome *c*, are shown in Fig 1-13B.

For a redox titration of compound "X" using visible spectroscopy, the titrations are commonly made in a stirred cuvette specially fitted with (a) a redox combination electrode (Pt-Ag/AgCl), (b) a port to allow addition of a strong oxidant and strong reductant to change the potential (analogous to addition of acid and base in a pH titration, and (c) an entry and exit port for N_2 or Ar gas for anaerobic titrations of reducing systems with a negative (reducing) potential that can be readily oxidized by O_2. The solution in the cuvette with compound "X" will contain redox buffers (Table 1-6) whose E_{m7} values cover the range of E values over which the titration wil be measured. Redox buffers are analogous to the need for pH buffers when performing pH titrations. As a pH buffer that undergoes a 1 H^+ protonation-deprotonation will buffer over a range of ±1 pH unit around its pK, a redox buffer undergoing a 1 e^- oxidation-reduction will buffer over a range of ±59 mV around its E_m.



(A)



(B)

Figure 1-13. (A) Determination of the midpoint potential of cytochrome *c* by titration of the ambient potential. The Fe^{3+} -heme ⇔ Fe^{2+} - heme titration of cyt c involves the transfer of one electron (n = 1) in the Nernst equation (eqns 1-72 to 76). An n = 2 titration, e. g., for the H_2 electrode or quinone ⇔ quinol is also shown. (B) Reduced (peaks at 417 and 550 nm) and oxidized spectra of horse heart cytochrome *c*.

Table 1-6. Some redox buffers and their midpoint potentials.

Redox buffers	E_{m7} (mV)
Anthraquinone-2-sulfonate	-220
2-OH-1,4 naphthoquinone	-140
2,5-dihydroxy-1,4-benzoquinone	-60
1,4-naphthoquinone	+65
1,2-naphthoquinone	+135

Homework problem 20. (A) Using values of R and F in appropriate units, evaluate the coefficient 2.3RT/nF (in millivolts) at 5°C and 25°C for n = 1 and n = 2.
(B) Consider the half-cell oxidation-reduction reaction, of redox compound, r: r(ox) + e^- + H^+ → r(red). If the midpoint potential, E_m, for this reaction = 0.0 V at pH 7,
(a) What is the value of the potential, E, for the reaction at pH 7, when the quotient of concentrations (activities) is:
(i) $\frac{r(\text{red})}{r(\text{ox})} = 10$; (ii) $\frac{r(\text{red})}{r(\text{ox})} = 1000$ (iii) $\frac{r(\text{red})}{r(\text{ox})} = 10^{-1}$; (iv) $\frac{r(\text{red})}{r(\text{ox})} = 10^{-2}$.
(b) What is the E_m for the half-cell reaction at pH 4?

Homework problem 21. The half-reactions of cytochromes *b* and *c* are (n = 1):

$b(\text{ox}) + e^- \rightarrow b(\text{red})$ $\quad E^o = -0.035$ V
$c(\text{ox}) + e \rightarrow c(\text{red})$ $\quad E^o = +0.26$ V.

(a) If these two redox centers are electronically connected, what is the equilibrium constant for (a) the reduction of cytochrome *c* by cytochrome *b* ;(b) For reaction (a), what is the potential change, ΔE, for the reaction when the ratio of reduced to oxidized cytochrome is: *b*(red): *b*(ox) = *c*(ox): *c*(red) = 10:1, or (d) = 1:10? T = 25 °C.

Homework problem 22. How much energy, in eV and kJ, is stored at pH 7 in reducing equivalents when two electrons are transferred from the special pair bacteriochlorophyll (E_m = +470 mV) of the bacterial photosynthetic reaction center to the secondary quinone, Q_B, whose E_{m7} = + 50 mV?

12.9 Half-cell Potential for a Reaction Involving e^- (*n* = 2) and H^+ transfer; e.g., Reduction of Quinone to Quinol

The half cell-reaction for the reduction of the aromatic quinone to quinol (Fig. 1-14A) is:

$$\text{Quinone} + 2e^- + 2H^+ \rightarrow \text{Quinol } (QH_2);\ n = 2 \qquad (1\text{-}77)$$

