THERMODYNAMICS OF ELECTRON TRANSFER AND ITS COUPLING TO VECTORIAL PROCESSES IN BIOLOGICAL MEMBRANES

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ABSTRACT A method is developed to express the flux of an electron transfer reaction as a function of the conjugate force, the redox potential difference, throughout the nonlinear region. The flux can be expressed by a product of the hyperbolic sine of the force, a factor ("redox-poising parameter") determined by the redox potentials of subsystem (in certain cases by local pH's and p*K*'s of subsystems), and some constants. This is analogous to the expression of the flux of a diffusion process by the product of its force and the concentration of the diffusing species. The redox-poising parameter corresponds to the concentration term. The expression is applied to redox chains in which electron transfers are coupled to vectorial processes such as proton translocation or electric current.

INTRODUCTION

Recent studies in bioenergetics have shown that many of the chemical reactions that occur in biological membranes have spatial direction and, in this respect, are analogous to physical diffusion (Mitchell, 1970; Harold, 1977). In isotropic systems, such as those composed of soluble enzymes and metabolic intermediates, chemical reactions and physical diffusion are thermodynamically quite distinct processes. Chemical reactions are scalar processes, whereas diffusion is vectorial. A scalar process and a vectorial process cannot be coupled to each other unless the system is asymmetric (Curie's principle; see Bunow, 1978). Biomembranes, however, are heterogeneous, anisotropic systems where chemical reactions can have vectorial properties. Coupling of vectorial reactions and other vectorial processes, such as diffusion or electric current, can occur readily in such systems.

The idea of "chemiosmotic coupling" (Mitchell, 1970), the coupling of chemical processes and diffusion, is an important conceptual framework for the study of energy conversion in biological membranes. Various forms of chemiosmotic coupling have been observed in biomembranes (Harold, 1977; Skulachev, 1977). A particularly important example of this concept is the "chemiosmotic theory" that oxidative and photosynthetic phosphorylation are composed of two chemiosmotic couplings, the coupling of electron transfer to proton translocation, and of proton translocation to the phosphorylation of ADP (Mitchell, 1961). Macroscopically, mitochondria perform coupling of vectorial proton translocation and scalar oxygen consumption. The elementary processes of which the chemiosmotic coupling is composed are, however, directed electron or hydrogen transfer in the membrane (Mitchell,

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1961, 1970); these are vectorial chemical reactions. Asymmetric organization of vectorial reactions can lead to macroscopic chemiosmotic coupling.

In heterogeneous systems such as biological membranes, activity coefficients of diffusing molecules or ions are not necessarily uniform. Molecules can diffuse from a lower to a higher concentration region, if the latter region has a lower activity coefficient for the molecule. For example, if the membrane contains acid/base groups, the activity of hydrogen ions (a_H) and the chemical potential of hydrogen ions (μ_H) can be defined by:

$$\ln a_H = \frac{\mu_H}{RT} = \frac{\mu_H^0}{RT} + \ln \frac{(\text{acid})}{(\text{base})}. \tag{1}$$

This expression can be used even in the absence of hydonium ions. Here, R and T are the gas constant and absolute temperature, respectively, μ_H^0 is a constant, and $-\mu_H^0/2.3RT$ corresponds to the pK of the acid-base couple. μ_H and a_H can be functions of spatial position within a membrane, and a gradient of the chemical potential ($\nabla\mu_H$) can cause H^+ flux. The activity and chemical potential of electrons or chemical groups transferred in a chemical reaction can be defined in a manner similar to Eq. 1, and can also be functions of position. For example, the activity and chemical potential of electrons (a_e and μ_e, respectively) become (Dutton and Wilson, 1974):

$$\ln a_e = \frac{\mu_e}{RT} = \frac{\mu_e^0}{RT} + \frac{1}{z} \ln \frac{(\text{red})}{(\text{ox})}, \tag{2}$$

where (red) and (ox) stand for the activities of a redox couple at the position considered, in the reduced and oxidized states, respectively. z is the number of electrons transferred during oxidation or reduction, $-\mu_e/F$ and $-\mu_e^0/F$ correspond to the redox potential (E_h) and the standard redox potential (E_0) of the couple, and F is the Faraday constant. $\Delta\mu_e$, the difference of μ_e between two redox couples at different spatial coordinates, becomes a force for the redox reaction or for the flux of electrons.

The flux of a diffusion process can be represented as a linear function of the conjugate force, which is the gradient of the chemical potential of the diffusing species. The flux of the diffusion of species j, $\mathbf{J}_j$, becomes:

$$\mathbf{J}_j = -u_j c_j \nabla\mu_j, \tag{3a}$$

where c_j and μ_j are the concentration and chemical potential of j, respectively, and u_j is a constant called the mobility of species j. $-\nabla\mu_j$, the gradient of μ_j, is the force conjugate to the flux $\mathbf{J}_j$. Both the flux and the force are, generally, three-dimensional vectors. If the diffusing species is electrically charged, the diffusion is coupled with electric current and Eq. 3a must contain an electrical term:

$$\mathbf{J}_j = -u_j c_j \nabla(\mu_j + z'F\psi) = -u_j c_j \nabla\tilde{\mu}_j, \tag{3b}$$

where z' is the charge on the diffusing species. $\tilde{\mu}_j (\equiv \mu_j + z'F\psi)$ is called the electrochemical potential. Similarly, the flux and the force of chemical processes (the rate and the "affinity" of the reaction, respectively) are linearly related, as long as the system is close to equilibrium

(Prigogine, 1967). The flux of electrons (J_e) in a redox chain is a linear function of $\Delta\mu_e$ if $z\Delta\mu_e$ is small enough relative to the value RT:

$$J_e = L_e \Delta\mu_e. \tag{4a}$$

The coefficient L_e is determined by the rate constant of the reaction and the redox states of the redox couples. When μ_e is a function of position, the force and the flux of electrons are vectors within the same spatial coordinates as the diffusion processes to which the electron transfer is coupled (for an example, see Appendix B in Arata and Nishimura, 1979). When the reaction accompanies a movement of net charges, Eq. 4a contains an electrical term as Eq. 3b does:

$$J_e = L_e \Delta(\mu_e + z'F\psi) = L_e \Delta\tilde{\mu}_e. \tag{4b}$$

z' is equal to $-z$ if the electron is the only charged species that is transferred.

