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THE INFLUENCE OF MEMBRANE POTENTIALS ON REACTION RATES CONTROL IN FREE-ENERGY-TRANSDUCING SYSTEMS

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The influence of membrane potentials on the rates of reactions involving the translocation of charged species across the membrane has been studied. Depending on the location of the rate-limiting step relative to the potential gradient either the forward or the backward rate is most strongly influenced by the potential. The rate of a proton translocation process in general is thus not a unique function of the protonmotive force. It is essential to include an explicit potential dependence in the kinetic coefficients to obtain a realistic description of the dynamics.

Introduction

Electronic ion pumps acting across a membrane play a crucial role in many biochemical processes. Prime examples are the free-energy-transducing steps in chloroplasts, chromatophores and mitochondria. According to the chemiosmotic theory, the different reaction steps of the total process are coupled through electrical fields across membranes and through differences in proton entropy of mixing in the two compartments separated by the membrane. The free-energy relations between the different steps have been thoroughly discussed [1,2], although several points remain unclear. Less effort has been devoted to the study of the dynamic aspects. These add another dimension of complexity to an already complex problem both from an experimental and a theoretical point of view. The purpose of finding a dynamical description is not only to characterize the kinetics in detail, but also to elucidate the general mechanisms of control of the biological process. At the present stage of knowledge of the free-energy-transducing systems the latter aspect may in fact be considered as more important than the first.

In the present paper we analyze the particular kinetic features of a chemical transformation involving transport of ions across a membrane. It is shown that membrane potentials change the kinetic coefficient in a way that depends on the structural organization of the enzyme system. There is consequently a relation between control behaviour and the spatial enzyme organization in the membrane. To obtain a quantitative formulation of these aspects we present an elementary analysis of the transmembrane electrical field, followed by kinetic analysis of a simple chemical reaction in an electrical field. An explicit model with a simple reaction coupled to a proton pump is then described in detail. We also present the Michaelis-Menten theory for an enzyme which acts as a proton pump, for example the H^+-ATPase. Finally we discuss some of the implications for the control of energy-transducing systems.

Results

The transmembrane electrical field

The transport of ions, actively or passively, from one compartment to another across a membrane leads to a charge separation and the development of an electrical field in the membrane. The total transmembrane potential $\Delta\psi$ is a function not only of transported charge and membrane capacitance but it also depends on surface charges, electrolyte concentrations in the compartments and dipolar potentials at membrane surfaces (Fig. 1a). For the sake of simplicity, we will ignore the complications due to surface and dipolar potentials and consider the simple system of Fig. 1b, where only a linear potential gradient is present in the membrane.

In terms of molecular forces, the membrane potential is generated by Coulomb interactions between ions on either side of the membrane. Due to the virtual absence of mobile charges in the membrane the Coulomb interaction is not screened as in a bulk solution. When an ion moves through the membrane it is thus affacted not only by short-range forces as in a bulk system but also by unscreened Coulomb interactions. Clearly this will influence both the equilibrium as well as the dynamical properties of the process.

For a hypothetical reaction:

$$S_1^{m+} \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} P_2^{m+} \qquad \text{(Scheme I)}$$

where 1 and 2 denote the two compartments, a transmembrane potential will affect the equilibrium if $m \neq 0$. From the relation $\Delta G^0 = RT \ln K_{eq}$ we find that the potential dependent equilibrium constant can be written:

$$K_{eq}(\Delta\psi) = K_0 \exp[-me\Delta\psi/kT] \qquad (1)$$

where $\Delta\psi = \psi_2 - \psi_1$ is the difference between the bulk potentials in the compartments and K_0 is the equilibrium constant in a homogeneous system.