Using the standard potential at pH = 0 as a reference, (from 1-75, 1-75a)

$$E = E^o - \frac{59}{2}\log\frac{\{QH_2\}}{\{Q\}\{H^+\}^2}, \qquad (1\text{-}78)$$

or

$$E = E^o + \frac{59}{2}\log\{H^+\}^2 - \frac{59}{2}\log\frac{\{QH_2\}}{\{Q\}};$$

then,

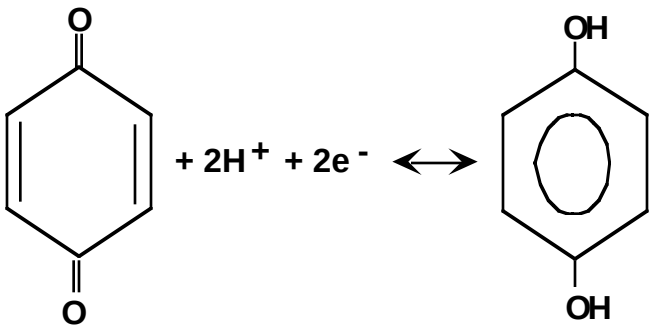
$$E = (E^{o} - 59\ \mathrm{pH}) - \frac{59}{2}\log\frac{\{QH_2\}}{\{Q\}}$$

and

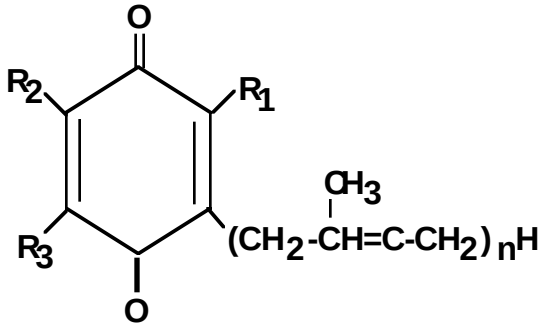
$$E = E_{mh} - \frac{59}{2}\log\frac{\{QH_2\}}{\{Q\}}, \text{ where} \qquad (1\text{-}79)$$

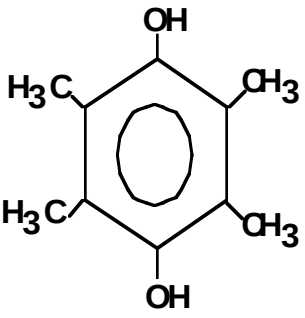
$$(E_{mh} = E^{o} - 59\ \mathrm{pH}), \text{ and} \qquad (1\text{-}80)$$

E_{mh} is the midpoint potential at a particular pH value. Notes: (a) The standard potential, E^{o}, of the hydrogen electrode is defined as 0.0 V at pH 0, 1 atm pressure, and 20°C. (b) The slope of the n = 2, $Q \leftrightarrow QH_2$, titration is steeper than that for n = 1. Thus, whereas the potentials (in mV), relative to the E_m, at which the n = 1 compound is ~90% oxidized or reduced are (E_m + 59) and (E_m – 59), respectively, the corresponding potentials for the n = 2 compound are (E_{mh} + 29.5) and (E_{mh} – 29.5) [cf., Fig. 1-13A].



(A)





(B) (C)

Fig. 1-14. (A) Reduction of benzoquinone to hydroquinone.

(B) Structure of the lipophilic ubiquinone (n = 6-10; $R_1 = CH_3$; R_2, $R_3 = CH_30$), found in mitochondrial and bacterial membranes, or plastoquinone (n = 9; $R_1 = H$; R_2, $R_3 = CH_3$) found in chloroplast and other oxygenic photosynthetic membranes. (C) The tetramethyl-substituted quinol (duroquinol) with an E_m = +480 mV.

1-12.9.1 Total pH dependence of E_m of a redox prosthetic group

The above expression (1-80) for reduction of a quinone to quinol is valid throughout the physiological range. However, at extremes of low and high pH, the E_m will not obey eqn (1-80) and will become pH-independent (Fig. 1-15). This is because at the low and high pH extrema, respectively, (a) the oxidized form of the redox compound is protonated, or (b) the reduced form in deprotonated. Thus, at the low and high pH extrema, the E_m reaches asymptotic constant values of E_{mAcid} and E_{mBase} (Fig. 1-15). At intermediate pH values, the E_{mh} depends on pH as in eqn (1-80). The protonation-deprotonation of the oxidized and reduced forms at low and high pH, respectively, is described by pK values, pK_{ox} and pK_{red}.

