

## THE DYNAMICS OF ELECTROSTATIC INTERACTIONS BETWEEN MEMBRANE PROTEINS

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(Received March 7, 1988; accepted April 15, 1988)

### Summary

The interactions of electrostatic fields with proteins in general, and with biological membranes in particular, are reviewed. Electrostatic fields can modulate protein conformational equilibria, due to differential interactions between the electric field and permanent and induced dipoles, which latter have different values in different protein conformational states. If the relevant conformational states with different dipole moments are part of an enzyme catalytic cycle, then modulation of the electrical potential will affect the enzyme's catalytic activity (for thermodynamically favourable chemical reactions). If the electrical field is non-stationary, for instance a sinusoidally modulated field or even "random" electrical noise, enzymes can transduce (or "harvest") free energy from the field for the performance of chemical or transport work. If two apposed membrane proteins exhibit reversible behaviour of this type (in which conformational transitions coupled to chemical or binding reactions are linked to changes in dipole moments), then energy coupling between chemical reactions catalysed by such proteins is possible, mediated purely via coulombic interactions. Such a model can account for ATP synthesis under conditions in which the time-averaged electrochemical potential difference of protons across an energy-coupling membrane is zero.

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### 1. Introduction

#### *1.1 Membrane-mediated coupling. An elementary step in cellular organization*

Historically, biology has approached living organisms from two sides. On the one hand, organisms were studied completely intact, usually by morphol-

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ological means. On the other hand, the fundamental elements of living systems, such as enzymes, and later nucleic acids, were studied after isolation and purification. Both approaches have been rather successful in that they have led to deep understanding of living systems at the level at which they were studied. Yet, although the two approaches are merely looking at the same problem from two directions, i.e., inward and outward, the “middle” elements of the problem are often invisible from either point of view and elude us. For instance, although the details of the kinetics of an important enzyme like hexokinase are known, their implications for the metabolism of the intact liver cell, or even the entire eukaryote, are ill-understood.

In trying to relate the molecular biology of the cell to its physiology one is severely hindered by the complex mathematics of the problem; typically, enzyme kinetics are non-linear, so that the solution of the set of differential equations that would describe a complete metabolic system would come down to solving a set of highly non-linear (and often quite “stiff”) differential equations. Using computers this can, of course, be done and impressive results have been obtained [1]. However, all the parameters of all the enzymes must be known rather precisely for this approach and, although system behavior can be related numerically to the enzyme properties, this does not lead to a true analytical “understanding” of those relations.

Approaches that aim at understanding the general principles of the relationships between enzyme properties and system behavior have had to sacrifice some exactness in favor of simplified mathematics. As one example we find Mosaic Non Equilibrium Thermodynamics [2]. This approach writes simplified relationships between reaction and transmembrane fluxes and the free-energy differences that drive them in order to clarify the relationships between free energy and metabolism. At the other end of the spectrum of methods there is the Metabolic Control Theory [3–5], which limits itself to the effect of small changes on metabolism and is thus able to come up with mathematically exact analyses of metabolic control.

In this article we shall not discuss these methods in any detail. Instead we shall discuss the first step on the way from the kinetics of individual enzymes to the integrated metabolic system: the coupling of two enzymatic reactions. Although “soluble” enzymes can also operate in a coupled fashion [6–8], there are more mechanisms of coupling two enzymes embedded in the same membrane. In this chapter we shall review mechanisms of coupling of membrane enzymes with special emphasis on newly discovered, electrodynamic coupling mechanisms.

## *1.2 Membranes and electric fields*

### *1.2.1 The role of membranes in metabolism*

Although there are some indications that the so-called “soluble” fraction of the cell sap is structurally stable by itself [9], the generally accepted paradigm

is that cells are surrounded by a plasma membrane, which prevents diffusion of low-molecular-weight metabolites and of high-molecular-weight enzymes and nucleic acids out of the cell. Because the plasma membrane contains uptake systems that are selective for metabolically important substances, it serves to discriminate undesired from desired substances. Probably, nerve cells were the first eukaryotic cells for which it was realized that the plasma membrane may play a more active role than just being a sieve; an electric potential may be generated across it, which can serve as a means to transmit information laterally [10], or to drive accumulation of substances into the cell. Indeed the plasma membranes of both eukaryotic and prokaryotic cells invariably have electric potentials across them.

In eukaryotic cells additional, topologically closed, membranes serve to divide the cell into metabolic compartments, such as the nucleus, the mitochondria and the lysosomes. One function is to keep mutually incompatible parts of metabolism (such as catabolism and anabolism; or protein synthesis and lysosomal proteolysis) separated. A second is to allow for higher concentrations of enzymes belonging to a metabolic pathway. A third is to allow for an extra control point of metabolic pathways at the transmembrane transport reactions. Since Mitchell's proposal for the mechanism of coupling of oxidative phosphorylation [11], we know that there is a fourth: to allow for coupling between otherwise unrelated enzyme reactions (cf. Section 4.1).

### 1.2.2 Electric fields in passive systems

An electric field arises when charges are distributed unevenly over space, such that in some areas there is excess positive (or negative) charge. The simplest case is that of a point charge for which Coulomb's law states [12]:

$$E(r) = D(r)/\epsilon = Q/4\pi\epsilon r^2 \quad (1)$$

Here  $D$  is the electric induction (in units  $C/m^2$ ) and  $E$  is the field strength that would actually be felt by a test charge at a distance  $r$  from the charge;  $\epsilon$  is the dielectric constant (permittivity) of the medium surrounding the test charge and  $Q$  is the magnitude of the charge that generates the field. Coulomb's law has been generalized to yield Gauss' law [12]:

$$\iint \mathbf{E} \cdot d\mathbf{S} = Q/\epsilon = \iiint (\rho/\epsilon) dV \quad (2)$$

Although this equation may look unattractive, its meaning is simple and readily applicable: it states that if we place a box around a part of space, then the integral of the electric field strength (taken normal to the surface of the box) over the closed surface of the box (or, in other words, the total number of field lines emanating from the box) is equal to the total charge within the box divided by the dielectric constant.

Figure 1 shows a beaker (which delineates our system) with in its middle a membrane. The total net charge of the contents of the beaker is zero. Yet,

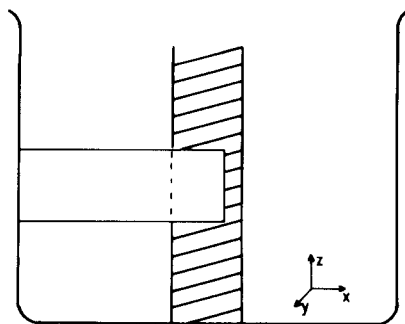


Fig. 1. A system ("beaker") with a membrane (shaded) that divides it into two compartments between which charge may have been transferred. The white box encompasses part of the left-hand solution and part of the membrane.

within the beaker charge separation may have taken place such that certain areas have net positive charge, whereas others have net negative charge. For simplicity we shall assume that properties in the system are independent of the  $y$  and  $z$ -coordinates. Consequently, the field strengths in the  $y$  and  $z$ -directions, as well as the field strengths at the sides of the beaker are zero. If we now apply eqn. (2) to the box depicted in Fig. 1, then we find that  $E_m$ , the electric field in the membrane, must be equal to the total amount of charge on the left of the membrane ( $Q$ ) divided by the surface area ( $S$ ) of the face of the box that is within the membrane (and divided by the dielectric constant):

$$E_m = Q/S\epsilon \quad (3)$$

Consequently, the electric field in the membrane is dictated *solely* by the amount of charge translocated per unit surface area and the dielectric constant within the membrane.  $\Delta_m\psi$ , the transmembrane electric potential difference, is closely related to the electric field within the membrane:

$$\Delta_m\psi = \int E \, dx = Q/S\epsilon d \quad (4)$$

Here  $d$  represents the thickness of the membrane. To stress that this is the electric potential across the membrane itself, we have given the subscript  $m$  to  $\Delta\psi$ .

At physiological ionic strength, there is no electric field in the aqueous phases bordering the membrane (for exceptions and the low-ionic-strength case, see Ref. [13]). As a consequence, one can speak in terms of a single electric potential difference, which is also dictated solely by the amount of charge translocated across the membrane and the dielectric constant of the membrane (at a given thickness of the membrane).

The important corollary is that a transmembrane electric potential difference can only be caused if net charge exists or has been translocated across that membrane. Because this charge translocation is "uphill" in terms of free

energy (if the charge is positive it will generate a positive electric potential where it goes, which will tend to drive it back) there must be a driving force for this charge translocation. Apart from equilibrium, Donnan potentials, there are two types of driving force which, in biological systems, generate transmembrane electric potential differences.

Electric potentials called “diffusion potentials” have a concentration difference as their driving force. If, say, the  $K^+$ -concentration on the left exceeds the concentration on the right, more  $K^+$ -ions will cross the membrane from left to right than from right to left (in the presence of an appropriate “channel”) such that net positive charge will start to accumulate in the right-hand compartment. As soon as such net charge translocation has occurred, there will be an electric potential. This electric potential will accelerate  $K^+$ -movement from right to left and will impede further  $K^+$ -movement from left to right. Ultimately, an equilibrium may be attained with the reverse driving force of the transmembrane electric potential exactly balancing the thermodynamic driving force from the concentration difference:

$$0 = \Delta \tilde{\mu}_k = F\Delta\psi + RT \ln ([K^+]_{in} / [K^+]_{out}) \quad (5)$$

It should be noted that in this scenario the ion ( $K^+$ ) always moves down its electrochemical gradient (by virtue of the fact that its chemical gradient exceeds the electric component of its electrochemical potential difference). Also, there should ultimately be another source for the electric potential difference, i.e., the process that generates (or maintains) the concentration difference for the ion.

### 1.2.3 Electric fields in active systems

The second type of driving force is provided by a chemical reaction that pushes an ion across a membrane. Membranous enzymes that can translocate an ion against its electrochemical potential difference are called “pumps” (cf. Section 1.3.1). The  $\Delta\psi$  across the inner mitochondrial membrane is widely believed to be caused by the pumping of protons by the electron transfer chain [14].

In this case, the pH inside the organelle is alkaline relative to the pH outside and the electric potential is negative on the inside. Hence, the proton movement must be driven by something else than its own concentration gradient. In the case of the electric potential difference across the plasma membrane of eukaryotic cells, the situation is more confusing. Again the electric potential difference is positive on the outside. The  $K^+$ -concentration in the cell is much higher than outside. The significant passive permeability of the plasma membrane for  $K^+$  would thus cause a transmembrane electrical potential difference of the diffusion type. The  $K^+$ -gradient is maintained by the  $Na^+ - K^+$ -ATPase [15], a pump that hydrolyzes ATP and pumps 3  $Na^+$  outward and 2  $K^+$  inward. This charge imbalance between the  $Na^+$  and the  $K^+$  also gives the pump

the possibility to contribute directly to the generation of the transmembrane electrical potential difference. The extent to which it actually contributes in this manner depends on the activity of the pump relative to the  $K^+$ -permeability of the membrane. It can be ascertained by measuring the immediate effect of inhibition of the  $Na^+-K^+$ -ATPase on the transmembrane electric potential difference. In different tissues, the contribution of the electrogenicity is different [16].

If there is a pump in a membrane which is able to generate and sustain an electric potential difference, then other ions to which the membrane is permeable will respond and redistribute until their electrochemical potential difference becomes zero. The ion gradient may reach the magnitude, again given by eqn. (5), at which it is in equilibrium with the electrical potential. Hence, solely from a comparison of the transmembrane electric potential difference and the concentration gradient of the ion, it is not possible to determine whether the electric potential has been generated by the ion gradient, or by some ion pump which is itself the cause of the ion gradient. Perhaps as a consequence of this indistinguishability at equilibrium, some schools of thought have long maintained that electric potentials could only be diffusion potentials. For the case of mitochondrial oxidative phosphorylation these schools proposed that the observed  $K^+$ -gradient must be the result of the activity of an inward  $K^+$ -pump driven by electron transfer. The  $K^+$ -gradient would then cause the transmembrane electric potential difference according to the diffusion potential scenario. The snag here is that it was not specified that the pumping of  $K^+$  would be electrogenic in the first place and would generate an electric potential of the opposite orientation.