The linear expression for chemical processes is usually valid only in a very limited region, close to equilibrium where the reaction affinity is much smaller than RT. The purpose of this paper is to express electron transfer as a nonlinear function relating the flux and the force. An important problem of the nonlinear region is that the process is not necessarily reciprocal.

Eq. 3b implies a reciprocity in the coupling of the diffusion and the electric current:

$$\frac{\partial \mathbf{J}_j}{\partial(\nabla\psi)} = \frac{\partial \mathbf{i}}{\partial(\nabla\mu_j)}, \tag{5}$$

where $\mathbf{i}$ ($= z'F\mathbf{J}_j$) stands for the electric current density. Eq. 4b implies a similar reciprocal relation:

$$\frac{\partial J_e}{\partial(\Delta\psi)} = \frac{\partial \mathbf{i}}{\partial(\Delta\mu_e)}. \tag{6}$$

The reciprocity is always true at near equilibrium (Prigogine, 1967) and is well known as Onsager's relation. However, it is not necessarily true far from equilibrium. Another nonreciprocity arises in chemical processes. A perturbation of the reaction affinity caused by changing the concentration of the reactant does not produce the same effect on the reaction rate as does a perturbation caused by changing the concentration of the product (Oster et al., 1973). This means that the rate of a chemical reaction is not necessarily a unique function of the conjugate force. Our expression will demonstrate the nonreciprocity as a common feature of chemiosmotic processes.

We are concerned with the thermodynamics of the elementary processes constituting an electron transfer chain. To characterize the elementary processes is important, because the thermodynamics of the overall processes are restricted by those of the elementary processes; for example, any combinations of linear processes are linear and any combinations of reciprocal processes are reciprocal (Oster et al., 1973).

ELECTRON TRANSFER IN THE ABSENCE OF ANY ENERGETICAL COUPLING

We first consider an electron transfer independent of other processes. The situation is idealized because an electron transfer always involves either movement of the charge carried by electrons or movement of other charged species, for example, protons, or both. However, it can approximate an electron transfer which does not involve the movement of any other charged species, under conditions where the electric field component of $\Delta\tilde{\mu}_e$ is negligible. This condition is always approximately valid if the reaction is in a direction parallel to the membrane. It is also realized by dissipating the transmembrane potential using ionophores if the reaction is perpendicular to the membrane. In the following sections, we shall consider electron transfer coupled with proton translocation or that coupled with electric current.

Consider a simple electron transfer reaction between molecules A and B:

$$A_R \cdot B_O \underset{k_-}{\overset{k_+}{\rightleftharpoons}} A_O \cdot B_R \tag{7}$$

Subscripts R and O represent reduced and oxidized states, respectively. Electron transfer from A to B in the complex $A_R \cdot B_O$ occurs with rate constant k_+, and reverse electron transfer in $A_O \cdot B_R$ occurs with rate constant k_-. The net rate of the reaction J, the "flux" of the process, becomes:

$$J = k_+(A_R \cdot B_O) - k_-(A_O \cdot B_R). \tag{8}$$

The conjugate force to J, the affinity $\mathcal{A}$, is:

$$\mathcal{A} = \mathcal{A}_0 + RT \ln \frac{\Pr(A_R \cdot B_O)}{\Pr(A_O \cdot B_R)}$$

$$\mathcal{A}_0 = RT \ln \frac{k_+}{k_-}, \tag{9}$$

where Pr means the probability of the state indicated in the parentheses. Our first problem is to represent J as a function of $\mathcal{A}$.

Eq. 8 can be rewritten as:

$$J = kN\left[\exp\left(\frac{\mathcal{A}_0}{2RT}\right) \cdot \Pr(A_R \cdot B_O) - \exp\left(-\frac{\mathcal{A}_0}{2RT}\right) \cdot \Pr(A_O \cdot B_R)\right], \tag{10}$$

where k is $\sqrt{k_+ \cdot k_-}$ and N is the total number of the A-B pairs. We now introduce two new parameters, α and $\Delta\mathcal{A}$. α is the fraction of the A-B pairs that are in the reactive states $A_R \cdot B_O$ and $A_O \cdot B_R$. $\Delta\mathcal{A}$ is the degree of departure of $\mathcal{A}$ from $\mathcal{A}_0$:

$$\alpha \equiv \Pr(A_R \cdot B_O) + \Pr(A_O \cdot B_R) \tag{11}$$

$$\Delta\mathcal{A} \equiv \mathcal{A} - \mathcal{A}_0 = RT \ln \frac{\Pr(A_R \cdot B_O)}{\Pr(A_O \cdot B_R)}. \tag{12}$$

Using these parameters, Eq. 10 can be rewritten as:

$$\frac{J}{kN\alpha} = \frac{\exp\left(\frac{\mathcal{A}_0}{2RT}\right)}{1 + \exp\left(-\frac{\Delta\mathcal{A}}{RT}\right)} - \frac{\exp\left(-\frac{\mathcal{A}_0}{2RT}\right)}{1 + \exp\left(\frac{\Delta\mathcal{A}}{RT}\right)}$$