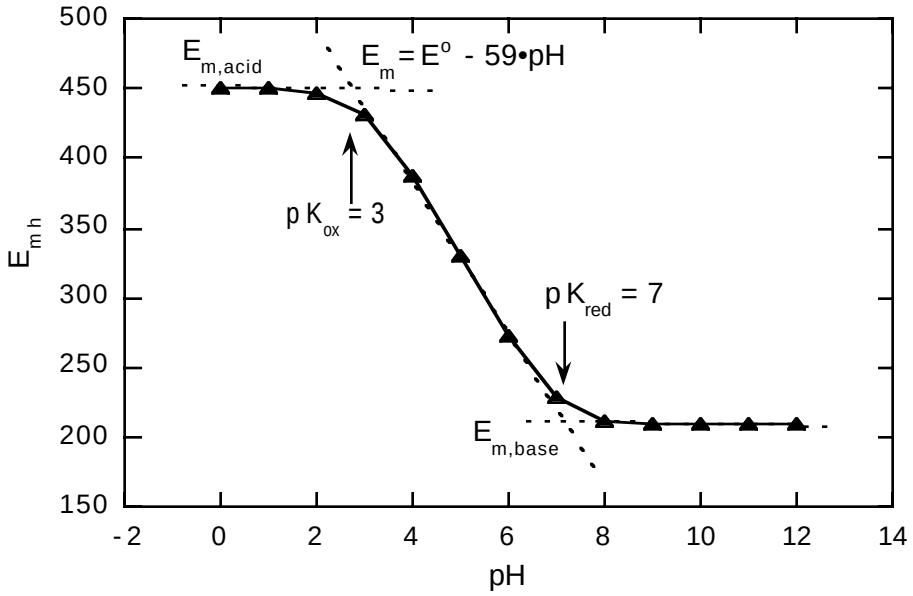


Fig. 1-15 pH dependence of E_m of redox prosthetic group including effects of pK_{ox} and pK_{red} (pK_{red}>> pK_{ox}) [modified from (Dutton, 1978)].

The pH cycle of proton and electron transfer reactions can then be described by the scheme in Fig. 1-16.

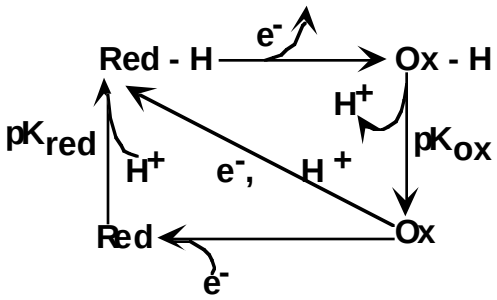


Fig. 1-16. Cycle of pH-dependent electron transfer reactions. The top and bottom lines in the cycle describe the redox reaction at the extreme of low and high pH, respectively. The pH-dependent reaction in the diagonal operates at intermediate pH values. Some groups whose protonation-deprotonation are linked to an oxidation-reduction are histidine ligands of hemes and iron-sulfur proteins, and the heme propionate side chain. (modified from (Dutton, 1978).

The analytical expression for the complete pH dependence of the E_m can be derived as follows:

$$\frac{\{ox\}\{H^+\}}{\{oxH\}} = K_{ox}; \quad \frac{\{red\}\{H^+\}}{\{redH\}} = K_{red} \tag{1-81}$$

$$E_h = E_{mh} - 59\,log\frac{\{total_{red}\}}{\{total_{ox}\}} \tag{1-81a}$$

$$= E_{mh} - 59\,log\frac{\{red + redH\}}{\{ox + oxH\}} \tag{1-81b}$$

since $\{total_{red}\} = \{red\} + \{red \cdot H^+\}$, and
$\{total_{ox}\} = \{ox\} + \{ox \cdot H^+\}$; then, from (1-81),

$$\{red\} = K_{red}\frac{\{redH\}}{\{H^+\}}$$

$\{ox\} = K_{ox}\frac{\{oxH\}}{\{H^+\}}$; then, substituting into (1-81b),

$$E_h = E_{mh} - 59\log\frac{redH \cdot \frac{K_{red}}{H^+} + redH}{oxH \cdot \frac{K_{ox}}{H^+} + oxH} \tag{1-81c}$$

then, factoring,
$$E_h = E_{mh} - 59\log\frac{redH}{oxH}\ \frac{(1\ +\ \frac{K_{red}}{H^+})}{(1+\frac{K_{ox}}{H^+})} \tag{1-81d}$$

$$= E_{mh} - 59\log\frac{redH}{oxH} - 59\log\frac{1+\frac{K_{red}}{H^+}}{1+\frac{K_{ox}}{H^+}} \qquad (1\text{-}81e)$$

For pH << pK_{ox}, pK_{red}, $[H^+]$>> K_{ox}, K_{red}, $E_h = E_{macid} - 59\log\frac{\{redH\}}{\{oxH\}}$ and

$$-59\log\frac{\{redH\}}{\{oxH\}} = E_h - E_{macid}\text{; substituting into (1-81e)}$$

$$E_h = E_h + E_{mh} - E_{macid} - 59\,log\frac{1+\frac{K_{red}}{H^+}}{1+\frac{K_{ox}}{H^+}} \qquad (1\text{-}81f)$$