The reaction rate

The electrostatic potential $\Delta\psi$ has an influence on the rate constants of Scheme I. This is apparent from the general relation:

$$K_{eq} = k_1/k_{-1} \qquad (2)$$

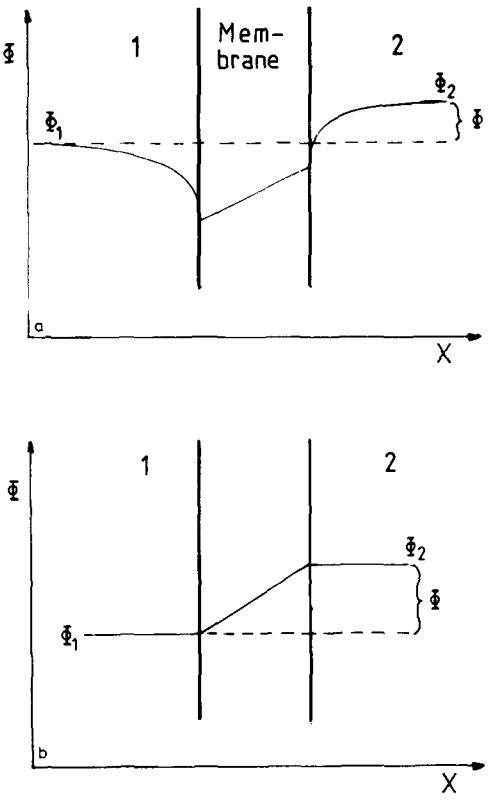

Fig. 1. Electrostatic potential profile across the membrane. (a) The total membrane potential $\Delta\psi = \psi_2 - \psi_1$ has contributions due to surface charges creating a surface potential, due to surface dipoles creating dipolar potentials and due to transported charges giving a field inside the membrane. (b) In the simplified model discussed in the text the only contribution to $\Delta\psi$ is the one caused by transported charges.

since K_{eq} is $\Delta\psi$-dependent. An interesting and significant difference between the equilibrium constant and the rate constants is that we can predict the $\Delta\psi$ dependence for K_{eq} from simple considerations whereas this is not true for the rate constants. Eqns. 1 and 2 demonstrate a restriction on the ratio between the rate constants. Thus we can write, in accordance with the Butler-Volmer equation for electrode reactions (see Refs. 3–6):

$$k_1 = k_{10} \exp[-\alpha me\Delta\psi/kT] \qquad (3)$$

$$k_{-1} = k_{-10} \exp[(1-\alpha) me\Delta\psi/kT] \qquad (4)$$

where α is an undetermined parameter with a value typically, but not necessarily, between zero

and one, while k_{10} and k_{-10} are the rate constants for $\Delta\psi = 0$. The analysis of the equilibrium conditions does not reveal if it is the forward or the backward reaction that are most influenced by the potential. For $\alpha = 1$ only the forward and for $\alpha = 0$ only the backward reaction is affected.

A molecular interpretation of the parameter α can, for example, be obtained within the conceptual framework of the transition state theory. The value of α depends on the spatial location of the transition state in the electrical field. If it is at the reactant side of the membrane, α has a small value; α is close to unity, when the transition state is on the product side of the membrane. For a charged reactant in an electrical field an extra energy term contributes to the free energy. The value of α depends on the spatial location of the transition state relative to the reactant and the product.

Non-equilibrium thermodynamics is often used to treat the dynamical aspects of energy-transducing systems [7–13] and it is thus of interest to investigate the role of $\Delta\psi$ within this formalism. It is a customary approximation to consider only a linear regime where the reaction rate V can be written:

$$V = LA + \text{constant} \tag{5}$$

Here L is the kinetic coefficients and A is the affinity of the reaction. If the linearization is made around the equilibrium point, the constant is zero. A comparison with the kinetic Scheme I reveals that [14]:

$$L = S_{eq} k_1 / kT \tag{6}$$

In the presence of a potential both the equilibrium concentration S_{eq} and the rate constant k_1 will be independently potential dependent and L is in general a function of $\Delta\psi$. When Eqn. 5 is used for a situation where $\Delta\psi$ is changed from the value chosen for the equilibrium point, not only the affinity A will change but also the kinetic coefficient L.