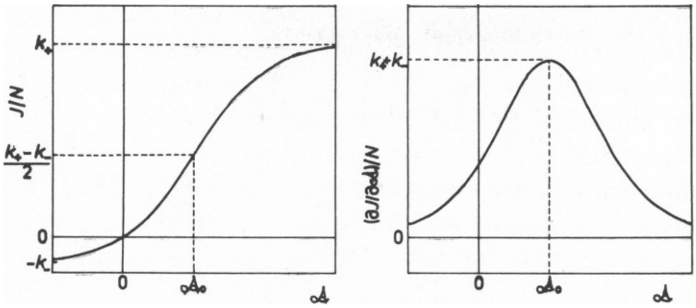

FIGURE 1 Flux (left) and dynamic conductance (right) of the electron transfer process as a function of affinity according to Eq. 11, at constant α.

or

$$\frac{J}{kN\alpha} = \frac{\sinh\left(\frac{\mathcal{A}}{2RT}\right)}{\cosh\left(\frac{\Delta\mathcal{A}}{2RT}\right)}. \tag{13}$$

In Fig. 1 (left), J is shown as a function of $\mathcal{A}$ at constant α. The maximum and minimum asymptotes of J correspond to the forward reaction rate in the absence of the back reaction, and to the back reaction rate in the absence of the forward reaction, respectively. If $\mathcal{A}_0$ is large and positive, the flux remains small until the affinity $\mathcal{A}$ becomes positive and approaches $\mathcal{A}_0$. The derivative of J with respect to $\mathcal{A}$, which can be called "incremental conductance" (Oster et al., 1971, 1973) or "dynamic conductance" (Schonfeld and Neuman, 1977), is shown in Fig. 1 (right). It has a maximum value when $\mathcal{A} = \mathcal{A}_0$.

α is always 1 if the redox reactions of A and B are completely dependent on each other, i.e., if the reduction of A necessarily accompanies the oxidation of B and *vice versa*. This situation applies when A and B cannot exchange electrons with other redox components. The equilibrium then can be disturbed only if charge separation between A and B is driven by an external energy source such as light. Ross (1977) has considered this situation in an analysis of the relationship between rate constants and free-energy loss (force necessary to give a particular flux). Our α corresponds to his $(1 - \alpha)$. In the completely dependent case, J is determined uniquely by $\mathcal{A}$. The reaction is essentially the same as the isomerization of a "single" species. In most cases, however, α is not equal to 1, and is not even constant.

Although J is a unique function of $\mathcal{A}$ in Eq. 11 at constant α, the numerator and denominator have quite different dependencies on $\mathcal{A}$. The numerator is a simple increasing (but nonlinear) function of $\mathcal{A}$. In contrast, the denominator has a minimum value at $\mathcal{A} = \mathcal{A}_0$.

Another limiting case concerning α is for the redox states of A and B to be completely independent:

$$\begin{aligned} \Pr(A_O \cdot B_R) &= \Pr(A_O) \cdot \Pr(B_R) \\ \Pr(A_R \cdot B_O) &= \Pr(A_R) \cdot \Pr(B_O). \end{aligned} \tag{14}$$

This situation applies when A and B rapidly and independently exchange electrons with their environments (Hill and Chance, 1978). In this case, α becomes:

$$\begin{aligned}\alpha &= \Pr(A_O) \cdot \Pr(B_R) + \Pr(A_R) \cdot \Pr(B_O)\\ &= \frac{1}{\exp(-a)+1} \cdot \frac{1}{\exp(b)+1} + \frac{1}{\exp(a)+1} \cdot \frac{1}{\exp(-b)+1},\end{aligned} \tag{15}$$

where

$$\begin{aligned} a &\equiv \ln \frac{\Pr(A_R)}{\Pr(A_O)} = \frac{z}{RT}\left[\mu_e(A) - \mu_{e0}(A)\right]\\ b &\equiv \ln \frac{\Pr(B_R)}{\Pr(B_O)} = \frac{z}{RT}\left[\mu_e(B) - \mu_{e0}(B)\right].\end{aligned} \tag{16}$$

The chemical potentials of electrons in A and B [$\mu_e(A)$ and $\mu_e(B)$, respectively] can be measured from their redox potentials of the respective environments.

Now we introduce two parameters. Let $\langle \bar{\mu}_e \rangle$ represent the degree of departure of the mean potential from the mean of the standard potentials:

$$\langle \bar{\mu}_e \rangle \equiv \frac{\mu_e(A) + \mu_e(B)}{2} - \frac{\mu_e^0(A) + \mu_e^0(B)}{2} = \frac{RT}{z} \cdot \frac{a+b}{2}. \tag{17}$$

Let $\langle \Delta\mu_e \rangle$ represent the degree of departure of the potential difference from the standard potential difference:

$$\langle \Delta\mu_e \rangle \equiv \frac{\mu_e(A) - \mu_e(B)}{2} - \frac{\mu_e^0(A) + \mu_e^0(B)}{2} = \frac{RT}{z} \cdot \frac{a-b}{2}. \tag{18}$$

$\langle \Delta\mu_e \rangle$ equals $\Delta\mathcal{A}/2z$. By using Eqs. 17 and 18, Eq. 15 can be rewritten:

$$\alpha = \frac{\cosh\left(\frac{z}{RT}\langle \Delta\mu_e \rangle\right)}{\cosh\left(\frac{z}{RT}\langle \Delta\mu_e \rangle\right) + \cosh\left(\frac{z}{RT}\langle \bar{\mu}_e \rangle\right)}. \tag{19}$$