#### *1.2.4 Electroneutrality*

Confusion sometimes arises as to the requirement that transmembrane transport be electroneutral. Would not such electroneutrality imply the absence of net charge transport and hence that there can be no electric potential difference between two compartments? The electric capacitance of biological membranes is typically some  $10 \text{ mF/m}^2$ . In a spherical organelle of  $1 \mu\text{m}$  diameter the pumping of  $0.1 \text{ mM}$  charges (based on the internal volume) will generate an electric potential of  $200 \text{ mV}$ . Since the concentration of bulk ions like  $K^+$  is usually of the order of  $150 \text{ mM}$ , electric potentials tend to be generated long before there is a noticeable change in ion concentration. This also holds true for protons; their free plus buffered concentration usually corresponds to some  $20 \text{ mM}$  [17]. For ion concentrations to change appreciably (say by more than  $1 \text{ mM}$ ), either the potential would have to become higher than  $1 \text{ V}$ , the maximum value that biological membranes can sustain [18], or compensating movement of other ions would have to occur. In the usual systems such high electric potentials would enforce such compensatory ion movements, or would, through a "back-pressure effect" [19] inhibit the primary

pumping process. Thus, if we examine transport at the resolution of 1 mM and above, it must be electroneutral. On the other hand, there may be charge imbalance in transport in terms of up to tens of micromolars and it is such imbalance that is responsible for the generation of electric potential differences.

### 1.2.5 Donnan potentials

Donnan potentials are a kind of diffusion potentials. Let us suppose that inside the organelle there is a solution of, say 150 mM,  $K^+P^-$ , whereas on the outside there is a solution of, say 150 mM,  $K^+Cl^-$ . The membrane is readily permeable to  $Cl^-$ , but impermeable to  $P^-$ . Let us first consider the case where the membrane is impermeable to  $K^+$  as well. Due to the driving force of its concentration gradient,  $Cl^-$  will diffuse into the organelle and will generate an electric potential across its membrane. This electric potential will continue to increase until it has become equal to the driving force imposed by the  $Cl^-$  concentration gradient. The  $Cl^-$  concentration on the outside will change only little. Since at the outset the  $Cl^-$  concentration inside is zero, that concentration will change appreciably, especially when considered in relative terms (or in log terms, such as in the chemical potential). The final situation corresponds to the inward translocation of 0.11 mM  $Cl^-$  and a transmembrane electrical potential difference of 187 mV.

In the typical case of a Donnan potential the cation is also permeable. Thus,  $K^+$ , though initially present at equal concentrations inside and outside, will respond to the electric potential generated by the  $Cl^-$  diffusion and will move inward until it reaches electrochemical equilibrium. At this point the transmembrane electrochemical potential differences of both  $Cl^-$  and  $K^+$  must be equal to zero:

$$0.060 \log_{10} ([Cl^-]_o / [Cl^-]_i) = \Delta\psi \quad (6)$$

and:

$$0.060 \log_{10} ([K^+]_i / [K^+]_o) = \Delta\psi \quad (7)$$

A similar equation for  $P^-$  is not valid, because  $P^-$  is impermeant. Since the external volume is much larger than the internal volume, the external concentrations of  $Cl^-$  and  $K^+$  are essentially constant. Thus we have two equations with three unknowns, i.e.,  $[Cl^-]_i$ ,  $[K^+]_i$  and  $\Delta\psi$ . Use of the electroneutrality condition (cf. Section 1.2.4) now makes it easy to generate the third equation necessary to calculate the final equilibrium state. Inside the organelle the total concentration of positive ions must equal the total concentration of negative ions:

$$[P^-]_i + [Cl^-]_i = [K^+]_i \quad (8)$$

The solution of these three equations gives an electric potential of 13 mV (negative inside), an internal chloride concentration of 92 mM and an internal

potassium concentration of 242 mM. It may be noted that, at the 1 mM accuracy level, electroneutrality applies (i.e.,  $92 + 150 = 242$ ), whereas there is an electric potential difference. At lower external ionic strengths Donnan potentials are typically much higher. For instance if the external concentration of KCl were 15 mM instead of 150 mM, the electric potential would amount to 60.2 mV, the internal  $\text{Cl}^-$  concentration would be 1.5 mM and the internal  $\text{K}^+$  concentration would be 151.5 mM.

### 1.2.6 Debye-Hückel screening

An electric potential tends to be attenuated by polarization of the medium. Macroscopically this effect is accounted for by allowing the dielectric constant  $\epsilon$  to differ (by a factor  $\epsilon_r$ ) from its value *in vacuo* ( $\epsilon_0$ ). Importantly, in this method of correction, the distance-dependence of the electric potential remains  $1/r$ . If the medium contains mobile charges (such as in a salt solution), this approximation is no longer valid for short distances [20], basically because the polarization of the medium no longer depends linearly on the electric potential. At low ionic strength, the  $1/r$  dependence of the electric potential around a charge is modified by: (i) a factor  $\exp(\kappa a)/(1 + \kappa a)$ , where  $a$  is the radius of the ion that generates the primary charge; and (ii) an exponential factor  $\exp(-\kappa r)$  which decays by 73% for every “Debye-Hückel length”  $1/\kappa$ . The value of  $1/\kappa$  depends only upon the ionic strength and the dielectric constant of the medium. Typically, for a univalent salt solution,  $1/\kappa$  would be 1 nm at 100 mM and 10 nm at 1 mM. The physical picture is that, if the primary charge is positive, negative salt ions will move close to the positive ion and screen its charge from its environment. At higher ionic strength there are more ions to do this. The screening is not 100% because this would require very high local concentrations of the anions and would be counteracted by their tendency to diffuse to regions of lower concentration.

When an ion is pumped across a membrane, its first location is the interface between membrane and aqueous medium. Subsequently, the charge will tend to diffuse away from the surface because its local surface concentration exceeds that in the bulk aqueous phase. On the other hand, the ion is attracted electrostatically to the left-hand side of the membrane. Again we have a case of ions tending to screen what now is “the other side of the membrane” counteracted by a driving force resulting from the accumulation of ions. There is however an important difference with the situation of the single ion described above; given the amount of translocated charge, the potential at the surface of the membrane is not affected by the polarization of the aqueous medium bordering the membrane. However, the electric potential difference between the bulk aqueous phases is affected by the screening. Paradoxically, it is *increased*, by a factor  $1 + \epsilon_m/\epsilon_w \kappa d$ , which amounts to some 5% at physiological ionic strength [13]. The difference between the electric potential at a distance  $x$  from the membrane and the electric potential out in the bulk phase decays again as



$\exp(-\kappa x)$ . Thus, for the simplest model of biological membranes, we do not have to bother distinguishing (the active component of; traditional surface potentials may be substantial [21]) the electric potential at the surface from the electric potential in the adjacent bulk phase. For the possibility that due to special properties of the membrane–water interface, this conclusion may not apply to all biological membranes, we refer to the work by Kamp et al. [13].

### 1.3 Proteins and electric fields

Characteristic of transmembrane proteins (enzymes) is the inhomogeneity of their environment. The thermodynamic parameters experienced by the outside (extracellular) part of the protein are generally not the same as those felt by the inside (intracellular) portion. Also, transmembrane proteins “feel” the transmembrane electric potential difference,  $\Delta\psi$ . This  $\Delta\psi$  typically ranges between 10 and 200 mV, corresponding to the very sizable field *strengths* of 2–40 MV/m. Since most membrane-spanning protein segments contain net charges at positions that may not be completely fixed, and consist of  $\alpha$ -helices, which constitute large dipoles [22], we are led to speculate that modulation of the  $\Delta\psi$  may be one mechanism by which a cell regulates the structure and, thereby, activity of its membrane-bound enzymes. In the present article we shall discuss the possible relevance of this type of interaction for catalysis and free-energy transduction.

#### 1.3.1 Channels, translocators and pumps

The ionic concentrations found in the extracellular milieu are generally not conducive to life, and consequently, most cells must use up a great deal of their total available energy towards maintaining acceptable levels of various ions ( $\text{Ca}^{2+}$ ,  $\text{K}^+$ ,  $\text{Cl}^-$ , etc.). The proteins responsible for performing these functions are known either as channels, as translocators, or as pumps. A channel is a protein that catalyzes the electrochemical equilibration of a substance by forming a “pore” in the membrane. A translocator catalyzes electrochemical equilibration of a substance by binding it, undergoing a conformational change that translocates the binding site to the other side of the membrane, and releasing the substance again. Characteristics for channels are high maximum turnover numbers, diffusion-like kinetics and a noise-power spectrum extending to the sub-millisecond domain. Characteristics for translocators are their specificity, low turnover number, noise spectrum only below 1 kHz, and enzyme kinetics. A pump is a translocator (or perhaps [23] a channel) that is competent to move an ion up its electrochemical gradient at the expense of (photo)chemical free energy. As explained in Section 1.2.3, electrogenic pumping causes the build-up of a transmembrane potential. This potential difference changes with changes in the activities of the pumps. All of the membrane proteins will experience this field of varying intensity and modulation of their

properties may therefore be expected even for proteins not classically labeled as “voltage-gated”.

### 1.3.2 Interaction of electric fields with membrane proteins

There are many ways in which an electric field can interact with a protein and affect the “basic” (see below) free energies of its conformational states. With respect to the catalytic properties of a transmembrane enzyme, we are not so much interested in the change in basic free energy that all the enzyme states have in common, as we are in changes in the basic free-energy difference between states.

(i) *Proteins carrying net charge.* If a membrane protein carries a net charge its energy will depend on the position of that charge in any external electric field. For instance, if the transmembrane electric field would be homogenous and two states of the enzyme would differ in the position of the charge, such that in the second state the positive charge would be removed  $\Delta x$  farther from the interphase, then the two states would differ in electrostatic potential energy by:

$$\Delta G^b = E\Delta x/d \quad (9)$$

where  $d$  represents the thickness of the membrane.

(ii) *Dipole moments.* Net electric charge is not the only property that gives a substance a potential energy in an electric field. If a substance has a permanent dipole moment, then its energy will depend on the orientation and the magnitude of that dipole moment. The simplest case is that where the end of a protein that bathes in the left-hand aqueous phase would carry a net negative charge whereas the other end of the protein, bathing in the right-hand solution, would carry the same charge but positive. If the two elementary charges were removed by 5 nm this would bestow upon the protein a dipole moment of  $0.8 \times 10^{-27}$  C m or 240 Debye (1 Debye corresponds to  $3.33 \times 10^{-30}$  C m). But even in the absence of such explicit charges one would expect protein molecules to have a dipole moment. Transmembrane segments of proteins typically are composed of  $\alpha$ -helices [24]. The hydrogen bonding between the oxo group of one amino acid residue and the amino group four residues ahead in the chain generates a dipole moment which is almost parallel to the helix axis. Consequently, the  $\alpha$ -helix structure is the structure that has the largest dipole moment attainable by the polymerization of amino acids. Other possible protein conformations such as  $\beta$ -sheet or random coil have much smaller dipole moments [22]. Typically, a single peptide unit has a dipole moment (as projected onto the axis of the  $\alpha$ -helix) of some  $12 \times 10^{-30}$  C m (3.5 Debye). The overall dipole moment of an  $\alpha$ -helix can be obtained by multiplying the number of peptide units in the chain by  $12 \times 10^{-30}$  C m [22]. For the usual 20–25 amino-

acid transmembrane 224 helix [24], this amounts to some 75 Debye. Many transmembrane proteins traverse the membrane more than once. The orientations of the subsequent  $\alpha$ -helices alternate however, so that the total dipole moment of the protein will subside to zero or to the value characteristic of a single  $\alpha$ -helix. Bacteriorhodopsin, which traverses the membrane seven times, has a dipole moment of some 60–90 Debye [25,26]. That its seven  $\alpha$ -helices have alternating dipole moments may not be without further implications; a transmembrane electric field would tend to differentially rotate the  $\alpha$ -helices (see below) and such a conformational change could interfere with the catalytic mechanism. Similar effects may be relevant for the acetylcholine receptor.

If two protein states differ in the magnitude, or the orientation of their dipole moment in the presence of an external electric field (induction)  $D$ , their difference in basic free energy is given by:

$$\Delta G^b = D\Delta(\mu_0 \cos \Theta) \quad (10)$$

Here  $\Theta$  is the angle between the electric field,  $D$ , and the dipole moment,  $\mu_0$ . In contrast to the case of the electric charge, the energy content of the dipole does not change when its position changes; it depends solely on its orientation and its magnitude.