Then, rearranging terms in (1-81f):

$$E_{mh} = E_{macid} + 59\log\frac{1+\frac{K_{red}}{H^+}}{1+\frac{K_{ox}}{H^+}}; \qquad (1\text{-}81g)$$

$$E_{mh} = E_{macid} - 59\,log\frac{1+\frac{K_{ox}}{H^+}}{1+\frac{K_{red}}{H^+}} \qquad (1\text{-}82)$$

Formula (1-82) describes the complete pH dependence of the E_m (Dutton, 1978). It can be seen that E_{mh} has the following values or pH dependence for the three regions of pH (a–c below)

(a) pH << pK_{ox} << pK_{red}: $E_{mh}=E_{macid}$ (1-82a)

(b) pH >> pK_{red} >> pK_{ox}: $E_{mh} = E_{macid} - 59\log\frac{K_{ox}}{K_{red}}$ (1-82b)

(c) pK_{ox} << pH <<pK_{red}: $E_{mh} = E_{acid} - 59\log\frac{K_{ox}}{\{H^+\}} = E_{macid} - 59\,pH - 59\log K_{ox}$ (1-82c)

Homework problem 23. If the pK_{ox} and pK_{red} of cytochrome *x* are 3 and 10, respectively, and E_{macid} = +300 mV, what is the E_{m7} of the cytochrome?

Homework problem 24. (A) If E_{m7} = +65 mV for the mitochondrial n = 2 ubiquinone/ubiquinol couple, what is the value of E at pH = 7 at which it is (a) 50% reduced, (b) 90% reduced, (c) 9% reduced? (B) If the E_{m7} = +260 mV of benzo-hydroquinone (fig. 1-14A), what is its E^o?

Homework problem 25. (a) Calculate the E_{m7} of the two semiquinone redox pairs that interact on the p-side quinone binding niche of the mitochondrial membrane if the semiquinone formation constant = 10^{-2} and the E_{m7} of the UQ/UQH_2 couple is =65 mV. (b) What is the semiquinone formation constant that will allow formation of a semiquinone whose E_{m1} at pH 7 equals that of one of the hemes with E_{m7} = -112 mV of the integral membrane *b* cytochrome in which the quinone niiche is embedded? [Thus, the semiquinone can readily reduce the *b* heme, which is a demonstration of "oxidant-induced reduction"].

Homework problem 26. If the redox span of the mitochondrial respiratory chain extends from –320 mV [E_{m7} ($NAD^+/NADH$)] to + 815 mV [E_{m7} (O_2/H_2O)], (a) calculate the ΔG made available from the transfer of 2 electrons from NADH to O_2 with the electron donor and acceptor operating at their mid-point potentials. (b) Calculate the number of ATP molecules that can be synthesized per pair of electrons transferred down the respiratory chain from NADH to O_2 if $\Delta G^o{}_{ATP}$ = 33.5 kJ/mol, the ATP/ADP ratio = 100, and the phosphate concentration is 10^{-2} M.

1-12.10 Redox Properties of Semiquinones

In addition to the two-electron oxidation-reduction of quinines (1-77), one-electron reactions involving the half-reduced semiquinone form of the quinone-quinol couple also occur (Rich and Bendall, 1980).

If the two electron quinone reaction is:

$$Q + 2e^- + 2H^+ \leftrightarrow QH_2 \quad \text{for the neutral quinol, and} \tag{1-83a}$$

$$Q + 2e^- + H^+ \leftrightarrow QH^-, \text{ for the anionic quinol} \tag{1-83b}$$

with midpoint potential $\equiv E_{mh}$, then the reactions involving the semiquinone are:

$$Q + e^- + H^+ \leftrightarrow QH^\bullet; \text{ neutral semiquinone} \tag{1-83c}$$

$$Q + e^- \leftrightarrow Q^{\dot{-}}; \text{ anionic semiquinone} \tag{1-83d}$$

with midpoint potential, E_{m1}, and

$$QH^\bullet + e^- + H^+ \leftrightarrow QH_2, \tag{1-83e}$$

$$Q^{\dot{-}} + e^- + H^+ \leftrightarrow QH^- \tag{1-83f}$$

with midpoint potential $\equiv E_{m2}$.