Therefore,

$$J = kN \frac{\sinh \frac{\mathcal{A}}{2RT}}{\cosh\left(\frac{z}{RT}\langle \Delta\mu_e \rangle\right) + \cosh\left(\frac{z}{RT}\langle \bar{\mu}_e \rangle\right)} \tag{20a}$$

or

$$J_e = zkN \cdot \theta \cdot \sinh \frac{z\Delta\mu_e}{2RT}, \tag{20b}$$

where

$$\theta^{-1} = \cosh\left(\frac{z}{RT}\langle\Delta\mu_e\rangle\right) + \cosh\left(\frac{z}{RT}\langle\bar{\mu}_e\rangle\right). \tag{21}$$

J_e is the flux of electrons and $\Delta\mu_e[= \mu_e(A) - \mu_e(B)]$ corresponds to the conjugate force. zkN is a constant for the system. The hyperbolic-sine expression in Eq. 20b represents the nonlinear relationship between flux and force. The "redox-poising parameter," θ, is the factor that determines the "degree of operation" of the system, and is set by the redox potentials of the two electron transfer subsystems. θ has a maximum value when $\mu_e(A) = \mu_e^0(A)$ and $\mu_e(B) = \mu_e^0(B)$. This expresses the fact that electron transfer reactions become very slow if too many electrons are put into the system, so that all of the redox couples are reduced, or if too many electrons are removed, so that all are oxidized. Eq. 20a is a function of two independent variables, $\Delta\mu_e$ and $\langle\bar{\mu}_e\rangle$; $\langle\Delta\mu_e\rangle$ and $\Delta\mu_e$ are not independent of each other.

In the completely dependent case (Eq. 13 with $\alpha = 1$), $1/\cosh(\Delta\mathcal{A}/2RT)$ corresponds to θ. Since the mean redox potential is always constant in this case, a term depending on $\langle\bar{\mu}_e\rangle$ does not appear in θ.

In certain limiting cases, θ becomes proportional to the number of electrons in the system and corresponds to c_j in Eq. 3a. Suppose $\mu_e^0(A) = \mu_e^0(B)$. At very low redox potentials $[\mu_e(A) < \mu_e^0(A)$ and $|\mu_e(A) - \mu_e^0(A)| \gg |\Delta\mu_e|]$, we can regard $a = b$ and $\Pr(A_O) = 1$. Then,

$$\cosh\left(\frac{z}{RT}\langle\Delta\mu_e\rangle\right) \simeq \cosh(0) = 1$$

$$\cosh\left(\frac{z}{RT}\langle\bar{\mu}_e\rangle\right) \simeq \cosh(a) \simeq \frac{1}{2\Pr(A_R)}.$$

Since $\Pr(A_R)$ is very small relative to 1,

$$\theta \simeq 2\Pr(A_R). \tag{22}$$

$N \cdot \Pr(A_R)$ is the number of electrons in the system. At near equilibrium, the hyperbolic-sine term in Eq. 18 can be approximated by a first-order term:

$$\begin{aligned} J_e &\simeq zkN \cdot 2\Pr(A_R)\frac{z}{2RT}\Delta\mu_e \\ &= u_e c_e \cdot \Delta\mu_e, \end{aligned} \tag{23}$$

where $u_e = z^2k/RT$ and $c_e = N \cdot \Pr(A_R)$. Eq. 23 is same as Eq. 3. Thermodynamically, a diffusion process is a limiting case of a chemical process: a uniform system which has an infinitely large number of "binding sites" for the diffusing species.

COUPLING TO PROTON TRANSLOCATION

In the respiratory and photosynthetic redox chains, electron transfer is coupled to proton translocation. Mitchell (1970, 1976) has proposed that the reduced forms of some of the redox components bind protons as well as electrons and are responsible for proton-electron symport across the membrane. In Mitchell's (1961) original scheme, hydrogen-carrying redox components such as quinones diffuse in the membrane to carry protons and electrons together. A possible modification of this mechanism is the transfer of hydrogen atoms between two (or more) redox molecules (components) that are fixed in position in the membrane (Witt, 1971; Dutton and Prince, 1977). We shall consider the latter case in this paper.

Suppose that reduced A and reduced B both bind z_1 electrons and z_2 protons, so that the process in Eq. 7 includes a translocation of z_1 electrons and z_2 protons. If z_1 and z_2 are not the same, the reaction carries electric current. However, for simplicity, we shall neglect the coupling with electric events in this section, just as we did in the previous section. Under conditions that allow the "independence approximation," the ratios of the oxidized and the reduced states of A and B are determined by the chemical potentials of electrons and hydrogen ions in the corresponding environments:

$$a\left[= \ln \frac{\Pr(A_H)}{\Pr(A_O)}\right] = \frac{1}{RT}\{z_1[\mu_e(A) - \mu_e^0(A)] + z_2[\mu_H(A) - \mu_H^0(A)]\}$$

$$b\left[= \ln \frac{\Pr(B_H)}{\Pr(B_O)}\right] = \frac{1}{RT}\{z_1[\mu_e(B) - \mu_e^0(B)] + z_2[\mu_H(B) - \mu_H^0(B)]\}. \tag{24}$$

Here, subscript H stands for the protonated reduced states. μ_H and μ_H^0 are the chemical potential and standard chemical potential, respectively, of hydrogen ions in the subsystems; these can be defined in the same manner as μ_e and μ_e^0. $-\mu_e^0/F$ corresponds to the half reduction potential without protonation and $-\mu_H^0/2.3RT$ corresponds to the pK of the reduced state. The affinity of the reduction becomes:

$$\mathcal{A} = \mathcal{A}_0 + RT \ln \frac{\Pr(A_R) \cdot \Pr(B_O)}{\Pr(A_O) \cdot \Pr(B_R)} = z_1 \Delta\mu_e + z_2 \Delta\mu_H$$

$$\mathcal{A}_0 = z_1 \Delta\mu_e^0 + z_2 \Delta\mu_H^0. \tag{25}$$

The reaction rate J has exactly the same form as in the case without proton symport:

$$J = kN\theta \sinh\left(\frac{\mathcal{A}}{2RT}\right), \tag{26}$$

where the redox- and pH-poise parameter θ becomes:

$$\theta^{-1} = \cosh\left(\frac{z_1\langle\Delta\mu_e\rangle + z_2\langle\Delta\mu_H\rangle}{RT}\right) + \cosh\left(\frac{z_1\langle\bar{\mu}_e\rangle + z_2\langle\bar{\mu}_H\rangle}{RT}\right). \tag{27}$$