A reaction coupled to a transmembrane proton pump

Consider a transformation $S \rightleftarrows P$ coupled to a transmembrane proton pump so that the total process is:

$$S + nH_1^+ \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} P + nH_2^+ \qquad \text{(Scheme II)}$$

where only the protons move across the membrane. At equilibrium:

$$\frac{[P][H^+]_2^n}{[S][H^+]_1^n} = K_0 \exp(-ne\Delta\psi/kT) \tag{7}$$

in analogy with Eqn. 1. Scheme II applies both to a transformation ($S \rightarrow P$) driven by a proton electrochemical gradient so that $K_0 \ll 1$ and $\Delta\psi$ is negative and to a chemically driven proton pump where $K_0 \gg 1$ and $\Delta\psi$ is positive. In biological systems reactions such as those in Scheme II are catalyzed by enzymes. However, for sake of simplicity we will ignore the complications of the kinetics of enzyme catalysed reactions in this section, and discuss them separately in the next section.

Scheme II combined with Eqns. 3 and 4 leads to the rate expression:

$$V = \frac{d[P]}{dt} = k_{10}[S][H^+]_1^n \exp\frac{-\alpha ne\Delta\psi}{kT} - k_{-10}[P][H^+]_2^n \exp\frac{(1-\alpha)ne\Delta\psi}{kT} \tag{8}$$

Introducing the affinity A_h for the homogeneous reaction $S \rightleftarrows P$:

$$A_h = kT \ln([S] \cdot K_0 / [P]) \tag{9}$$

and the difference in proton electrochemical potential:

$$\Delta\tilde{\mu}_H = \tilde{\mu}_H(2) - \tilde{\mu}_H(1) = kT \ln([H^+]_2/[H^+]_1) + e\Delta\psi \tag{10}$$

Eqn. 8 can be rewritten as:

$$V = k_{10}[S][H^+]_1^n \exp(-\alpha ne\Delta\psi/kT) \times (1 - \exp[(n\Delta\tilde{\mu}_H - A_h)/kT]) \tag{11}$$

Under typical conditions in a biological system, the concentrations of the reactants and the product show only small variations and can be considered constant in a first approximation. Further-

more, the conditions are usually such that the pH can be considered constant on one side of the membrane so that either $[H^+]_1$ or $[H^+]_2$ has a fixed value. The variables in Eqn. 11 are $\Delta\psi$, $\Delta\tilde{\mu}_H$ and possibly $[H^+]_{1,2}$. The behavior of the rate V in Eq. 11 is shown in Fig. 2a and b for the two cases where $[H^+]_1$ and $[H^+]_2$ are constant, respectively, with $\alpha = 0$ and A_h positive. Fig. 2c and d show the rates under the same conditions but with $\alpha = 1$. Fig. 2a and d indicate that the rate is the same whether the protonmotive force is generated by the electrical potential or by the proton gradient. Fig. 2a also shows a strong control close to equilibrium, while for the conditions of Fig. 2d a good control is never achieved (by strong control we mean a steep relation between V and $\Delta\tilde{\mu}$). Fig. 2b and c show that the control in these cases is dependent upon how the protonmotive force is generated. We can also see that the steepness of the rise is dependent on the number of protons, n. The other case with A_h negative is related to plots 2a–d by symmetry. In the regime where there is a net backward reaction, the rate V grows in an unlimited way towards negative values. This effect is caused by the pushing effect of the potential and the pH difference. Under realistic conditions the unlimited growth of V will be stopped by saturation effects or by another process becoming rate limiting.