The other reaction linking the fully reduced (quinol), oxidized (quinone), and half-reduced (semiquinone) components is that of semiquinone "dismutation" into the oxidized quinone, Q, and the reduced quinol, QH_2:

$$Q + QH_2 \leftrightarrow 2QH^\bullet, \tag{1-83g}$$

$$Q + QH^- \leftrightarrow 2Q^{\dot{-}} + H^+ \tag{1-83h}$$

The equlibrium constant, K_s, for semiquinone formation,

$$K_s = \left\{ \frac{\left(QH^\bullet\right)^2}{(Q)(QH_2)} \right\}_{eq}, \text{or} \tag{1-83i}$$

$$K_s = \left\{ \frac{\left(Q^{\dot{-}}\right)^2 \left(H^+\right)}{(Q)\left(QH^-\right)} \right\}_{eq} \tag{1-83j}$$

is a measure of the stability of the semiquinone.

The pK values of ($QH_2 \rightarrow QH^- + H^+$) and ($QH^{\cdot} \rightarrow Q^{\dot{-}} + H^+$) are approximately 11 and 5–6 respectively, for duroquinol and durosemiquinone (Fig. 1-14c).

The midpoint potential of the two electron quinone reduction is, in general, not equal to those of the individual one electron reactions, i. e., ,$E_{mh} \neq E_{m1} \neq E_{m2}$, for the different quinone redox reactions because the affinity of the quinone (Q) for one electron reduction to the semiquinone ($QH^{\cdot}$) is different from its affinity for cooperative reduction to the quinol (QH_2) by two electrons, and also different from the affinity of the semiquinone for one electron reduction to the quinol. If the E_m of the ($Q/QH^{\cdot}$) reaction decreases, then that of the ($QH^{\cdot}/QH_2$) reaction must increase to balance the free energy change for the net (Q/QH_2) reaction. This follows from the fact that the pathway from the quinone through the semiquinone to the quinol is a second route for quinone reduction to the quinol through two one-electron reactions, from a common initial state (quinone) to the same final state (quinol). In addition, the more unstable the $QH^{\cdot}$ (smaller value of K_S), the stronger the reducing nature of $QH^{\cdot}$ or $Q^{\dot{-}}$, and therefore the more negative the E_m of $Q/QH^{\cdot}$. Thus, there should be some definite relations between E_{mh}, E_{m1}, E_{m2}, and K_S.

To derive the relationships between E_{m1}, E_{m2}, and K_2, let E_1 and E_2 be the working potentials of the one electron reactions reactions (1-83d) and (1-83f). Then,

$$E_1 = E_{m1} - 59 \log \frac{\left\{Q^{\dot{-}}\right\}}{\{Q\}}$$

$$E_2 = E_{m2} - 59 \log \frac{\left\{QH^-\right\}}{\left\{Q^{\dot{-}}\right\}\left\{H^+\right\}}.$$

Using a condition at which a relationship can be found between the constants, at equilibrium, $E_1 = E_2$,

and, $E_{m1} - 59 \log \left| \frac{\left\{Q^{\dot{-}}\right\}}{\{Q\}} \right|_{eq} = E_{m2} - 59 \log \left| \frac{\left\{QH^-\right\}}{\left\{Q^{\dot{-}}\right\}\left\{H^+\right\}} \right|_{eq}$; rearranging terms,

$$E_{m1} - E_{m2} = 59 \log \left| \frac{\left\{Q^{\dot{-}}\right\}^2 \left\{H^+\right\}}{\{Q\}\left\{QH^-\right\}} \right|_{eq} = 59 \log K_s, \tag{1-84}$$

Where K_S is the semiquinone formation constant.

If $K_S > 1$, indicating that the semiquinone species is stable, $E_{m1} > E_{m2}$, and the couple $\left(Q/Q^{\dot{-}}\right)$ operates at a more oxidizing (more positive) potential than the couple $QH^{\cdot}/QH_2$. However, if $K_S < 1$, and the semiquinone is unstable, then $E_{m1} < E_{m2}$, and the $\left(Q/Q^{\dot{-}}\right)$ couple provides a stronger reductant than the $QH^{\cdot}/QH_2$ couple. In the case of semiquinone species thought to interact with proteins, the K_S has been found to be 10^{-1}–10^{-2} in mitochondria and

chromatophores, which corresponds to E_{m1}–E_{m2} = –60 to –120 mV and a significant concentration of semiquinone.