(iii) *Polarizability.* If the protein is substantially polarizable, an electric field can induce a dipole moment in the protein and interact with it. While the direction of the energy shift upon application of an electric field to a permanent dipole would depend on the sign of the field, and the rotational state of the dipole, the effect due to the polarizability of the protein is always to lower the basic free energy of the protein. The difference in basic free energy between two enzyme states that differ in their polarizability  $\alpha$  is given by:

$$\Delta G^b = \frac{1}{2} D^2 \Delta \alpha \quad (11)$$

This equation supposes that the polarizability of the protein is isotropic, or that the protein is allowed to rotate rapidly [20]. For a protein fixed in a membrane not only a change in its conformation, but also a change in its orientation, can induce a change in its polarizability in the direction of the applied electric field.

(iv) *The second Wien effect.* Most proteins bear charges at physiological pH. These charges are due to protonation of amino groups, or deprotonation of carboxylate or thiol groups. In view of the high electric field strengths in membranes, this allows for a special type of polarization, i.e., the second Wien effect (for references see Ref. [27]). In this case, the reaction may be written



In the case of transmembrane proteins acted on by transmembrane electric

fields, the dissociation reaction would be internal to the protein, as in the removal of a proton from an internal carboxyl group. Here the direct influence of the field is to further the forward second step. However, an electric field across a solution of macromolecules can result in the rapid induction of large transient dipoles leading to increased *adsorption* of ions [27]. For certain molecular symmetries, the prevalent effect of the field may actually be to further the reverse of step two in eqn. (12), especially if  $A^+$  represents a protein with a large dipole moment.

### 1.3.3 Influence of an electric field on an enzyme conformational equilibrium

Possible protein conformational changes in which the electric properties of the protein would change in one of the above senses, are shown in Fig. 2. Taking

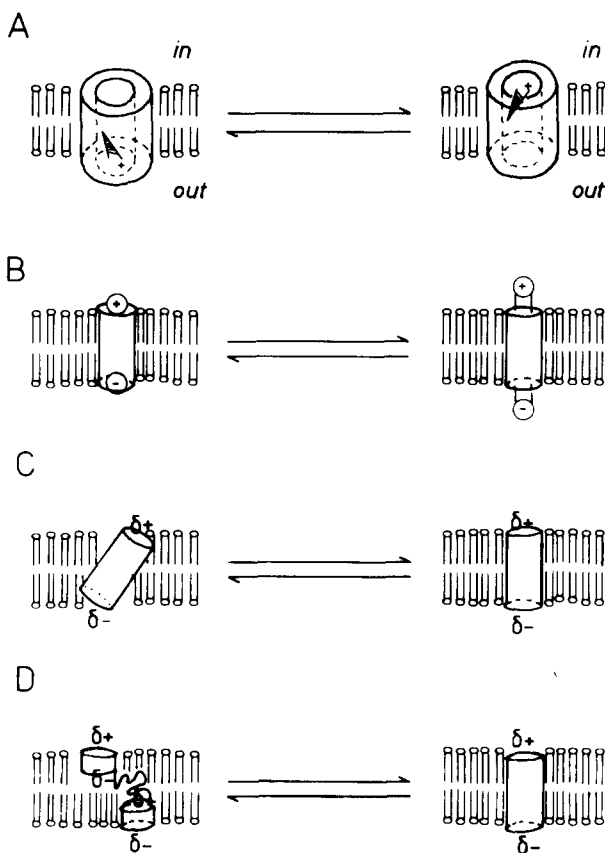


Fig. 2. Ways in which a transition of a protein between two states can comprise a transition in electric properties. (A) A charge arm of the protein is translocated across part of the membrane. (B) An  $\alpha$ -helix of the protein is stretched. (C) An  $\alpha$ -helix is rotated. (D) Some random coil assumes the  $\alpha$ -helical structure.

one possibility, rotation of  $\alpha$ -helices, we show in Fig. 3 a schematic representation of the basic free energy versus the extent of reaction. Through eqn. (10), we note that the presence of a transmembrane electric potential of some 150 mV would differentially change the basic free energies of the two states by some 6 kJ/mol. Due to the relation [28,2]:

$$RT \ln(K_{\text{eq}}) = \Delta G^b \quad (13)$$

the equilibrium constant of the transition between the two states in Fig. 3 would be changed by a factor of 11 in the presence of the transmembrane electric potential. In the case that the two states of the protein would differ by the position of a single charge, in the sense that the charge would either be on the right-hand, or on the left-hand side of the membrane, this would become a factor of 300. It is clear that the presence of an electric field can significantly stabilize one conformation of a protein relative to another and hence can cause the protein to change its net conformation.

In this respect, two conformations differing in the location of a charge are equivalent to two conformations differing in dipole moment: the situation with the shifted charge can be taken as the situation with the charge in its original position except that a dipole has been added such that its negative charge coincides with the initial position of the, say, positive charge and its positive charge with the new position of the protein charge.

Macroscopically, the phenomenon that an electric field can change the position of charge in the protein or that it can change its dipole moment could be measured as (increased) polarizability. Fröhlich [29] has also suggested that transmembrane electric fields might polarize components of biological membranes. He stressed the possibility that the dipole moments of the membrane components would be large and close enough to very strongly interact between themselves, such that changes in the external electric field (or in other parameters that would be related; microwave irradiation was proposed to be a potential trigger) could induce large all-or-none "phase" transitions; the dipoles would then enter a mode of coherent vibration. There would have to be a sustained metabolic feeder reaction, which would keep the oscillations in the molecules subliminal until the external trigger would bring about the coherent mode. For such coherent modes far-from-equilibrium conditions are necessary; the polarization must depend on higher than first order terms in the field. We will not further discuss these phenomena. We shall confine ourselves to the near-to-equilibrium domain where the polarization depends linearly on the electrical field (see below).

There are important characteristics shared between "coherent excitations" [29] and the non-coherent transitions of individual proteins at issue here. One is that both should be detectable through dielectric dispersion measurements, [30]. The transitions we here discuss however lie in the frequency range of catalytic turnover, i.e., 0.1–10 kHz. In this region of the dielectric dispersion

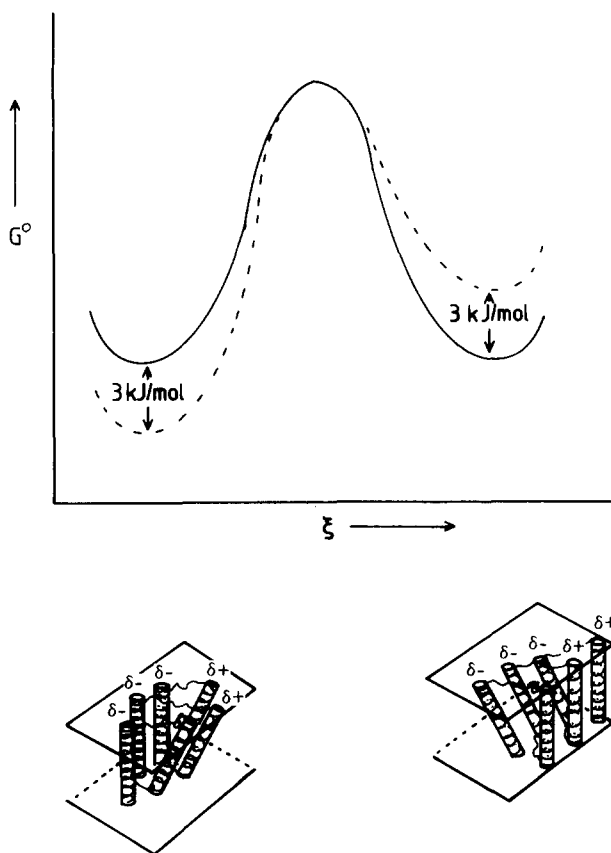


Fig. 3. An example of electroconformational coupling: the basic free energy profile of a protein transition in the absence (—) and presence (---) of a transmembrane potential difference of 150 mV. As illustrated at the bottom of the figure, six  $\alpha$ -helices of 20 amino acid residues are assumed to span the 5-nm membrane. In the conformation on the left, the three that screw upward (clockwise drive of the continental screw) are parallel to the field, the three that screw downward make an angle of some  $40^\circ$  with the electric field. In the conformation on the right this has been reversed. The concomitant change in dipole moment per  $\alpha$ -helix normal to the membrane would be  $20 \times 3.5 \times (1 - \cos 40.3^\circ) = 17$  Debye. The difference in basic free energy of the two conformations would thus correspond to that of a 100 Debye dipole in an electric field of  $0.15/5 \times 10^{-9}$  V/m, i.e., 6.0 kJ/mol. For the transition state (not shown) it was assumed that all  $\alpha$ -helices have the same orientation with respect to the membrane. As a consequence, the forward and the reverse unidirectional rate constants would be affected by the factors 3.3 and  $1/3.3$  respectively; the equilibrium constant by the factor 11.

spectrum, biological membranes exhibit much absorption that should be regarded as largely irrelevant background.

The equilibrium constant for the transition between two protein states is equal to the ratio of the forward to the reverse transition rate constants. Con-

sequently, if the equilibrium constant depends on the electric field, at least one of these two rate constants must also depend on the electric field. In the absence of further detailed knowledge, the electric field-dependence is often attributed equally to the forward and the reverse rate constants [28].

## 1.4 Energetics of enzyme catalysis

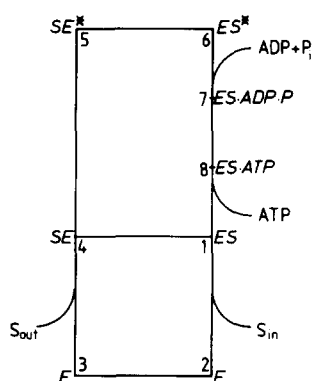
### 1.4.1 Diagrams, cycles, fluxes, forces

We shall be interested in structural transitions that affect the catalytic properties of enzymes. A convenient way to discuss the energetics of catalysis is the diagram method for calculating enzyme state probabilities initiated by King and Altman [31] and extended for the purpose of discussing cycles, incomplete coupling and free-energy transduction by Hill [32], cf. [28,2]. Figure 4 shows a diagram representation of a (fictitious) pump of the neutral substance S driven by the hydrolysis of ATP. Catalysis is visualized as a series of discrete transitions between thermodynamically well-defined enzyme states (1 through 8 in Fig. 4); each state has a basic free energy [28] consisting of the standard free energy of the enzyme in that state plus the chemical potential of the free ligands. Since catalysis implies that the protein returns to its initial state, one need only consider the three possible cycles shown in Fig. 4B. Cycle *a* just hydrolyzes ATP. Cycle *b* “leaks” S. In its counterclockwise mode of operation cycle *c* pumps S against its electrochemical potential difference, because it is driven by ATP hydrolysis. In its clockwise mode of operation it catalyzes ATP synthesis, usually against an opposing free energy of hydrolysis of ATP, driven by S movement down its electrochemical potential difference.

The catalytic activity of a pump may be enhanced by differential binding affinity. For the outward S pump of Fig. 4, this would mean that state 1 relative to state 2 is energetically much more stable (i.e., has a lower standard free energy) than state 4 relative to state 3. Such an asymmetry allows substance S on the low-concentration (in)side to bind with equal (or even greater) probability than S on the high-concentration side. Thermodynamically, this necessitates that the conformational transition describing transport of the chemical species from the low- to the high-concentration side (transition 1 to 4 in Fig. 4) becomes energetically unfavorable. This is often taken to imply that this is the step in which free energy is to be “absorbed” from the environment, to promote the complex to an “energized” form. In Fig. 4A this free-energy absorption would consist of the free energy “liberated” in the hydrolysis of ATP as the enzyme goes from state 1 to state 6. The latter state would then correspond to an “energized state” of the enzyme if compared with state 1.