Here $\langle\bar{\mu}_H\rangle$ and $\langle\Delta\mu_H\rangle$ are similar to $\langle\bar{\mu}_e\rangle$ and $\langle\Delta\mu_e\rangle$, respectively:

$$\langle\bar{\mu}_H\rangle = \frac{\mu_H(A) + \mu_H(B)}{2} - \frac{\mu_H^0(A) + \mu_H^0(B)}{2} \tag{28}$$

$$\langle\Delta\mu_H\rangle = \frac{\mu_H(A) - \mu_H(B)}{2} - \frac{\mu_H^0(A) - \mu_H^0(B)}{2}. \tag{29}$$

The fluxes of electrons (J_e) and protons (J_H) become:

$$J_e = z_1 kN\theta \sinh \frac{z_1 \Delta\mu_e + z_2 \Delta\mu_H}{2RT} \tag{30a}$$

$$J_H = z_2 kN\theta \sinh \frac{z_1 \Delta\mu_e + z_2 \Delta\mu_H}{2RT}. \tag{30b}$$

Eqs. 30a and b are the "phenomenological equations" expanded to the nonlinear region. θ is a factor modulated by the chemical potentials of electrons and hydrogen ions, i.e., by the local redox potentials and the local pH's in the system.

In some cases, electron transfer and proton translocation will not be tightly coupled. For example, if the reduced components have pK's near physiological pH, they will be incompletely protonated. One then must consider three states for each redox component (oxidized, reduced, and protonated reduced), and three reactions (electron transfer, proton transfer, and hydrogen transfer):

$$A_R \cdot B_O \rightleftharpoons A_O \cdot B_R \tag{31a}$$

$$A_H \cdot B_R \rightleftharpoons A_R \cdot B_H \tag{31b}$$

$$A_H \cdot B_O \rightleftharpoons A_O \cdot B_H \tag{31c}$$

The fluxes for the three processes in Eq. 31 can be written:

$$J_i = k_i N\theta_i \sinh \frac{\mathcal{A}_i}{2RT} \quad (i = 1, 2, 3), \tag{32}$$

where the subscript i = 1 represents the electron transfer reaction (Eq. 31a), i = 2 represents proton transfer (Eq. 31b), and i = 3 represents hydrogen transfer (Eq. 31c). Calculation of three θ's is somewhat troublesome, but the process is essentially the same as before:

$$\begin{aligned}\theta_1^{-1} = {} & \cosh\left(\frac{z_1}{RT}\langle\Delta\mu_e\rangle\right) + \cosh\left(\frac{z_1}{RT}\langle\bar{\mu}_e\rangle\right) \\ & + \exp\left(-\frac{z_2}{RT}\langle\bar{\mu}_H\rangle\right)\cosh\left(\frac{1}{RT}\langle\Delta\mu\rangle\right) + \exp\left(-\frac{1}{RT}\langle\bar{\mu}\rangle\right)\cosh\left(\frac{z_2}{RT}\langle\Delta\mu_H\rangle\right) \\ & + \frac{1}{2}\exp\left(-\frac{1}{RT}\langle\bar{\mu}_H\rangle\right)\exp\left(-\frac{z_2}{RT}\langle\bar{\mu}_H\rangle\right)\end{aligned} \tag{33a}$$

$$\begin{aligned}\theta_2^{-1} = {} & \cosh\left(\frac{z_2}{RT}\langle\Delta\mu_H\rangle\right) + \cosh\left(\frac{z_2}{RT}\langle\bar{\mu}_H\rangle\right) \\ & + \exp\left(\frac{1}{RT}\langle\bar{\mu}\rangle\right)\cosh\left(\frac{z_1}{RT}\langle\Delta\mu_e\rangle\right) + \exp\left(\frac{z_1}{RT}\langle\bar{\mu}_e\rangle\right)\cosh\left(\frac{1}{RT}\langle\Delta\mu\rangle\right) \\ & + \frac{1}{2}\exp\left(\frac{1}{RT}\langle\bar{\mu}\rangle\right)\exp\left(\frac{z_1}{RT}\langle\bar{\mu}_e\rangle\right)\end{aligned} \tag{33b}$$

$$\theta_3^{-1} = \cosh\left(\frac{1}{RT}\langle\Delta\mu\rangle\right) + \cosh\left(\frac{1}{RT}\langle\bar{\mu}\rangle\right)$$
$$+ \exp\left(-\frac{z_1}{RT}\langle\bar{\mu}_e\rangle\right)\cosh\left(\frac{z_2}{RT}\langle\Delta\mu_H\rangle\right) + \exp\left(\frac{z_2}{RT}\langle\bar{\mu}_H\rangle\right)\cosh\left(\frac{z_1}{RT}\langle\Delta\mu_e\rangle\right)$$
$$+ \frac{1}{2}\exp\left(\frac{z_2}{RT}\langle\bar{\mu}_H\rangle\right)\exp\left(-\frac{z_1}{RT}\langle\bar{\mu}_e\rangle\right) \tag{33c}$$

$$\frac{\theta_3}{\theta_1} = \exp\left(-z_2\langle\bar{\mu}_H\rangle\right), \quad \frac{\theta_3}{\theta_2} = \exp\left(z_1\langle\bar{\mu}_e\rangle\right) \tag{34}$$

where

$$\langle\Delta\mu\rangle \equiv z_1\langle\Delta\mu_e\rangle + z_2\langle\Delta\mu_H\rangle \tag{35}$$

$$\langle\bar{\mu}\rangle \equiv z_1\langle\bar{\mu}_e\rangle + z_2\langle\bar{\mu}_H\rangle. \tag{36}$$

Eq. 34 means that the coupling between proton and electron flux becomes loose at high pH, because of the electron transfer reaction, and at low redox potential, because of the proton transfer reaction. We have observed the former case (Arata and Nishimura, 1978, 1979) in the cyclic electron transfer system in photosynthetic bacteria.