1-12.10.1 Relationship of Midpoint Potentials of the Quinone/Quinol *n* = 2 Reaction (E_{mh}) and the Semiquinone *n* = 1 Reactions (E_{m1}, E_{m2})

To determine the relationship of the midpoint potentials of the reactions described in Eqns. (1-83a-f) , note that free energy changes, ΔG_1 and ΔG_2 are associated wih the one electron reactions,

$$Q + e^- \leftrightarrow Q^{\dot{-}} \quad (1\text{-}83d)$$

$$Q^{\dot{-}} + e^- + \mathrm{H}^+ \leftrightarrow QH^-, \quad (1\text{-}83f)$$

Because the two electron reaction described in Eqn (1-83b) is the sum of (1-83d) and (1-83f),

$$Q + 2e^- + H^+ \leftrightarrow QH^- \quad (1\text{-}83b)$$

$$\Delta G(Q/QH^-) = -nF \cdot E(Q/QH^-) = -2F \cdot E(Q/QH^-)$$

$$= -F \cdot E(Q/Q^{\dot{-}}) - F \cdot E(Q^{\dot{-}}/QH^-)$$

$$= \Delta G\left(Q/Q^{\dot{-}}\right) + \Delta G\left(Q^{\dot{-}}/QH^-\right)$$

$$= -F\left(E_{m1} - 59\log_{10}\frac{\{Q^{\dot{-}}\}}{\{Q\}}\right) - F\left(E_{m2} - 59\log\frac{\{QH^-\}}{\{H^+\}\{Q^{\dot{-}}\}}\right),$$

and combining the arguments of both log terms,

$$-2F \cdot E(Q/QH^-) = -F(E_{m1} + E_{m2}) + 59F \log\frac{\{QH^-\}}{\{Q\}\{H^+\}}$$; dividing both sides by -2F,

$$E(Q/QH^-) = \frac{E_{m1} + E_{m2}}{2} - \frac{59}{2}\log\frac{\{QH^-\}}{\{Q\}\{H^+\}}; \quad (1\text{-}85)$$

The relation between the different midpoint potentials is then:

$$E_m(Q/QH_2) = \frac{(E_{m1} + E_{m2})}{2}; \quad (1\text{-}86)$$

the midpoint potential of the two electron reaction is then the average of the two one-electron semiquinone reactions.

Then, eqns. (1-84) and (1-86) are the two equations that allow determination of the two unknowns, E_{m1} and E_{m2} in terms of E_m, the midpoint potential of the 2-electron quinone reduction, and K_s, the semiquinone formation constant. The two cases are:

(A) K_S <1, then from Eq. 71, $E_{m1} < E_m < E_m$ (1-87)

so that the strength of the reductant in the semiquinone/quinone couple > that of quinol/quinone > that of quinol/semiquinone. Similarly,

$$\text{(B) } K_S > 1, \quad E_{m1} > E_m > E_{m2}. \tag{1-88}$$

From Eqns. (1-84) and [1-(86-88)], it can be seen that the splitting of E_{m1} and E_{m2} around E_m is symmetric, so that the two cases described in Eqns.[1-(88-89)] can be arranged in an E_m level diagram (Fig. 1-17). The more reducing potential of E_{m1} for the $(Q/Q^{\dot{-}})$ couple when Ks < 1 (Fig 1-16A) has biological importance because such a reducing semiquinone species is generated when ubi- or plastoquinol is oxidized by the cytochrome bc_1 or b_6f complex in respiratory and photosynthetic electron transport (Mitchell, 1976).

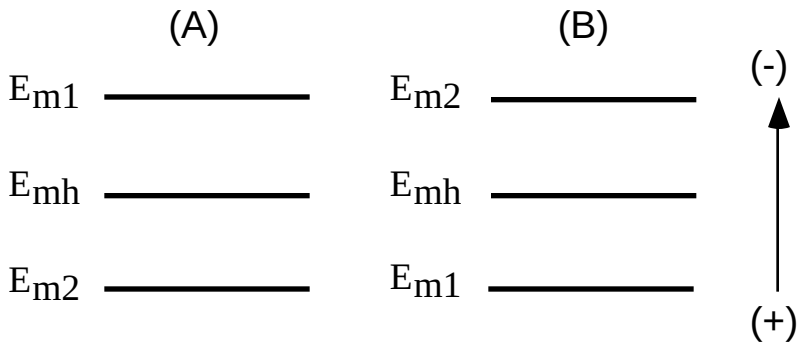


Figure 1-17. Relative E_m values for $K_S < 1$ (A) and $K_S > 1$ (B); $|K_S|$ the same in (A), (B).