The dissection of the catalytic cycle in terms of a set of steps (1–8–7–6 in Fig. 4) that completely hydrolyze ATP and do nothing but bring the enzyme from one state to another conformational state and a set of steps (6–5–4–3–2–1 in Fig. 4) that translocate S, is not always possible however. Often the ATP

A



B

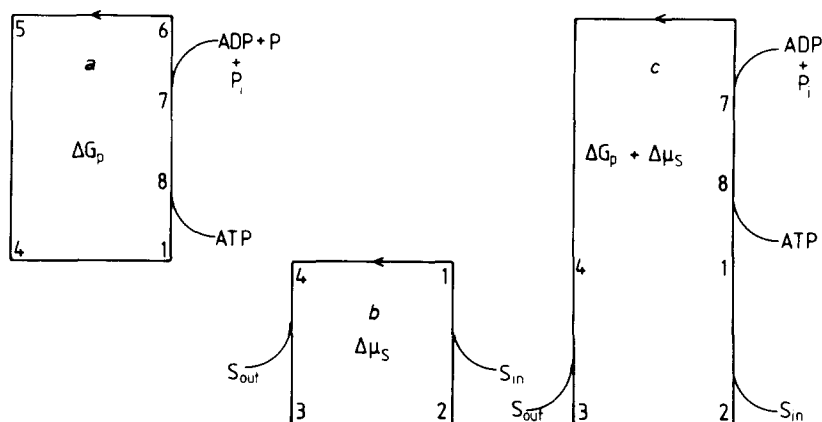


Fig. 4. Hill diagram for an enzyme that can hydrolyze ATP coupled to the translocation of a substance S across a membrane. (A) Definition of the enzyme states (in italics). States on the left have the proton-binding group on the external side of the membrane, states on the right have it on the intravesicular side.  $ES^*$  differs from  $ES$  in conformation. (B) The three cycles possible in (A), with their driving forces written inside.  $\Delta G_p$  is the phosphate potential, or free energy of ATP hydrolysis;  $\Delta \mu_S$  is the transmembrane electrochemical potential difference for the substance S.

hydrolysis reaction and the free-energy consuming reactions (uphill S translocation in this example) are more interwoven (e.g., the enzyme may translocate S before it dissociates the ADP). In fact, as pointed out by Hill and Eisenberg [33], it is in general not useful to dissect the sequence of catalytic events into events that catalyze the free-energy-yielding reaction and events that catalyze the free-energy-requiring reaction. In line with the very characteristic of catalysis itself, one should concentrate on discussing complete cycles;



the enzyme must return to its original state. The relevant questions then become: What for each cycle is the thermodynamic driving force? and: Which of the cycles is the most probable?

The driving force for any cycle equals the free-energy difference of the reaction it catalyzes. In Fig. 4B we have indicated these thermodynamic driving forces. The probability of a given enzyme state,  $P_i$ , is proportional to the sum, called  $\Sigma_i$ , of all products of the unidirectional rate constants along any route in the diagram that leads to that state.  $P_i$  is obtained by dividing  $\Sigma_i$  by the sum,  $\Sigma$ , of all the analogous sums for all the states of the enzyme:

$$P_i = \Sigma_i / \Sigma \quad (14)$$

Hill [32,28] recognized that one may define and calculate cycle probabilities,  $P_\kappa$ , in much the same way. The net flux around cycle  $\kappa$  can be obtained by multiplying the enzyme concentration ( $[E_{\text{tot}}]$ ), by the cycle probability ( $P_\kappa$ ), by the product of the unidirectional rate constants along the cycle in the reverse direction ( $\prod_{\kappa-}$ ) and by the factor  $(\exp(X_\kappa/RT) - 1)$ , where  $X_\kappa$  is the thermodynamic driving force of the cycle  $\kappa$  [28,2]:

$$J = [E_{\text{tot}}] P_\kappa \prod_{\kappa-} (\exp(X_\kappa/RT) - 1) \quad (15)$$

Most importantly, the direction of the flow along the cycle is solely determined by the magnitude of the driving force ( $X_\kappa$ ) of the cycle, hence is always thermodynamically downhill. As a consequence, in determining the direction of flow it is irrelevant where in cycle  $c$  of Fig. 4 ATP is hydrolyzed, or where ATP associates with, or ADP dissociates from, the enzyme. The only essential thing is that somewhere along cycle  $c$  all the events necessary for ATP hydrolysis happen.

One may consider ATP hydrolysis in terms of providing an environment in which the pumping of S (in the example of Fig. 4) is not energetically uphill but downhill. Also, from this point of view, attempts to isolate a single step as the energy-requiring process seem doomed to failure. This does not mean that kinetically distinct steps cannot be revealed, but only that no one (or group) of them is the sole free-energy-absorbing process. In fact, kinetics play an important role in the free-energy-transducing process. First, they determine the magnitude of the term  $\prod_{\kappa-}$  in eqn. (15), and hence the actual catalytic capacity of the enzyme. Second, through their effect on the different cycle probabilities  $P_\kappa$ , they are important in favoring the coupled cycle  $c$  (cf. Fig. 4) over the uncoupled cycles  $a$  and  $b$  [34,2,35,36].

#### 1.4.2 Mechanisms of coupling at the enzyme level

The above discussion has demonstrated that for coupling between two processes to occur, they have to be interwoven in some fashion in a single catalytic cycle. In Fig. 4B the driving force of the cycle  $b$  is such that the latter will only work down the electrochemical gradient of S. Cycle  $c$  may pump S in the ther-

modynamically uphill direction, because some additional process is involved in cycle *c* which runs thermodynamically downhill. Or, in other words, there is a process that pushes our enzyme (over the top, see Fig. 4) from state 3 to state 6. We may now ask which types of processes have been documented in the literature and which others may be feasible in addition to that. Figure 4 shows the first: coupling to a chemical reaction.

A second type of coupling would occur if ATP and ADP in Fig. 4 would be replaced by  $P_{in}$  and  $P_{out}$ , respectively. Translocation of *S* would then be coupled to the translocation of substance *P*. This type of coupling is amply documented in the literature. The ionophore nigericin, which exchanges protons for  $K^+$  across biological membranes, and lactose permease of *E. coli* are but two examples. If *P* carries a charge, not only the concentration gradient of *P* across the membrane enters the equation for the driving force, but also the transmembrane electric potential difference. Thus we here find one way in which the transmembrane electric potential difference can enter the driving force for an enzyme cycle. However, the time-independent transmembrane electrical potential always enters the driving force *as part of* an electrochemical potential of an ion or electron [37] that is translocated during the cycle. The reason is that there is no way in which in a cyclic process net charge can be transferred across the membrane without the simultaneous transfer of an ion or electron.

One might propose that, if part of the enzyme were electrically charged and if that part would be translocated across the membrane in part of the catalytic cycle, a dependence on transmembrane electrical potential would arise. As we shall show below, this is indeed the case; rate constants can become dependent on the electric potential and so can the absolute magnitude of the rate. However, for its complete catalytic activity the enzyme has to return to its original state, which implies that its charged part must translocate back across the membrane. The consequence is that the electric potential drops out of the thermodynamic driving force for the cycle and cannot (see however below) drive the cycle flux into a direction it would otherwise not go.

A third way in which our enzyme could be pushed from state 1 to state 4 would be by a force exerted by another enzyme in its vicinity. One could visualize such conformational interaction of two proteins as simple "pushing" through steric (Van der Waals) contact, through electrostatic interaction, or even through hydrophobic interaction. Although conformational changes of proteins are well documented, the role of conformational interactions in enzyme catalysis is much less so. Also, it is not completely clear what properties such conformational interactions should have. To specify one: the interaction must be conditional, i.e., it must only push the enzyme from right to left if the enzyme has *S* bound; if it would push equally well if the enzyme is devoid of *S*, no cycling would result [36]. From this rule it may seem that the pushing enzyme would have to know what state the pushed enzyme is in. One of the

surprising aspects of this and two related papers is that rather the opposite is true.

## 2. The role of stationary transmembrane electric fields in catalysis

### 2.1 (In)activation of an enzyme by an electric field

An electric field across an enzyme can cause that enzyme to undergo a conformational change (Section 1.2). This suggests that a transmembrane electric potential may modulate the activity of an enzyme that is present in that membrane, if only one conformation can carry out its catalytic function. Figure 5 shows results of calculations for the case where the two conformations differ in permanent dipole moment (cf. the bottom of Fig. 2; with the left-hand con-

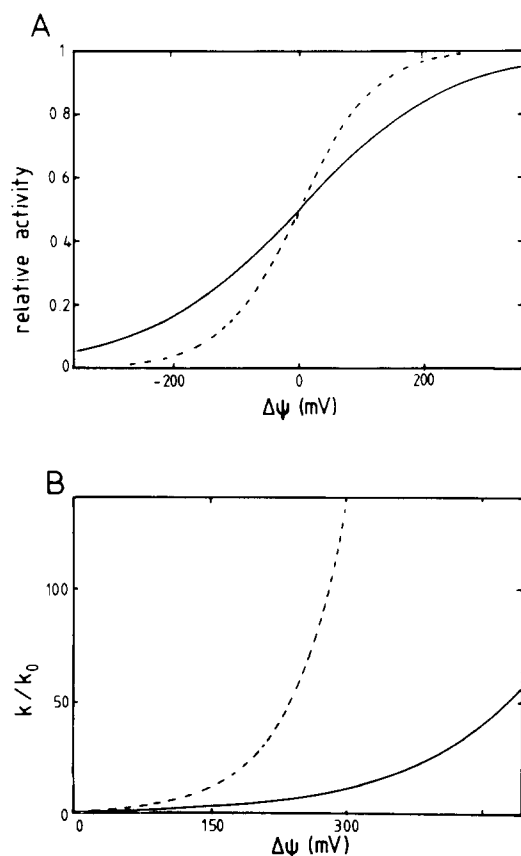


Fig. 5. (In)activation of catalytic activity by an electric field. (A) Fractional activity of the enzyme. (B) Effect on the unidirectional rate constant. The enzyme was assumed to have an active and an inactive conformation, which differed by (—) 50, or (---) 100 Debye in dipole moment normal to the membrane surface, but were otherwise equal in basic free energy (cf. Fig. 3). Membrane thickness: 5 nm.

formation catalytically inactive and the right-hand conformation active). In the absence of an electric field the two conformations were taken to have equal free energy. Hence, at zero field the enzyme has 50% of its maximum activity (cf. Fig. 5A). At high fields in the one orientation, the enzyme is forced into its inactive conformation. With the field in the other orientation, the activity of the enzyme is increased. These effects are the stronger the larger the difference in dipole moment between the two states. Considering that a dipole moment of 100 Debye is quite reasonable for membrane proteins, and transmembrane potentials of 200 mV are possible, we conclude that these (in)activation effects of the transmembrane electric potential may be quite relevant for the regulation of the activity of membranous enzymes in general. In this respect the regulation of the chloroplast  $H^+$ -ATPase by the transmembrane (or the local) electric potential may be a potential example; here the position of an inhibitor protein has been thought to regulate the activity of the enzyme such that it would not hydrolyze in the dark the ATP it makes in the light [38]. In the light of the above, it may correspond to an attached protein factor, the shifting of which may change the dipole moment of the protein. The phenomenon may also play a role in the gating of channels, such as the sodium channel, or the acetylcholine receptor.

## 2.2 Static electric fields as driving forces for the catalytic cycle

In the case we just discussed, the static electric field did not enter a catalytic cycle as part of the driving force. Rather it favored one catalytic cycle over another. In contrast, the driving force for cycle *c* in Fig. 4 contains the transmembrane electrochemical potential difference of S,  $\Delta\tilde{\mu}_S$ , which is composed of a concentration term and the transmembrane electric potential difference,  $\Delta\psi$ :

$$\Delta\tilde{\mu}_S = RT \ln([S]_{in}/[S]_{out}) + z_S F \Delta\psi \quad (16)$$

Here  $z_S$  is the number of elementary charges carried by S. Up to this point we have considered S to be neutral ( $z_S=0$ ), such that the electric potential term was inconsequential. If we substitute  $H^+$  for S in Fig. 4, then the electric potential does enter the driving force. A transmembrane potential, say positive on the outside, would push the enzyme towards state 6, whenever it would be in state 5. Since no similar pushing would occur at the 3–2 transition, this would have the effect of the electric potential driving the cycle *c* in the clockwise direction. If the hydrolytic free energy of ATP hydrolysis is smaller than the free energy harvested from the electric field by the transmembrane charge movement, ATP synthesis could result. This, of course is the (by now conventional) way of looking at ATP synthesis by proton-motivated ATP synthases [28,39,2]. Dependencies of ATP synthetic rates on  $\Delta\psi$  have been calculated for model systems like that of Fig. 4 and compared to experimentally determined flow–force relationships [39,2].