Since

$$J_e = z_1(J_1 + J_3), \quad J_H = z_2(J_2 + J_3)$$

$$\mathcal{A}_1 = z_1\Delta\mu_e, \quad \mathcal{A}_2 = z_2\Delta\mu_H, \quad \mathcal{A}_3 = z_1\Delta\mu_e + z_2\Delta\mu_H,$$

the "phenomenological equations" for this system become:

$$J_e = z_1 k_1 \theta_1 \sinh\left(\frac{z_1\Delta\mu_e}{2RT}\right) + z_1 k_3 \theta_3 \sinh\left(\frac{z_1\Delta\mu_e + z_2\Delta\mu_H}{2RT}\right) \tag{37a}$$

$$J_H = z_2 k_2 \theta_2 \sinh\left(\frac{z_2\Delta\mu_H}{2RT}\right) + z_2 k_3 \theta_3 \sinh\left(\frac{z_1\Delta\mu_e + z_2\Delta\mu_H}{2RT}\right). \tag{37b}$$

COUPLING TO ELECTRIC CURRENT

Since the electron transfer system is spatially organized (Racker, 1970; Witt, 1971; Trebst, 1974; Dutton and Prince, 1977; Boyer et al., 1977), the oxidation-reduction reactions are coupled to an electric current. In this section we shall consider the electron transfer coupled to electric current but not to movement of any other species. Suppose that electron transfer occurs between A and B in the presence of an electric potential difference, $\Delta\psi$, between the position of molecules A and B. Since we have supposed that the reaction is not coupled with movement of any charged species, the number of charges moving from A to B per reaction is $-z$. The driving force of the reaction is, in this case, the sum of the affinity, $\mathcal{A}$, and an electrical term, $-zF\Delta\psi$. At equilibrium, both flux and force of the reaction must become zero:

$$\mathcal{A} - zF\Delta\psi = z\Delta\mu_e^0 + RT\ln\frac{\Pr(A_R)\cdot\Pr(B_O)}{\Pr(A_O)\cdot\Pr(B_R)} - zF\Delta\psi = 0 \tag{38}$$

$$J = N[k_+ \Pr(A_R) \cdot \Pr(B_O) - k_- \Pr(A_O) \cdot \Pr(B_R)] = 0. \tag{39}$$

To satisfy Eqs. 38 and 39, the ratio of the forward and backward reaction rate constants must be:

$$\frac{RT}{z} \ln \frac{k_+}{k_-} = \Delta\mu_e^0 - F\Delta\psi. \tag{40}$$

If one assumes (*a*) that the relation in Eq. 40 continues to hold when the system is far from equilibrium, and (*b*) that the electric field does not change the value of $k(= \sqrt{k \cdot k_-})$, the relation between the flux and the force can be derived as was obtained in Eq. 20b:

$$\begin{aligned} J_e &= kN\theta \sinh \left[\frac{z}{RT}(\Delta\mu_e - F\Delta\psi)\right] \\ \theta^{-1} &= \cosh\left(\frac{z}{RT}\langle\Delta\mu_e\rangle\right) + \cosh\left(\frac{z}{RT}\langle\bar{\mu}_e\rangle\right). \end{aligned} \tag{41}$$

Note that although the electric potential difference $\Delta\psi$ contributes to the force for the reaction in the same manner as the redox potential difference, it does not contribute to the value of θ. Assumptions *a* and *b* are not obvious and need further rationalization. (Assumption *b* corresponds to the case where the "symmetry factor" is equal to ½ in the Butler-Volmer equation for electrode current density; cf. Kell, 1979). However, even if modification of Eq. 41 is needed, it is clear that one expects $\Delta\mu_e$ and $\Delta\psi$ to affect the flux differently.

The relation in Eq. 41 is shown in Fig. 2. z, $\langle\bar{\mu}_e\rangle$, and $\Delta\mu_e^0$ are supposed to be 1, 0, and $3RT$, respectively. In Fig. 2 *A*, J is plotted against the force, $\Delta\mu_e - F\Delta\psi$, as $\Delta\mu_e$ varies for several fixed values of $\Delta\psi$. In Fig. 2 *B*, J_e is plotted against $F\Delta\psi$ for several fixed values of the force. A

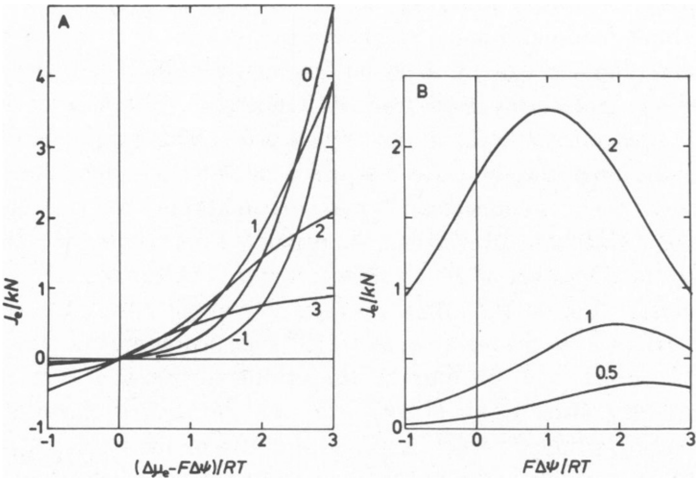

FIGURE 2 Effect of electric field on the electron transfer coupled with electric current. The figure is drawn according to Eq. 39. z, $\langle\bar{\mu}_e\rangle$, and $\Delta\mu_e^0$ are supposed to be 1, 0, and $3RT$, respectively. (*A*) The flux of electrons as a function of the force, with several fixed values of $F\Delta\psi/RT$ indicated in the figure. (*B*) The flux of electrons as a function of $F\Delta\psi$, with several fixed values of the force, $(\Delta\mu_e - F\Delta\psi)/\mathrm{RT}$, indicated in the figure.