Homework Problem 27. Determine the midpoint potential of a mitochondrial cytochrome *b* heme if it was found through titration of its optical spectrum at 25 °C to be 90 % and 99 % reduced at potentials of –150 mV and – 209 mV, and 90% and 99 % oxidized at –32 mV and +27 mV.

Homework Problem 28. Calculate the E_{m7} of the two semiquinone redox pairs that interact at the p-side plasto quinone binding site of the chloroplast membrane if the semiquinone dismutation constant = 10^{-2} and the E_{m7} of the PQ/PQH_2 redox couple is + 80 mV; temperature = 25°C. Will the E_{m1} of the $PQ/PQ^{\dot{-}}$ be favorable for reduction of the p-side heme of cytochrome b_6 whose E_{m7} = -50mV?

Homework Problem 29. The synthesis of ATP in mitochondrial membranes can be coupled to the oxidation of the anionic quinol [E_m ($QH^{\cdot}/QH^-$) = + 209 mV] by the high potential iron-sulfur protein, and subsequent electron transfer to a metal center of cytochrome oxidase, [E_m = 491 mV]. If all of the free energy made available in the electron transfer from the quinol to the oxidase is converted to a proton electrochemical potential, $\Delta\tilde{\mu}_{H+}$

(i) What is the value of the ΔG for consecutive transfer (n =1) of *two* electrons, with a ratio of reduced: oxidized quinone = 10:1, and of reduced: oxidized oxidase = 1:10?

(ii) Calculate the efficiency for conversion of redox energy to ATP synthesis at 25° C, made available from the transfer of **two** electrons, if the ATP/ADP poise is 100, the phosphate concentration is 10^{-2} M, and ΔG^{o}_{ATP} = + 8 kcal/mol.

1-13. Free Energy of Activation for Electron Transfer

Consider the transfer of an electron from a donor, *D*, to an acceptor, *A*: $D^- + A \rightarrow (D^- – A) \rightarrow (D – A^-) \rightarrow D + A^-$, where the docked donor (reactant) and acceptor (product) complex marked in parentheses are included in the reaction. An activation energy for the electron transfer arises because (a) the electron in the donor complex has a different potential and free energy than the acceptor complex; (b) the transfer of the electron results in a change of the internuclear distances in the atoms that surround the electron, and (c) a "reorganization energy". As shown in Fig 1-18, the reorganization energy, λ, is the energy that is required to change the average internuclear distance, X_D^o, of the reactant, to that X_A^o, of the product complex without electron transfer. This can be described in a diagram showing the energy of the electron in the donor and acceptor complex, which moves in the electrostatic field created by the nuclei, as a function of the average internuclear distance in the donor (*D*) and acceptor (*A*) complex (Figure 1-18).

The G_D and G_A curves in Fig 1-18 are assumed to be equal parabolae corresponding to oscillators with the same frequency. The equation of the two curves are (DeVault, 1984):

$$G_D = k_H \frac{\left(X - X_D^o\right)^2}{2} + G_D^o \qquad (1\text{-}89a)$$

$$G_A = k_H \frac{\left(X - X_A^o\right)^2}{2} + G_A^o, \qquad (1\text{-}89b)$$

with k_H = force constant (Hooke's law) of the parabolae. From Figure 1-18, measuring from X_D^o

(i) $$\lambda = k_H \frac{\left(\Delta X^o\right)^2}{2}, \qquad (1\text{-}90)$$

and (ii) $$\Delta G^{\ddagger} = k_H \frac{\left(\Delta X^{\ddagger}\right)^2}{2}; \qquad (1\text{-}91a)$$

then, measuring from X_A^o, (iii) $$\Delta G^{\ddagger} = k_H \frac{\left(\Delta X^o - \Delta X^{\ddagger}\right)^2}{2} + \Delta G^o \qquad (1\text{-}91b)$$

with $\Delta G = G_A^o - G_D^o$. Combining (i), (ii), and (iii) gives

(iv) $$\Delta G^{\ddagger} = \frac{\left(\lambda + \Delta G^o\right)^2}{4\lambda}. \qquad (1\text{-}92),$$

the activation energy, sometimes called the Franck-Condon term.