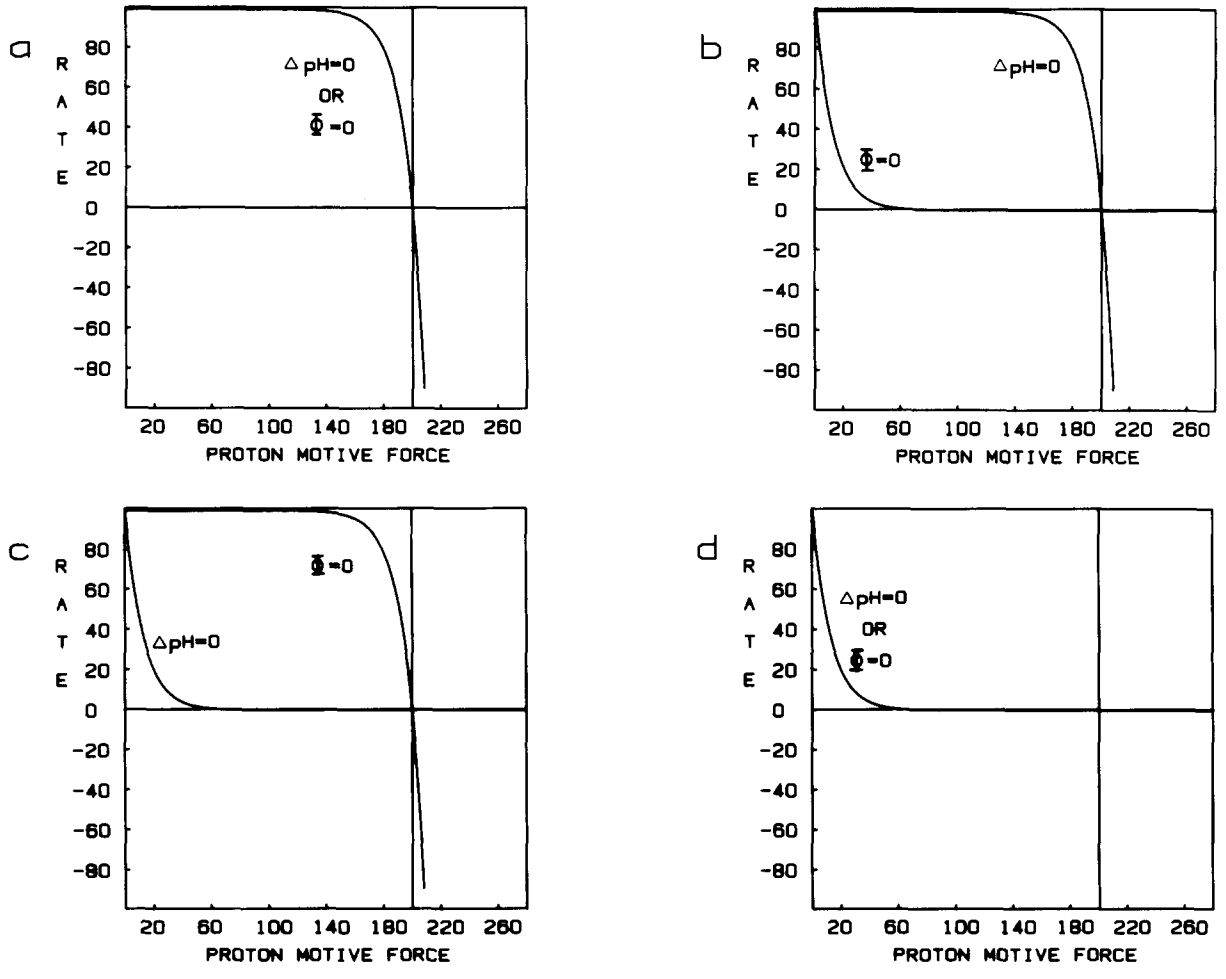

Fig. 2. Relation between reaction rate and proton motive force. (a) $\alpha = 0$, positive affinity and $[H^+]_1$ constant. (b) $\alpha = 0$, positive affinity and $[H^+]_2$ constant. (c) and (d) As in (a) and (b), but with $\alpha = 1$.

Michaelis-Menten kinetics for a reversible reaction

The reaction in the Scheme II is in practice enzyme catalyzed. In the Michaelis-Menten scheme one has the simplest possible kinetic model:

$$E+S+nH_1^+ \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} EHnX \underset{k_2}{\overset{k_{-2}}{\rightleftarrows}} E+P+nH_2^+ \qquad \text{(Scheme III)}$$