change in the relative contribution of the chemical term and the electrical term in the force alters the flux. If the system is not too far from equilibrium, the flux is larger in the presence of positive $\Delta\psi$. In other words, less dissipation of free energy is necessary in the presence of the electric field to get the same flux as in the absence. Positive $\Delta\psi$ means the same direction of the field as that formed by the movement of the electrons. If $\Delta\mu_e^0$ has a negative value, negative $\Delta\psi$ reduces the expense of free energy.

DISCUSSION

Although the number of papers in which irreversible thermodynamics is used is increasing in the field of bioenergetics, little attention is payed to nonlinearity and nonreciprocity of the system. The validity of linear and reciprocal phenomenological equations is frequently supposed, but not always examined critically. A system can be approximated by linear equations relating the fluxes and forces if it is close to equilibrium. In this case, the reciprocity is always true (Prigogine, 1967; Mikulecky et al., 1977). However, linearity is not a fundamental property of chemical reactions.

Even at near equilibrium, where the linearity and the reciprocity hold true and the system can be well described by Onsager's symmetric set of coefficients, the constancy of the coefficients is only a local property. This is demonstrated in Rottenberg's (1973) discussion of enzyme-catalyzed reactions. When the affinity is changed by changing the concentration of the product, the dependence of the reaction rate on the affinity depends on the concentration of the substrate fixed, and the slope of the curve, $J/\mathcal{A}$, at $\mathcal{A} = 0$, is not constant.

Our expression using the "redox-poising parameter" gives another clear demonstration of the problem. The flux is not only a function of the force, but also a function of θ, which depends on the redox potential and, in some cases, pH. When $\langle \bar{\mu}_e \rangle$ is either decreased or increased, θ, and therefore $J_e/\Delta\mu_e$, approaches zero. When the electron transfer is coupled with electric current, $J_e/(\Delta\mu_e - F\Delta\psi)$ depends on the value of $\Delta\psi$ (Fig. 2 *A*). This is because the redox state that gives equilibrium depends on $\Delta\psi$.

A linear relationship can exist in a region far from equilibrium. In this case, however, reciprocity does not necessarily hold true. Rottenberg (1973) showed that the rate of enzyme-catalyzed reactions is linear in the affinity over a wide range (up to ± 1 kcal/mol), when the concentration of the substrate is of the same order of magnitude as the K_m of the reaction. This linear region is not necessarily close to equilibrium. If it is far from equilibrium, i.e., if the enzyme is kinetically irreversible, the reaction is not reciprocal. Perturbation of the product concentration does not affect the reaction rate, whereas the rate varies linearly in response to a perturbation of the affinity caused by changing substrate concentration. In network-thermodynamics terms (Oster et al., 1973), chemical processes must be expressed as "two-port." This means that the flux of the chemical processes is a function of two independent variables. Oster et al. (1973) expressed the rate of chemical reactions as a function of "forward affinity," which is the summation of the chemical potentials of the substrates, and "reverse affinity," which is the summation of the chemical potential of the products.

In Eq. 13, the rate of the reaction is uniquely determined by the affinity. This is simply because the system has only one degree of freedom. In Eq. 20a or b, where ΔE_h can be varied by changing the redox potential of either the donor or the acceptor, the rate of the reaction is a function of two independent variables, $\Delta\mu_e$ and $\langle\bar{\mu}_e\rangle$. $dJ_e/d(\Delta\mu_e)$ depends on how $\Delta\mu_e$ is perturbed:

$$\begin{aligned} dJ_e &= \frac{\partial J_e}{\partial \Delta\mu_e} d\Delta\mu_e + \frac{\partial J_e}{\partial\langle\bar{\mu}_e\rangle} d\langle\bar{\mu}_e\rangle \\ &= \frac{\partial J_e}{\partial \Delta\mu_e} d\Delta\mu_e + zkN\frac{\sinh(z\Delta\mu_e)}{2RT}\frac{\partial\theta}{\partial\langle\bar{\mu}_e\rangle} d\langle\bar{\mu}_e\rangle. \end{aligned} \tag{42}$$

$d\langle\bar{\mu}_e\rangle$ is positive if $\Delta\mu_e$ is raised by decreasing the redox potential of A, and negative if $\Delta\mu_e$ is raised by increasing the redox potential of B.

It may be worth pointing out that there are two regions where the process expressed by Eq. 20a is well-characterized as a unique function of $\Delta\mu_e$, i.e., the process is reciprocal. One of them is, of course, near-equilibrium: $\Delta\mu_e \simeq 0$. Since sinh (0) = 0, the second term in Eq. 42 becomes zero when $\Delta\mu_e = 0$:

$$dJ_e = \frac{\partial J_e}{\partial \Delta\mu_e} d\Delta\mu_e. \tag{43}$$

Another region where the process is reciprocal is around $\langle\bar{\mu}_e\rangle = 0$. When $\langle\bar{\mu}_e\rangle = 0$,

$$\frac{d}{d\langle\bar{\mu}_e\rangle}\cosh\left(\frac{z\langle\bar{\mu}_e\rangle}{RT}\right) = 0$$

Therefore,

$$\frac{\partial\theta}{\partial\langle\bar{\mu}_e\rangle} = 0,$$

and again, only the first term in Eq. 42 remains.