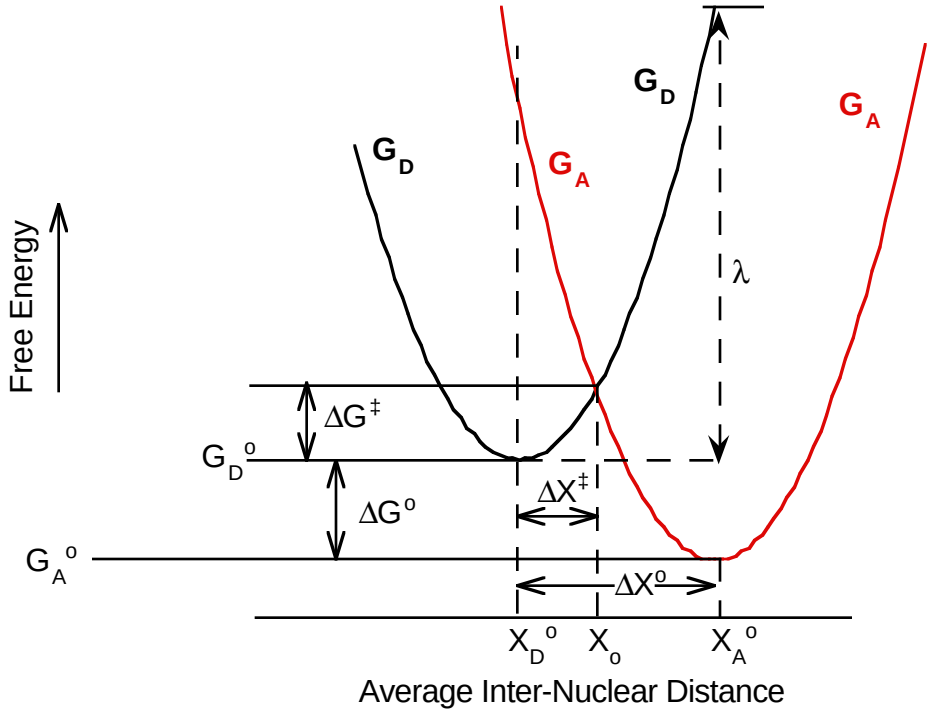


Figure 1-18. Free energy of the electron in the donor (D) and acceptor (A) complexes as a function of the average internuclear distance. X_D^o, X_A^o are the inter-nuclear distances for the donor and acceptor complexes at equilibrium, and $\Delta X^{\ddagger}$, the distance change associated with the gain of activation energy, $\Delta G^{\ddagger}$, by the donor complex. $\left(X_A^o - X_D^o\right) = \Delta X^o$; λ = reorganization energy. (modified from (DeVault, 1984)).

Activation-less electron transfer. It can be seen from Eqn (1-92) that $\Delta G^{\ddagger} = 0$, and the electron transfer is activation-less, when $\Delta G^o = -\lambda$. In principle, λ can be determined by measuring the rate of electron transfer as a function of ΔG^o that gives the maximum rate.

"Inverted region." When $-\Delta G^o > \lambda$, $\Delta G^{\ddagger} \neq 0$, and the cross-over coordinate X_o, of the G_A and G_D energy curves is at a position $X^o < X_D^o$.

Under conditions where the electronic coupling between donor and acceptor is relatively weak, the rate constant for electron transfer, k_{et}, is proportional to (a) the "Franck-Condon" term that includes the effect of the activation energy, Eqn (1-93), and as well (b) to a proportionality factor A(T) that is a function of temperature, and a function $e^{-\beta d}$, that describes the dependence on donor-acceptor distance, d. The distance decay constant, β, depends upon details of local structure. Thus,

$$k_{et} = A(T) \cdot \exp(-\beta d) \cdot \exp(-\Delta G^{\ddagger} / k_B T) \qquad (1\text{-}93)$$

where k_B = Boltzmann's constant, 1.38 x 10^{-23} J K^{-1}. Further reading on the above aspects of intraprotein electron transfer (Marcus and Sutin, 1985; Gray and Winkler, 1996; Page *et al.*, 1999).

Homework Problem 30. Tabulate or graph the dependence of the relative rate of electron transfer between electron donor and acceptor as a function of the ΔG^o for the transfer between them, varied from +0.1 eV to –1.0 eV, with the reorganization energy, λ, = 0.6 eV. Assume all other parameters of the electron transfer, including donor-acceptor distance, are constant. For what value of ΔG^o is the rate a maximum? Sketch approximate Marcus potential energy diagrams for reactant (R) and product (P) states for ΔG^o = + 0.1 eV, – 0.6 eV and – 1.0 eV. (The main parameter of interest is $\Delta G^{\ddagger}$).

Homework Problem 31. Calculate the activation energy for electron transfer (a) from the quinone, Q_A, to quinone, Q_B, if the $\Delta G^o/F$ for the reaction = +0.1V; and from bacteriopheophytin to Q_A if the $\Delta G^o/F$ = – 0.7 V. Assume that the reorganization energy, λ, in both cases = +0.6 V.

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