### 2.3 Catalytic effects of stationary electric fields

If, in the above example, the direction of the electric field were reversed, the driving force would drive cycle  $c$  in the direction of ATP hydrolysis. Thus, it would stimulate, rather than reverse, an already-ongoing process ( $\Delta G_p$  generally favors ATP hydrolysis over synthesis). With a catalytic effect of electric fields, we mean something different. Rather than an increase in  $J_c$  for cycle  $c$  (cf. eqn. 15) due to an increase in  $X_c$ , a catalytic effect of the electric potential refers to an increase in  $\Pi_{c-}$  at constant  $X_c$  and  $P_c$  (electric field effects on  $P_c$  are cases of (in)activation, an example of which was discussed in Section 2.1).

To investigate this possibility further, we return to the case where  $S$  was electrically neutral. Now however, we shall consider the  $S$ -binding site of the enzyme (which moves to and fro across the membrane during the catalytic cycle) to be negatively charged [cf. 40,41]. Because around any cycle the enzyme returns to its original state and there is no net transport of a charged species, the transmembrane electric potential will not enter the driving force in this case. For simplicity we shall also assume that the enzyme we consider cannot carry out the 5–6 transition, such that we are only left with cycle  $b$  of Fig. 4 (cf. Fig. 6A [40]). As an example we consider a case where the enzyme's rate constants are all equal to 1, but the internal concentration of  $S$  exceeds the external concentration of  $S$  appreciably. The upper line in Fig. 7A gives the dependence of the calculated translocation rate of  $S$  as a function of the transmembrane electric potential difference. There is quite a significant modulation of the activity of the enzyme by the stationary electric field. The optimum field is not quite at zero. If the gradient of  $S$  is reversed, the lower line in Fig. 7A is obtained. If we refrain from making the enzyme cycle at zero electric field and zero gradient of  $S$  completely symmetric, but introduce an asymmetry factor  $b$  (which makes states 4 and 2 energetically more favorable than states 1 and 3; for further special properties of such enzymes see below and Refs. [40,41]; for other asymmetry cases see Ref. [42]), one can obtain results as shown in Fig. 7B. Now, the catalytic activity is biased in the sense that it has a high optimum activity in transporting  $S$  down its electrochemical gradient if the latter is in the one orientation, but a low optimum activity if it is in the opposite orientation. Apparently the "diode" characteristics of catalysis [cf. 2], can be modulated by the transmembrane electric field. Finally, we would like to stress that (in contrast to cases with non-stationary electric fields which we shall discuss below), flux here is always down the chemical potential difference of  $S$ : the electric potential does not provide a driving force which could reverse the flux through the cycle.

## 3. Dynamic electric fields in catalysis and free-energy transduction

### 3.1 Oscillating electric fields driving work

Until rather recently, discussions of the roles of electric fields in bioenergetics were limited to static electric fields. Changes in a transmembrane electric

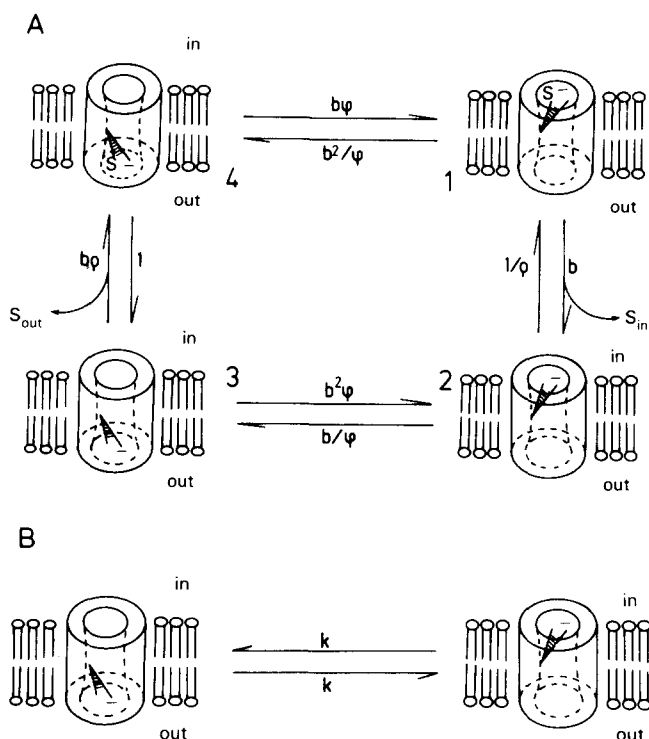


Fig. 6. Membrane proteins considered in view of catalytic or free-energy transduction effects of fluctuating electric fields. (A) A "translocator" protein corresponding to cycle  $b$  in Fig. 4. An arm of the protein, which now contains a negative charge in addition to a binding site for  $S$ , can flip-flop between two positions that expose it either to the inner or the outer surface of the membrane. (B) A "generator" protein with a negatively charged arm that fluctuates between a position on the external and a position on the internal surface of the membrane. Transition rate constants are indicated. The electric potential dependent factor  $\phi$  is defined by eqn. (17);  $\rho$  is defined as  $\sqrt{[S_{out}]/[S_{in}]}$ . Unless otherwise indicated parameter values will be:  $\phi = 16$  ( $\Delta\psi = 142$  mV),  $\rho = 0.35$ ,  $b = 500$ ,  $k = 7.4$ .

field were considered catalytically relevant only on time scales much slower than the catalytic turnover times of the enzymes. Resulting from a metabolic change, they in turn could affect other metabolic processes. In terms of their ability to serve as a free-energy source to do work, only their time-independent aspect was taken into account; if the time average of the field would be zero, no work would emanate.

From everyday experience we know that electric potentials with zero time average can be used to do work. Our use of the AC wall outlets is the demonstration. Yet, the possibility that the biological work-horses, the enzymes, might use oscillating electric fields as their input power, has received little attention, even though experimental effects of oscillating electric fields on biological tissue have been known for some time now [43].

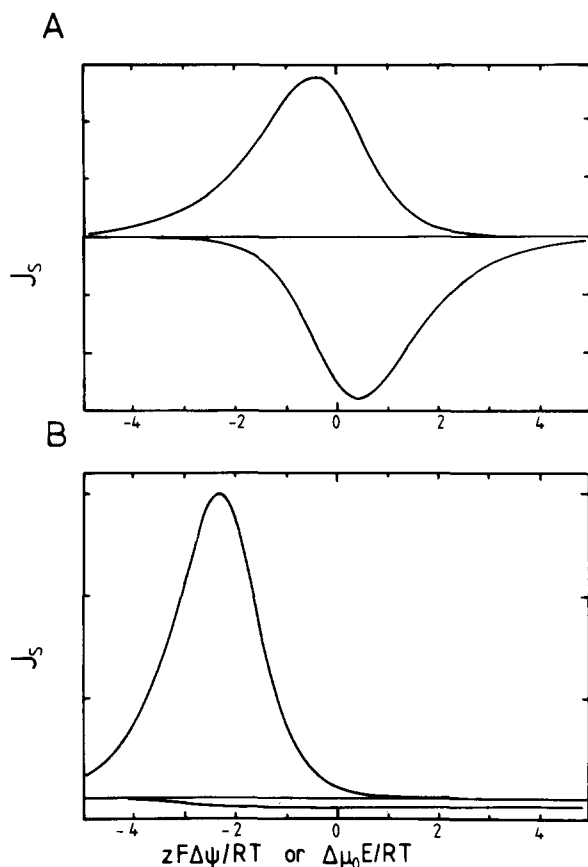


Fig. 7. Catalytic effects by time-independent transmembrane electric fields. The outward flux of S is given as a function of the time-independent transmembrane electric field (in units  $RT$ ) for the case where (upper lines) the internal concentration exceeds the external concentration by a factor  $10^{10}$  (think of ATP synthesis) and the case where the concentration gradient is the other way around. (A) symmetrical translocator ( $b=1$ ), (B) asymmetrical translocator ( $b=500$ ). The charge of the protein arm,  $z$ , is in units electron charge.

Serpersu and Tsong reported work done by the  $\text{Na}^+ - \text{K}^+$ -ATPase in the absence of substantial ATP hydrolysis, but in the presence of an oscillating electric field [44,45].  $\text{Rb}^+$ , which served as an analogue for  $\text{K}^+$ , was taken up by the erythrocytes. The effect depended on the magnitude of the field, and was reduced by the addition of ouabain.

In principle, simple enzyme systems, like the one illustrated in Fig. 6A, can indeed transduce free energy from an oscillating electric field to work [23,40,41]. Such work may consist either of transport of a substance against its electrochemical potential gradient, or of an endergonic chemical reaction, such as the synthesis of ATP. The calculation in Ref. [23] treats the case where states 1 and 3 differ from states 2 and 4 in their permanent dipole moment. The cal-

culations in Ref. [40] discuss the system in terms of differences in the position of an electrically charged part of the protein (see above). For the kinetic and thermodynamic arguments there is, in fact, no difference between the two cases, provided that it is realized that a change in dipole moment (in the direction of the field) of 240 D ( $0.8 \times 10^{-27}$  C m) is equivalent to the translocation of an elementary charge across a 5-nm membrane. In the following we shall discuss the topic in terms of the latter type of system, but it should be taken implied that similar arguments will hold for the variable dipole moment systems.

If subjected to an oscillating electric field, the system depicted in Fig. 6A can transport the electrically neutral substance S against its concentration gradient [40]. Clearly this is more than what a stationary electric field could do (Fig. 7). The physical picture behind it is that, when the enzyme is in state 4 (cf. Fig. 6A) and the electric field happens to be positive on the inside, this will make the negatively charged arm of the enzyme move inward, such that the enzyme assumes state 1. If the field would retain this orientation for a while, then the enzyme would re-equilibrate between states 1 and 2 (which both have the negative arm on the inside). In the case that the basic free energies of states 1 and 2 would be equal, states 1 and 2 would become equally populated. Inversion of the electric field would then cause equal transition fluxes from states 2 to 3 and 1 to 4 (we take the unidirectional rate constants and their electric field dependencies to be equal). If however, the basic free energy of state 2 would be lower than that of state 1, inversion of the field would cause more flux from the more densely populated state 2 (to state 3) than from state 1 (to state 4). If additionally the basic free energy of state 4 would be lower than the basic free energy of state 1, continued application of an alternating electric field would cause clockwise cycling of the enzyme through its four states.

To allow for a clear analysis of the work potential of the system of Fig. 6 with this type of asymmetry, we introduced the parameter  $b$  which exclusively reflects the higher stability of state 2 compared to state 1 and of state 4 compared to state 3. The electric potential dependence was distributed equally over the forward and the reverse unidirectional rate constants, by means of the factor  $\phi$  defined as:

$$\phi = \exp(F\Delta\psi/2RT) \quad (17)$$

The back pressure resulting from the transmembrane gradient of S was distributed equally over the inside part of the diagram and the outside part of the diagram through the factor  $\rho = \exp(\Delta\mu_S/2RT)$ . Thus, the system was chosen as symmetrical as possible, except for the asymmetry in the stabilities of states 4 and 2 versus states 3 and 1.