With the usual steady state assumption for the enzyme complex $[EH_nX]$ we obtain after standard manipulations:

$$\frac{d[P]}{dt} = V$$

$$= k_{-2}E_{tot}\frac{B}{1+B}\left(1-\frac{k_2[P][H^+]_2^n}{k_{-2}B}\right) \qquad (12)$$

where E_{tot} is the total enzyme content and the quantity B measuring the degree of saturation of the enzyme. B is defined by:

$$B = \frac{k_1[S][H^+]_1^n + k_2[P][H^+]_2^n}{k_{-1}+k_{-2}} \qquad (13)$$

Through manipulations analogous to the ones leading from Eqns. 8–11, Eqn. 12 can be rewritten:

$$V = k_{-2}E_{tot}\frac{B}{1+B}\left[1-\frac{\left(1+\frac{k_{-2}}{k_{-1}}\right)\exp\frac{n\Delta\tilde{\mu}_H - A_h}{kT}}{1+\left(\frac{k_{-2}}{k_{-1}}\right)\exp\frac{n\Delta\tilde{\mu}_H - A_h}{kT}}\right] \qquad (14)$$

In the limit when the enzyme is fully saturated with reactant S and product P so that $B \gg 1$ the reaction rate depends only on $\Delta\tilde{\mu}_H$ assuming that k_{-2} is independent of $\Delta\psi$. There is a rather good control since V reaches the V_{max} value with only a small change in $\Delta\tilde{\mu}_H$ from the equilibrium value, where $V = 0$. In the opposite limit, where only a small fraction of the enzyme molecules exists as enzyme substrate complex, $B \ll 1$ and the rate equation is reduced to a form similar to Eqn. 11. In general, the conditions will go from the unsaturated to the saturated case.

In the case of an enzyme catalyzed process it is possible to give an approximate structural interpretation of how the rate constants in Eqn. 13 defining B will depend on $\Delta\psi$. In the simpler cases, the rate limiting step involves a local transformation within the active site. Under such circumstances, the dissociation rate constants k_{-1} and k_{-2} are unaffected by the potential difference $\Delta\psi$ so that only the association rate constants k_1 and k_2 are dependent on $\Delta\psi$. Furthermore, the location of the active site in the membrane determines how the dependence on $\Delta\psi$ is partitioned between the two constants k_1 and k_2. The explicit $\Delta\psi$ dependent of B that determines the degree of saturation of the enzyme-reactant complex is now:

$$B = \frac{k_{10}\exp\frac{-\alpha n\Delta\psi}{kT}[S][H^+]_1^n + k_{20}\exp\frac{(1-\alpha)n\Delta\psi}{kT}[P][H^+]_2^n}{k_{-1}+k_{-2}} \qquad (15)$$

Due to the strong dependence on $\Delta\psi$ and $[H^+]$, B could change orders of magnitude for moderate variations in $\Delta\tilde{\mu}_H$, and the situation can clearly be fairly complex even in this simple model.

The H^+-ATPase — an example

The proton pumping ATP-ase enzyme complex in the free-energy-transducing systems of chloroplasts, chromatophores and mitochondria have a characteristic organization with a membrane integral part (F_0) and an external part with ATPase activity (F_1). The kinetics of this class of enzymes have been extensively investigated [15–17]. It is commonly believed that the rate-limiting step in the ATP synthesis (or hydrolysis) is a process located in the F_1-complex. From the point of view of the preceding discussion this is an interesting observation, since the location outside the membrane implies that the transmembrane potential influences the reaction rate in one direction only. We have thus a basis for discussing some of the consequences of this particular design of the enzyme complex.

In the reaction scheme:

$$E+ADP+Pi \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} E\cdot ADP\cdot Pi \underset{k_{-2}}{\overset{k_2}{\rightleftarrows}} E\cdot ATP \underset{nH_1^+}{\overset{nH_1^+}{\rightleftarrows}} E\cdot ATP\cdot H^+ \underset{k_{-4}}{\overset{k_4}{\rightleftarrows}} E+ATP+nH_2^+ \qquad \text{(Scheme IV)}$$

we assume that the rate limiting step is associated with the release of ATP implying equilibrium in the first three steps. The third step involves the

transduction of protons across the membrane and the equilibrium constant K_3 is thus dependent on the potential. For the forward and backward reaction rates, respectively, we can write:

$$V_f = k_4[E \cdot ATP \cdot H_n]$$

$$= k_4 K_1 K_2 K_3(\Delta\psi)[E][ADP][Pi][H^+]_1^n$$

$$V_b = k_{-4}[E][ATP][H^+]_2^n$$

We see that the potential acts to increase the concentration of the $E \cdot ATP \cdot H_n$-complex leading to an acceleration of the forward reaction, while the rate of the backward reaction is unaffected by $\Delta\psi$. This is clearly an effective way of generating a net forward reaction in the working system.