Rottenberg (1973) observed that, in mitochondrial respiration, the coupling of oxygen consumption and phosphorylation is reciprocal: the change in the rate of phosphorylation caused by a change in the oxidation affinity was the same as the change in the rate of oxygen uptake caused by a change in phosphorylation affinity. It is possible that the elementary steps are close to equilibrium, although the overall force is large (the difference between the phosphorylation affinity and the oxidation affinity was in the range of 44 to 49 kcal/mol). Any processes close to equilibrium are reciprocal and any combinations of reciprocal systems are reciprocal (Oster et al., 1973). Another possibility worth considering is that most of the free energy available is spent in a few "rate limiting" steps and these steps are still reciprocal far from equilibrium. The condition that $\langle\bar{\mu}_e\rangle = 0$ is perfectly physiological.

The situation is very similar in Eq. 30. The condition in which the system is reciprocal, in addition to near-equilibrium, is:

$$z_1\langle\bar{\mu}_e\rangle + z_2\langle\bar{\mu}_H\rangle = 0 \tag{44}$$

Let $x = z_1\langle\bar{\mu}_e\rangle + z_2\langle\bar{\mu}_H\rangle$. Since

$$\frac{\partial}{\partial x}\cosh\frac{x}{RT} = 0$$

when $x = 0$,

$$\frac{\partial J_e}{\partial \Delta\mu_H} = z_1\left(\frac{\partial J}{\partial \mathcal{A}}\frac{\partial \mathcal{A}}{\partial \Delta\mu_H} + \frac{\partial J}{\partial x}\frac{\partial x}{\partial \Delta\mu_H}\right)$$

$$= z_1\frac{\partial J}{\partial \mathcal{A}}\frac{\partial \mathcal{A}}{\partial \Delta\mu_H} = z_1 z_2\frac{\partial J}{\partial \mathcal{A}}$$

$$\frac{\partial J_H}{\partial \Delta\mu_e} = z_2\left(\frac{\partial J}{\partial \mathcal{A}}\frac{\partial \mathcal{A}}{\partial \Delta\mu_e} + \frac{\partial J}{\partial x}\frac{\partial x}{\partial \Delta\mu_e}\right)$$

$$= z_2\frac{\partial J}{\partial \mathcal{A}}\frac{\partial \mathcal{A}}{\partial \Delta\mu_e} = z_1 z_2\frac{\partial J}{\partial \mathcal{A}}.$$

Therefore,

$$\frac{\partial J_e}{\partial \Delta\mu_H} = \frac{\partial J_H}{\partial \Delta\mu_e}. \tag{45}$$

In the coupling with electric current (Eq. 41), the condition which gives reciprocity, besides near-equilibrium, is more stringent:

$$\langle \Delta\mu_e \rangle \simeq 0 \quad \text{and} \quad \langle \bar{\mu}_e \rangle \simeq 0. \tag{46}$$

Eq. 46 means that the redox potentials of the electron donor and the acceptor are about same as their respective midpoint potentials. Although this condition is still physiological, the electric coupling can easily cause results which cannot be predicted from the analysis of linear reciprocal phenomenological equations. In addition, the condition of Eq. 46 does not give reciprocity if assumption *b*, a symmetric effect of $\Delta\psi$ on the forward and the reverse rate constants, is not correct.

Melandri et al. (1978) observed that nigericin slightly increased the proton-motive-force created by illumination in chromatophores of *Rhodopseudomonas capsulata,* while valinomycin strongly decreased it. The increase of the proton-motive-force caused by introducing a dissipative process (electroneutral exchange of H^+ and K^+) cannot be interpreted by linear reciprocal thermodynamics. Westerhoff et al. (1979) showed that, in a linear reciprocal system composed of light-driven proton pump and closed membranes, small changes in the conductance of both K^+-leak (catalyzed by valinomycin) and K^+-H^+ exchange (catalyzed by nigericin) do not affect the total proton-motive-force in the steady state, and that high concentrations of these ionophores would decrease the steady state value of the proton-motive-force. The observed increase of the proton-motive-force caused by nigericin must, therefore, be a result of nonlinearity or nonreciprocity, if nigericin acts only as a K^+-H^+ exchanger. Nonlinearity seems unlikely to be the problem. One important point is that Westerhoff et al. (1979) supposed that the flux of the elementary process is a unique function of the total force, the summation of the chemical term and the electrical term, as in Eq. 3b or 4b. This may not be appropriate, depending on the experimental conditions, or on the sort of light-driven proton pump (purple membrane or electron transfer system). In chromatophore membranes, at least two electron transfer steps are associated with an electric current in a direction perpendicular to the membrane: the electron transfer from bacteriochlorophyll to the first quinone acceptor, and the electron transfer from cytochrome *c* to bacteriochlorophyll (Dutton and Prince, 1977). The couplings of the electron transfer and the electric current in these steps are not reciprocal. They possibly give the system nonreciprocity. Building-up of the transmembrane potential, part of which is $\Delta\psi$ between two electron carriers positioned perpendicular to the membrane, can reduce the expense of free energy at these steps.

Linear reciprocal irreversible thermodynamics is a powerful tool with which to analyze the biological energy-transforming system, provided that the experimental conditions are carefully chosen. However, we are also interested in states in which the system does not exhibit linearity and reciprocity. If we can handle nonlinear and nonreciprocal systems, irreversible thermodynamics will be more powerful and more informative.

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