Figure 8 gives the dependence of the capacity of this system to transduce work from an oscillating electric field upon various properties of the system



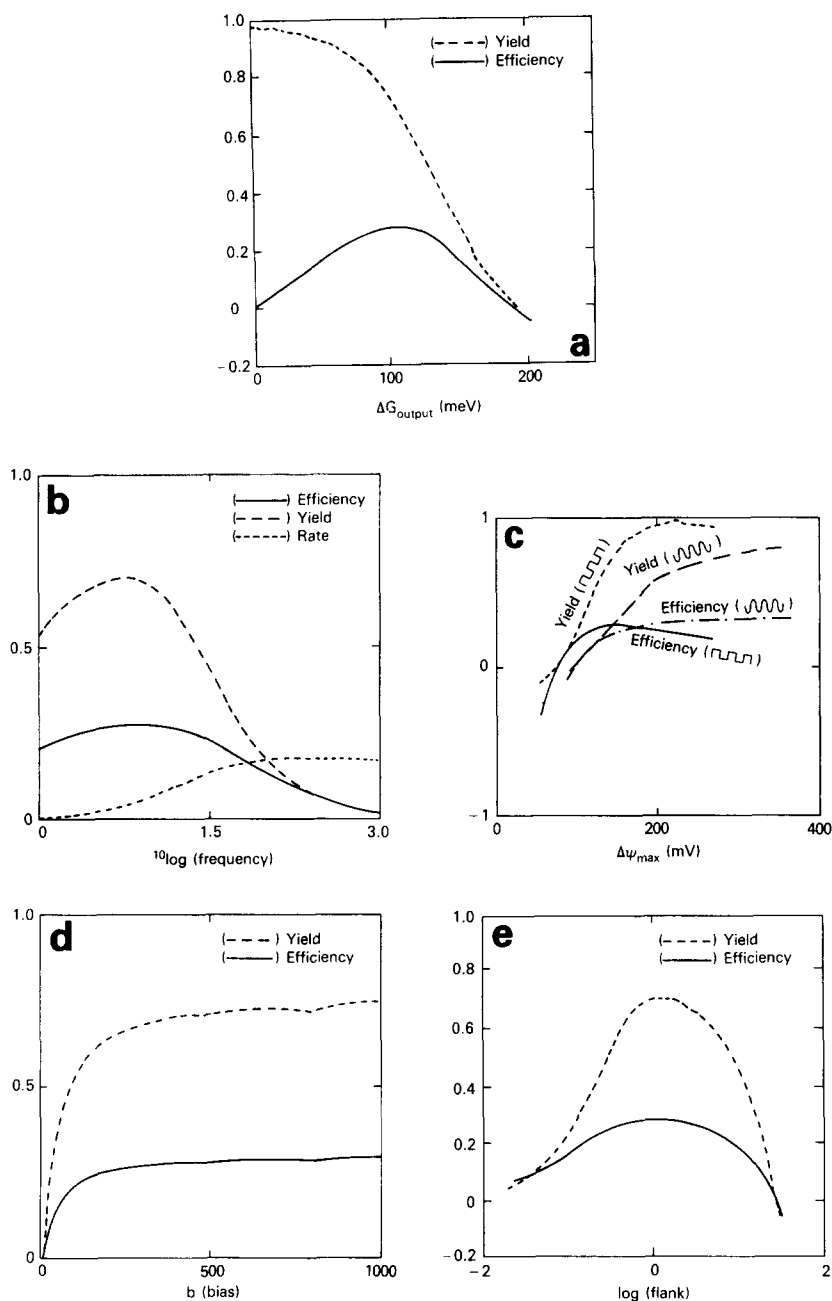


Fig. 8. Free-energy transduction performance of the model free-energy transducer of Fig. 6A in a square-wave oscillating electric field. Full lines refer to the thermodynamic efficiency, dashed lines to yield (in number of clockwise enzyme cycles per field cycle). For the rate in Fig. 8b, 0.5 on the ordinate axis corresponds to 50 cycles per unit time.

and the field. Figure 8d confirms that in our model system some asymmetry (in this case in terms of differential stability of enzyme states, for other cases see Ref. [42]) is essential for the transduction of free energy: if  $b$  equals 1, both the yield, in terms of number of molecules of S transported per complete field cycle, and the thermodynamic efficiency of the free-energy transduction (defined as the ratio between the rate at which free energy is retrieved in S being transported against its thermodynamic potential and the rate at which free energy is absorbed from the electric field [40,2]) is zero.

Yield and efficiency of free-energy transduction also depend on kinetic properties of the system. For instance, the magnitude of the unidirectional rate constants for the “lateral” (i.e.,  $1 \rightarrow 2$  and  $3 \rightarrow 4$ ) transitions should not be too disparate from the unidirectional rate constants of the transversal (i.e.,  $3 \rightarrow 2$  and  $1 \rightarrow 4$ ) transitions. Figure 8e shows the effect of multiplying all lateral unidirectional rate constants by the factor “flank”.

In Fig. 8a we see that at a low transmembrane gradient of S, the yield can be extremely high. With increasing output free energy  $\Delta G_{\text{out}}$  ( $\equiv \Delta \mu_S$ ), the yield decreases. On the other hand, the thermodynamic efficiency of the free energy transduction is zero when the gradient in [S] is small, increases with that gradient until it goes through a maximum and then decreases again.

The yield and efficiency of free-energy transduction as a function of frequency are given in Fig. 8b. The dashed line shows that at low frequencies the yield in enzyme (or work) cycles per field cycle increases with frequency. This demonstrates that it is indeed the oscillation of the field that is responsible for the free-energy transduction. At high frequencies however, the yield per field cycle decreases again. This is because, initially, the fluxes from state 4 to state 1 and from state 3 to state 2 tend to be equal. It is only after state 3 gets depleted that the flux from state 4 to state 1 exceeds that from state 3 to state 2. At very high frequencies, state 3 does not have the time to become depleted.

Because the duration of each cycle decreases with increasing frequency of the oscillating electric field, it could well be that with increasing frequency, the cycling flux per unit time would keep increasing. The dotted line in Fig. 8b gives this flux per unit time. At frequencies higher than 1000, the flux per unit time decreases with frequency; for instance, for a frequency of  $10^4$  it is only 10 and at  $2 \times 10^4$  only 6.

The other adjustable parameter in the alternating field is the field amplitude (i.e.,  $\Delta \psi_{\text{max}}$ ). Figure 8c shows (--- and --) that the yield increases monotonically with the amplitude of the applied field. The efficiencies (solid line and - · -) go through an optimum because at high field strength an increase in field strength does not lead to much increase in the yield, but does lead to an increase in free-energy absorption from the field.

We also studied the maximum output force that the model system described here could achieve. The “static head force-ratio” or “efficacy”, evaluated as  $\Delta G_{\text{out}}/\text{field amplitude}$  readily exceeded 1 and could come close to 2. This is

because the effective input force is actually the magnitude  $|\Delta\psi_{\max} - \Delta\psi_{\min}| = 2\Delta\psi_{\max}$ . Up to frequencies of 1000, this efficacy increased with frequency and with the asymmetry factor  $b$ .

### 3.2 Autonomous fluctuations in the electric field may also drive an enzyme into doing work

The electric fields considered in the previous section were oscillating in a regular manner: they had the form of a square wave (for sinusoidal wave forms see Ref. [40]). This may correspond to experimental conditions [44,45], but not so much to what is expected *in vivo*. There, fluctuations in the electric field are expected to be more irregular. To come closer to the *in vivo* situation, we [41] considered the situation illustrated by Figs. 6A and B: the enzyme ("translocator") that could translocate the neutral substance S if exposed to a regularly oscillating electric field, plus a "generator". The latter would generate a fluctuating transmembrane electric potential difference because its negatively charged arm can flip-flop between two positions on opposite sides of the membrane. We assumed [41] that the unidirectional rate constants of the transitions of the generator charge were equal and independent of the environment of the generator (i.e., the fluctuations were assumed to be "autonomous", see, however, below). The translocator was assumed to be close enough to the generator to experience electric fields due to the generator charge comparable to those resulting from 140 mV transmembrane electric potential differences.

Considering these two elements, close to one another in the same membrane, as a single system, one recognizes that this system can be in any of eight states. The states in the outer square of Fig. 9A have the generator charge (not shown) on the outside, whereas the states on the inside have the generator charge on the inside of the membrane. The rate constants for the transmembrane transitions (Fig. 9A) reflect this. For instance, in the outer square the inward translocation of the charged arm of the translocator is helped by the field factor  $\phi$  ( $\phi > 1$ ), whereas in the inner square this translocation is made less probable by the same factor. The factor  $\phi$  is again defined by eqn. (17), where  $\Delta\psi$  represents the difference in electric potential between the two positions the translocator charge (of  $z_t$  elementary charges) can assume. One can estimate that if the translocator charge would flip-flop across a 5-nm membrane and would be 1 nm away from the generator charge when both are on the same side, this would correspond to a  $\Delta\psi$  of some 230 mV.

The system depicted in Fig. 9A is formally identical to any 8-state enzyme with the same unidirectional rate constants and the same allowed transitions. This has the important corollary that the kinetic and thermodynamic properties of our system of translocator plus generator can be analyzed mathematically by the same methods that would be used for such an eight-state enzyme (cf. Section 1.3.1) [41]. We note that for any cycle  $\kappa$ , the driving force  $X_\kappa$  will

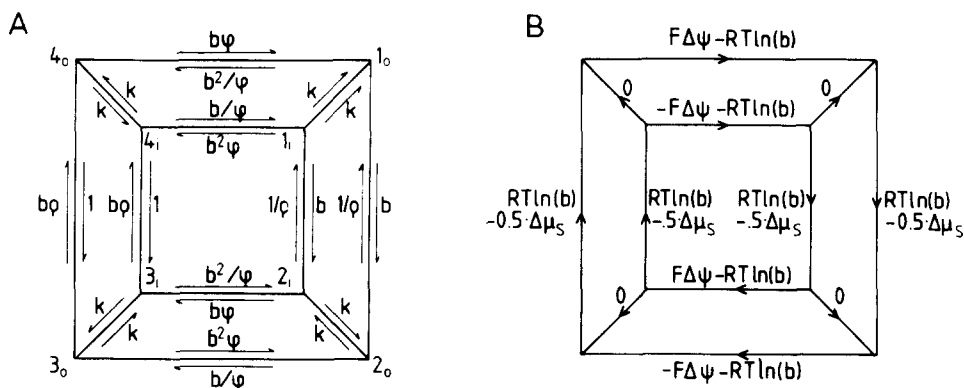


Fig. 9. A single diagram for the system consisting of translocator plus generator (i.e., Figs. 6A and B), close together in the same membrane. Subscripts  $o$  and  $i$  to the states refer to the position of the generator charge, i.e., at the outer and inner membrane surface respectively. The numbers refer to the state of the translocator (cf. Fig. 6A). In (A) the magnitudes of the unidirectional rate constants are given, in (B) the basic thermodynamic driving forces along the branches, in the direction of the arrow head.  $2F\Delta\psi = RT\ln(\phi)$ .

also be equal to the sum of the “basic driving forces” around the branches of that cycle, provided that these “basic driving forces” are defined as  $RT$  times the natural logarithm of the ratio of the forward to the reverse rate constant for that branch:

$$X_{i \rightarrow j} = RT \ln(\alpha_{i \rightarrow j} / \alpha_{j \rightarrow i}) \quad (18)$$

Thus, the basic driving force for a branch is equal to the basic free-energy difference between the two states bordering the branch. In Fig. 9B we give the basic driving forces for the branches of the translocator/generator system.

Figure 9B allows us to investigate which cycles have the electric potential generated by the generator system as part of their driving force and would thus be responsible for free-energy transduction. Some cycles in Fig. 9B are somewhat meaningless. For instance, the cycle  $4_i, 3_i, 3_o, 4_o$  corresponds to the oscillation of the translocator between two states on one side of the membrane, with the generator oscillating across the membrane and back. The driving force for this cycle equals zero, such that there will be no steady net cyclic flux. On the other hand, cycles like  $4_i, 1_o, 1_i, 4_i$  translocate the S-binding group across the membrane with concomitant oscillation of the generator, but no transport of S results because S does not associate or dissociate from the enzyme. This type of cycle does have a driving force, i.e.,  $2F\Delta\psi$ , and constitutes a “slip” of free energy (input slip) [34]. Our primary interest lies in cycles that do transport S. These are all possible cycles that encircle the central square in Fig. 9. Of these, neither the outer cycle nor the inner cycle, which correspond to turnover of the translocator without the generator charge changing position, have

the factor  $\phi$  in their driving force. Their sole driving force is  $\Delta\mu_S$ , such that these cycles will always work in the direction of transporting S down its chemical gradient (output slip). In the clockwise direction cycles transport S from out to in. The cycle  $4_o, 1_o, 1_i, 2_i, 3_i, 4_i, 4_o$  is a cycle that does this with a driving force  $(2F\Delta\psi - \Delta\mu_S)$ . There are four cycles like this, all with the same driving force. They have in common the  $4_o$  to  $1_o$  transition and the  $2_i$  to  $3_i$  transition. In other words, in these cycles, the negatively charged translocator arm swings inward when the generator charge is on the outside, and swings back when the generator charge is on the inside. Through these cycles S can be transported against its chemical gradient, provided that  $2F\Delta\psi$  exceeds  $\Delta\mu_S = RT \ln([S]_{in}/[S]_{out})$ . On the other hand, for each of these cycles there is a symmetrical cycle (for the present example the cycle  $3_o, 3_i, 4_i, 1_i, 2_i, 2_o, 3_o$ ) that will have the driving force  $-2F\Delta\psi - \Delta\mu_S$ , such that it will tend to operate in the reverse direction. An end result of uphill transport of S driven by the field generated by the fluctuations of the generator charge will only be attained if the probability times the kinetic capacity of the former set of cycles exceeds the probability times the kinetic capacity of the latter set plus the back slippage of S along the cycles corresponding to the outer and the inner square, respectively, of Fig. 9.