The effect of ΔpH on the reaction rates is dependent on the orientation of the enzyme complex. If $[H^+]_2$ is constant, such as in a submitochondrial particle the backward rate V_b is unaffected also by ΔpH and the forward rate is accelerated. In this case V_f is a unique function of $\Delta\tilde{\mu}_H$. For an orientation as in mitochondria an increased inner pH leads only to a decrease in the backward reaction rate. To accelerate the forward reaction it is thus favourable to maximize the electrostatic potential contribution to $\Delta\tilde{\mu}_H$.

Discussion

In the previous sections, we discussed how the transmembrane potential affects the dynamics of transmembrane ion transport processes. A number of simplifying assumptions were introduced due to the complexity of the problem, and one can see that additional complications arise if one attempts a more realistic description. However, one can expect that the qualitative effects found in the simpler system are also present as components of the more complex system.

What can we learn about the dynamics of energy-transducing systems from the model calculations?

(i) It is a common, tacit or explicit, assumption in the analysis of experiments on energy-transducing systems that the chemiosmotic theory implies that the overall rate is a unique function of the proton electrochemical potential gradient across the membrane at given substrate concentrations. This assumption can be partly justified using the linearized form of non-equilibrium thermodynamics. In the calculations reported above, we found that, for example, for a fully saturated enzyme obeying simple Michaelis-Menten kinetics such a relation existed between $\Delta\tilde{\mu}_H$ and the reaction rate V provided that the rate-limiting step involved a localized chemical transformation. However, in several other cases the rate V was independently dependent on the electrical and ΔpH components of $\Delta\tilde{\mu}_H$. Clearly, the experimental finding that V is not a unique function of $\Delta\tilde{\mu}_H$ cannot by itself be used as an argument to refute the chemiosmotic theory [18–20]. Conversely, a careful experimental study of the dependency of rate on $\Delta\psi$ and $\Delta\tilde{\mu}_H$ will add considerably to the understanding of the dynamics in the system.

(ii) The kinetic coefficients, whether in a conventional molecular kinetic scheme or in a general non-equilibrium thermodynamic formulation, in general carry a potential dependence. Obviously the kinetic coefficient can be considered constant over a sufficient narrow range of potentials, but in many applications it appears that this range is too narrow for the approximation to be useful. This potential dependence has largely been neglected in quantitative models of free energy transducing systems, with the notable exception of the kinetic treatment of the ATP-ADP translocator reaction in the model of Bohnensack [21]. The calculations also show that the kinetic response of a system to changes in $\Delta\psi$ and $\Delta\tilde{\mu}_H$ depends on the organization of the enzymatic system. Roughly, one can say that either it is the forward reaction accelerated or the backward reaction slowed down. Here we denote the forward direction as that occurring in the normal functioning biological system. One can possibly consider the parameter α as a design parameter in the evolution of the enzymatic complexes in the free energy transducing systems.

(iii) In experimental studies on respiratory control, one finds that a small variation in $\Delta\tilde{\mu}_H$ leads to a large change in respiration rate [18,22], when going from State 4 to State 3. Further changes of $\Delta\tilde{\mu}_H$ in State 3 have a smaller effect on the respiration rate. Such a sharp control is consistent with Eqn. 14 or with the plot in Fig. 2a. The linear regime can be very small even when the rate is a

unique function of $\Delta\tilde{\mu}_H$, and to understand the respiratory control one has to go beyond the linear approximation. In this respect our results are similar to those of Bohnensack [21].

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