Although the driving force and the kinetic capacity for the relevant cycles are easily calculated, the probabilities are quite complicated expressions in terms of all the rate constants. Consequently, the following approaches have been used to prove that in the system of generator plus translocator described by Fig. 9 the free energy contained in the electric field fluctuations generated by the generator can drive the translocator to transport the neutral substance S. First, the situation was considered in which no concentration gradient of S had yet developed. For the kinetic analysis of the system this results in two simplifications: (i) The two slip cycles, corresponding to the outer and the inner square of Fig. 9, respectively, can be left out of consideration, because their driving force reduces to zero. Second, the factors  $\rho$  in the equations vanish because they become equal to 1. Thus we were able to derive for the translocation rate of S,  $J_S$ , the following equation:

$$J_S \Sigma / (b^5 k^2) = (b-1)(\phi^2 - \phi^{-2})^2 \{b(b+1) + 2(b+2k+1)/(\phi + \phi^{-1})\} \quad (19)$$

Since  $(\phi^2 - \phi^{-2})^2$  is always positive if  $\phi \neq 1$ , it follows that S will be translocated from out to in whenever  $b$  exceeds 1 and from in to out whenever  $b$  is smaller than 1. Clearly, free-energy transduction from the fluctuating electric field is the general rather than the special case! Apparently, the stability ratio of the states 4 and 3 (2 and 1) provides the asymmetry that defines the direction of pumping.  $\Sigma$  is always positive [28].

Although the above equation proves that in the generator-translocator system under study the fluctuating transmembrane electric potential difference can drive the transport of S, it does not prove that free-energy transduction

can occur. For, in the absence of the concentration gradient of S, the free-energy difference across the output reaction is zero; no free-energy is recovered in the output reaction. Yet, by use of the above equation one can demonstrate that free-energy transduction is possible in this system. First, we note that at  $\rho=1$ ,  $J_S$  can have a value that is significantly different from zero. Second, we note that  $J_S$  is a continuous function of  $[S]_{\text{in}}$  and  $[S]_{\text{out}}$  and hence of  $\rho$  [28]. Consequently, there should be an interval around  $\rho=1$  in which  $J_S$  exceeds zero. In this interval free-energy transduction will take place.

Another way of proving that free-energy transduction can occur in the system under study is by the numerical calculation of examples. Indeed, this is feasible, because there is an alternative method to calculate  $J_S$ , i.e., the matrix inversion method: For each of the eight states of the system, one formulates the rate at which its concentration (in terms of fraction of the total number of systems) changes as a function of the concentration of all the states of the system. Here the rate constants given in Fig. 9 can be used. For state  $4_o$  this differential equation is:

$$dE_{4_o}/dt = (b^2/\phi)E_{1_o} + b\rho E_{3_o} - (k+1+b\phi)E_{4_o} + kE_{4_i} \quad (20)$$

Doing this for every state of the system, one obtains eight dependent equations. Using the relationship:

$$E_{4_i} = 1 - E_{4_o} - E_{1_o} - E_{2_o} - E_{3_o} - E_{1_i} - E_{2_i} - E_{3_i} \quad (21)$$

$E_{4_i}$  can be eliminated, leaving seven independent equations. Defining the column vector  $\mathbf{e}$  of length seven as consisting of the concentrations of the remaining seven system states, these equations can be formulated as:

$$d\mathbf{e}/dt = \mathbf{M} \cdot \mathbf{e} - \mathbf{q} \quad (22)$$

Here  $\mathbf{q}$  is a column vector of length seven. In the steady state the time average of  $d\mathbf{e}/dt$  must be equal to zero. Both matrix  $\mathbf{M}$  and vector  $\mathbf{q}$  are time-independent. As a consequence, the above equation can be solved for the time average of  $\mathbf{e}$  in the steady state:

$$\bar{\mathbf{e}} = \mathbf{M}^{-1} \cdot \mathbf{q} \quad (23)$$

Using this procedure we calculated the transmembrane flux of S for a number of cases and demonstrated that the fluctuating electric field emanating from the generator can indeed drive uphill transport of S [41]. For a ninefold concentration ratio of S and an electric potential term corresponding to 142 mV, Fig. 10a gives the rate of translocation of S (against its gradient) as a function of the transition rate constant of the generator charge at three different magnitudes of the asymmetry constant  $b$ . As in the calculation with the oscillating electric field (cf. Fig. 8), and in the experimental observations [23], there is an optimum frequency at which the electric field may oscillate. Figure 10b shows the flux of S as a function of the generator transition rate constant

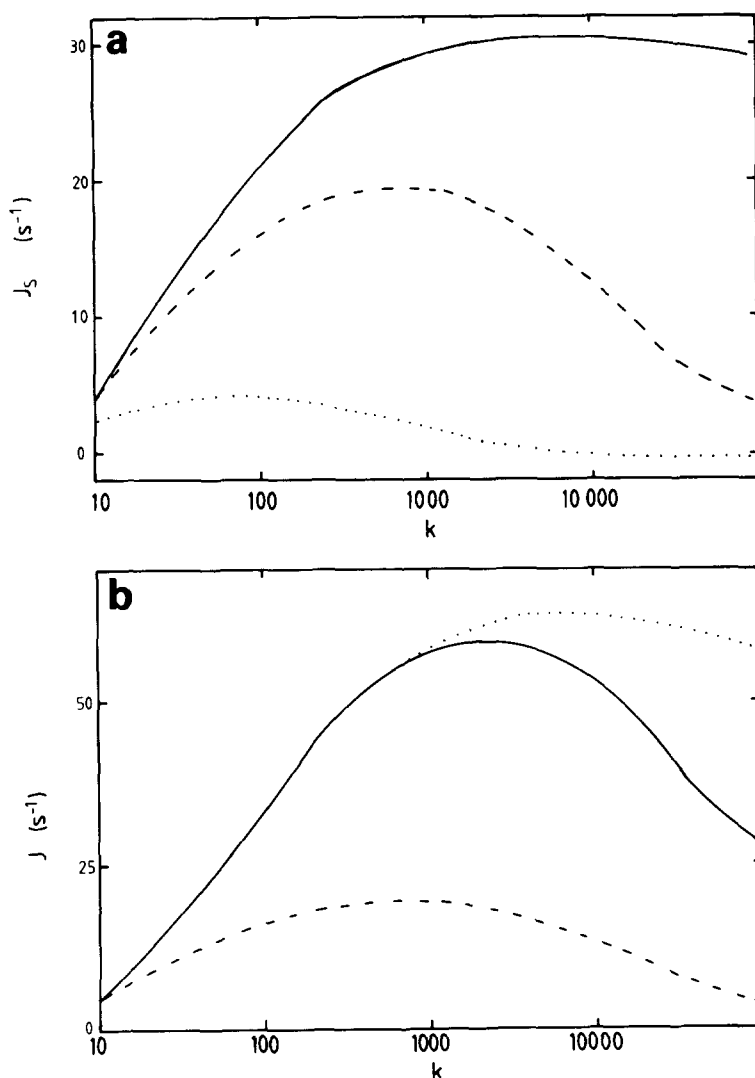


Fig. 10. Thermodynamically uphill transport of S driven by a fluctuating transmembrane electric potential as a function of the rate constant of the fluctuating charge. Calculated for the system of Figs. 6A and B, by applying the matrix inversion method to Fig. 9A. Standard parameter values except that in (a) ( $\cdots$ )  $b=50$  and (—)  $b=5000$ , in (b) (—)  $\phi=160$  and ( $\cdots$ )  $\phi=1600$ .

at three different magnitudes of the electric interaction between translocator and generator.

We also calculated the concentration ratio of S the system could maximally attain (i.e., at static head, defined by the translocation rate having readjusted to zero). These ratios could become quite appreciable and, again, were functions of the transition rate constant of the generator charge (Fig. 11a). Exper-

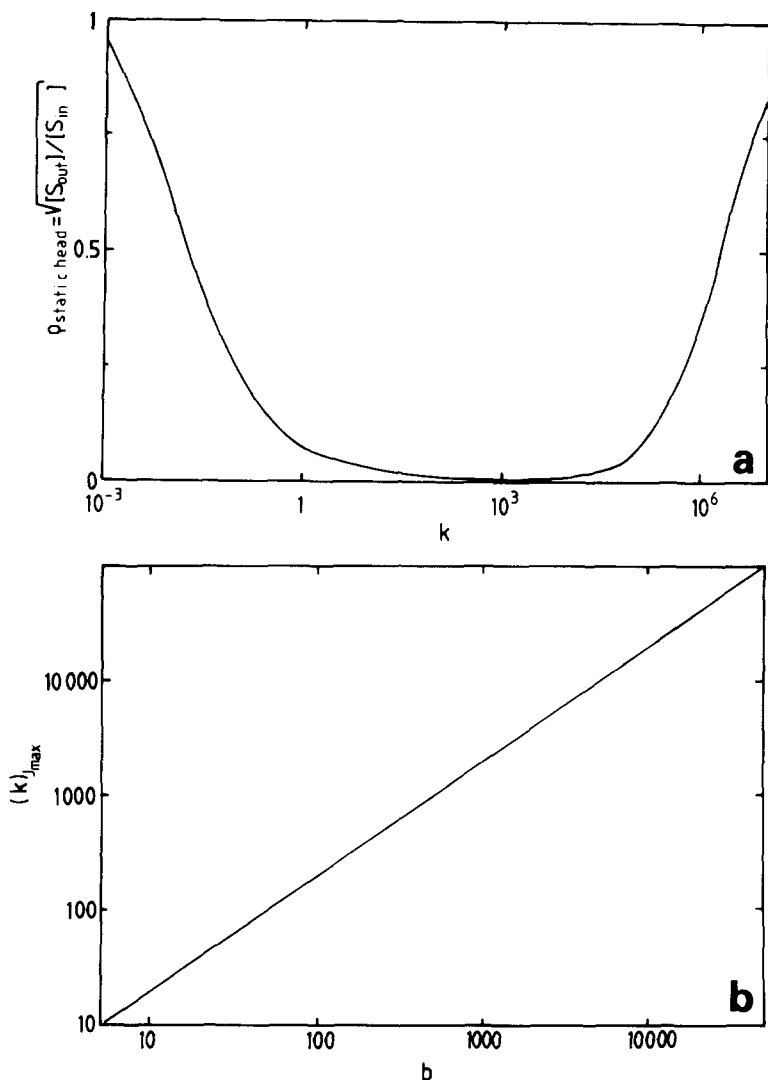


Fig. 11. Optimum characteristics of the free-energy transduction by the system of Figs. 6A and B. (a) The static head concentration ratio of S,  $\rho$ . At standard parameter values, but various magnitudes of  $k$ , the magnitude of the concentration ratio of S was varied until the translocation rate of S became equal to zero. The corresponding value of  $\rho$  is plotted for each value of  $k$ . (b) The optimum value of  $k$  for each extent of asymmetry (parameterized by  $b$ ) of the translocator. For standard parameter values, but various magnitudes of  $b$ ,  $k$  was varied until the highest values for the inward translocation rate of S was obtained. That value of  $k$  was plotted versus the values of  $b$ .



imental determination of the dependence of  $J_s$  as a function of the transition rate constant of the generator charge may be feasible if the generator charge is replaced by a macroscopic electric field with random switch. These dependences may then provide information about the kinetic characteristic of the translocator system. For instance, the transition rate constant at which the translocation rate is at its maximum depends strongly on the magnitude of the asymmetry constant  $b$  (Fig. 11b) [41,42].

### *3.3 Possible origins of the random fluctuations in the electric field*

In the calculations reported in the previous section the transition rate constant of the generator charge was assumed to be constant, i.e., independent of the environment of the generator system. This may be realistic for a case where the generator charge represents a macroscopic generator of an electric field. However, for an actual single charge that fluctuates between two binding sites across a membrane, this assumption is not realistic [41]: if the generator charge is sufficiently close to the translocator to influence the transitions of the latter, then the transitions of the generator charge will sense the electric field generated by the translocator. In the extreme case in which the mutual influence is reciprocal, no free-energy transduction remains [41]. Indeed, this is the situation if the generator charge is a fluctuating charge at equilibrium, i.e., without its fluctuations being driven by an additional driving force.

In biological membranes however, there are cases where the fluctuations of charge across the membrane may be driven by an additional reaction that, by itself, dissipates free energy. An example would be the case where there is an  $\text{Na}^+$  concentration gradient across a membrane that is nonpermeable to  $\text{Na}^+$  except for an  $\text{Na}^+$ -selective pore. If a lid covers this pore, and if this lid fluctuates between positions at which the pore is open and a position at which the pore is shut, then there can be fluctuations in an  $\text{Na}^+$ -induced diffusion potential across the membrane. Transmembrane enzymes that are subject to these fluctuations could harvest free energy from them.

In membranes specialized in catalyzing free-energy transduction, fluctuations in the electric field may be driven by a chemical reaction. As a potential example, the upper part of Fig. 12 (discussed below) shows a putative component of the mitochondrial electron-transfer chain, which catalyzes a redox reaction and translocates a charged group cyclically across the membrane. If this electron-transfer chain component would be brought close to the translocator element of Fig. 6, then its transitions would be influenced by the electric field generated by the translocator, but this influence would not be dominant; the transitions are also under the influence of the thermodynamic driving force of the redox reaction that drives the cycle. In such a case, free-energy transduction might again occur (see also below and in Ref. [41]).

## 4. Coupling at the organizational level

### 4.1 Chemiosmotic coupling

Although one does encounter electric potentials as the purpose of some biological processes, such as signal transmission by the nerve cell, in most other cases, the role of the potential is an energetic one, i.e., to serve as the driving force for transport or chemical reactions. The clearest example of how a transmembrane electric potential difference can serve as such a high free-energy intermediate may well be the chemiosmotic coupling scheme for oxidative phosphorylation proposed by Mitchell [11] and largely substantiated since then [46]. Electron transfer from substrates with low redox potential to substances with high redox potential is catalyzed by enzymes that are present in the inner mitochondrial membrane. Coupled to the electron transfer, these enzymes pump protons across that membrane, thus creating a pH difference (alkaline inside) and an electric membrane potential difference (minus inside). Figure 4 would become a prototype of such enzymes if the ATP hydrolysis reaction would be replaced with the aforementioned redox reaction and if S would be replaced with  $H^+$ . In the same membrane another enzyme is present, the  $H^+$ -ATPase. Driven by the hydrolysis of ATP, this enzyme pumps protons outward. Also for this enzyme Fig. 4 could serve as a prototype [2], again with S replaced by  $H^+$ . At sufficient activity of the redox enzymes a rather high membrane potential and pH gradient would be generated and these would then be able to drive the  $H^+$ -ATPase in the reverse direction (the clockwise direction in Fig. 4). Thus ATP synthesis would be ultimately driven by a redox reaction, with the transmembrane electrochemical potential difference for protons,  $\Delta\tilde{\mu}_{H^+}$  as the energetic intermediate.  $\Delta\tilde{\mu}_{H^+}$  consists of two parts, i.e., the pH difference between the two compartments and the electric potential difference between the two compartments,  $\Delta\psi$ :

$$\Delta\tilde{\mu}_{H^+} = -5.7 \Delta pH + 0.096 \Delta\psi \quad (24)$$

where  $\Delta\tilde{\mu}_{H^+}$  is in kJ/mol,  $\Delta\psi$  in volts and  $\Delta pH$  in pH units.

Consequently, a static transmembrane electric potential difference can serve as an energetic intermediate in free-energy transduction. Processes other than ATP synthesis, such as transport, or reverse electron transfer can take the place of ATP synthesis and processes other than redox reactions, such as ATP hydrolysis, transport can take the place of the redox reaction in generating  $\Delta\tilde{\mu}_{H^+}$ .

### 4.2 Other modes of coupling at the organizational level

Prior to the chemiosmotic coupling model, other ways of coupling reactions carried out by different enzymes had been elucidated. The first has very early roots and is indeed one of the most important ways of coupling: the reaction catalyzed by enzyme 1 (say the dephosphorylation of phospho enol pyruvate)

is coupled to the conversion of chemical A to chemical B. Reaction 2 (say the phosphorylation of glucose) is catalyzed by an enzyme (hexokinase) that couples that reaction to the conversion of B to A. The A–B couple, or rather the free energy difference between B and A plays the role of the energetic intermediate between reaction 1 and 2. The energetic nature of the intermediate is, perhaps, clearest in the ubiquitous cases where A and B correspond to ADP and ATP. However, other important, so-called coenzyme couples are  $\text{NAD}^+ - \text{NADH}_2$  and  $\text{NADP}^+ - \text{NADPH}_2$ , and creatine–creatine phosphate. This mode of coupling could be called “chemical coupling”.

In the absence of the identification of an A and B in oxidative phosphorylation, it was proposed that mitochondrial respiration would be coupled to a conformational change of either the entire mitochondrial membrane, or a protein component thereof [47] and that relaxation of the resulting conformation to the original one would drive ATP synthesis. Conformational changes are observed in the process of oxidative phosphorylations, as they are in so many unrelated enzyme-catalyzed reactions, but it remains difficult to assess their energetic importance as an intermediate. Once proton movements coupled to redox reactions, the generation of pH gradients and transmembrane electric potentials, and the driving of ATP synthesis by artificial such gradients had been demonstrated, the general preference shifted to the coupling scheme proposed by the chemiosmotic hypothesis.

#### 4.3 $\Delta\tilde{\mu}_{\text{H}^+}$ as the intermediate; delocalized or localized?

The measurement of pH gradients and electric potentials across biological membranes is subject to substantial random and systematic errors, such that it is somewhat difficult to analyze with definiteness whether the transmembrane electrochemical potential difference for protons behaves as the thermodynamically and kinetically competent intermediate in mitochondrial oxidative phosphorylation (and the analogous processes of membrane-mediated free-energy transduction). Yet, in as far as such analyses are carried out, they tend to yield observations that are hard to reconcile with the interpretation that the  $\Delta\tilde{\mu}_{\text{H}^+}$  that is relevant for mitochondrial oxidative phosphorylation is indeed the electrochemical potential difference for protons between an essentially homogeneous inner aqueous phase and a presumed homogeneous external aqueous phase [48–50]. Often the observed magnitude of  $\Delta\tilde{\mu}_{\text{H}^+}$  is too small to account for the free energy of the ATP that is being synthesized, protons that should be pumped across the membrane are not, or not quickly enough, seen in the aqueous bulk phase, redox-driven proton pump and  $\text{H}^+$ -ATPase behave as single units rather than as subsequent enzymes in a non-channelled metabolic pathway, and the rate of ATP synthesis is controlled by electron transfer more directly than through the magnitude of the  $\Delta\tilde{\mu}_{\text{H}^+}$  alone.

This is not the place to review and discuss the experimental data relevant to these points (for such reviews see Refs. [48–50]). What we wish to do however,

is to draw attention to an almost [48,51–54] overlooked point: The proposal that the proton is the coupling intermediate in membrane-linked free-energy transduction, does not necessarily mean the time and space average of the transmembrane electrochemical potential difference for protons is the free-energy intermediate. Indeed, several authors (reviewed [48,55]) have proposed that protons pumped by an electron transfer chain would not delocalize all over the bordering aqueous phase, but would remain close to that electron transfer chain and an  $H^+$ -ATPase that is continuously or transiently [49,56] close by. Thus these protons would be predestined to drive the ATP synthesis catalyzed by that specific  $H^+$ -ATPase. It has been shown [53] that such a scheme would exhibit rather peculiar kinetic properties, which could indeed account for the observations that were not consistent with delocalized chemiosmotic coupling. The disadvantage of this type of coupling scheme, is not that it is “nebulous” [57], as that is what the truth may be after all, but that it depends on the assumption that there must be something that prevents the proton pumped by the electron transfer chain from equilibrating with the adjacent aqueous bulk phase [48]. Since proton movement in aqueous phases and in proteinaceous phases tends to be much more rapid than the turnover times of the  $H^+$ -ATPase [48], a special structural element would have to be postulated. No direct experimental evidence for such a specialized structural barrier for proton diffusion exists, though all that is necessary to be postulated is that whilst the primary proton pump moves protons across the membrane, it does not *release* them to the bulk if they are then to drive phosphorylation.

#### 4.4 An electrodynamical coupling model

Against the background of Section 3 of this article, we are in the position to postulate an electrodynamical coupling model for oxidative phosphorylation, which accounts for most of the experimental observations, without the necessity to postulate a diffusion barrier for protons. Figure 12 contains the essence of the model. Close (either statically, or dynamically) to each other in the free-energy transducing membrane, there are (i) the electron-transfer chain that catalyzes the transfer of an electron coupled to a cyclic translocation of a charged group (or perhaps even the electron itself) across the membrane and (ii) the ATPase that catalyzes ATP hydrolysis coupled to a cyclic translocation of a charged group across the membrane. From the calculations by Kamp and colleagues [13] it can be gleaned that, provided that the two proteins are close (i.e., within 1 nm, see also Section 3.2), or the charged groups do not quite extend into the aqueous phases bordering the membrane, the ATPase will sense the electric field generated by the charge of the redox enzyme. As substantiated above the ATPase may well possess some of the wide range of possible asymmetry properties [40–42] required for the above-described free-energy transduction from a fluctuating electric field. It should then be able to extract free energy from the fluctuating local electric field generated by the turnover of the

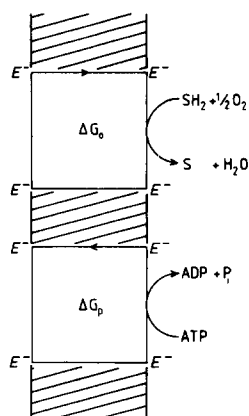


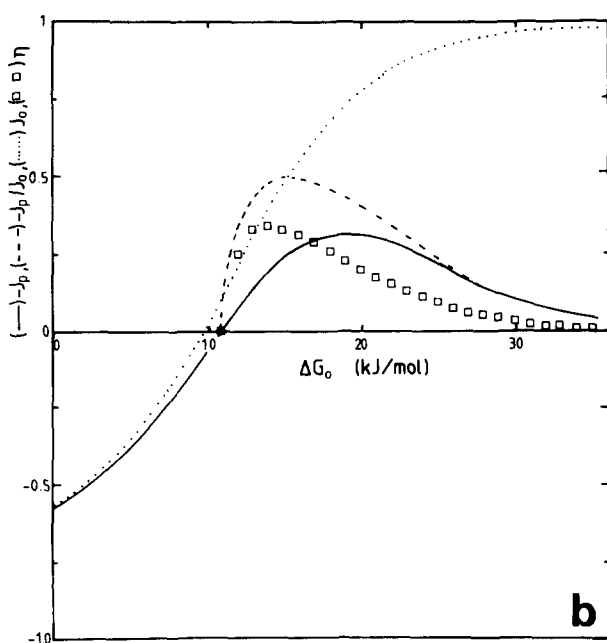
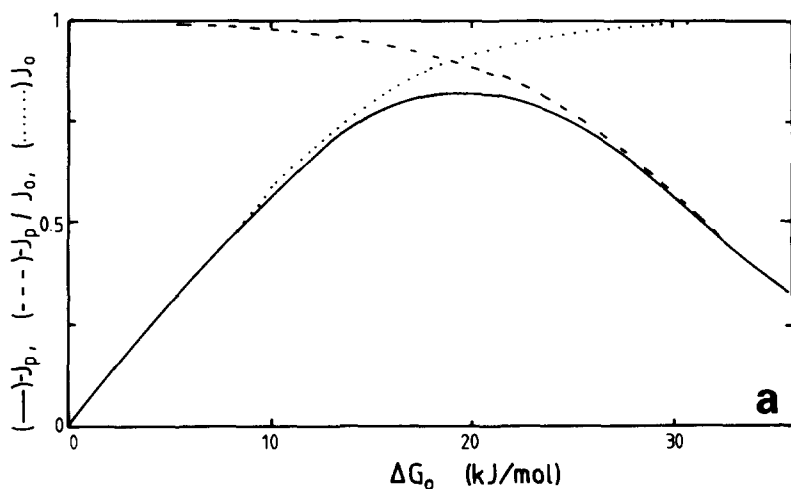
Fig. 12. Scheme for the electrodynamical coupling hypothesis for membrane-linked free-energy transduction. In its formulation for mitochondrial oxidative phosphorylation, an electron-transfer chain and an ATPase would be close together (either continuously, or transiently) in the inner mitochondrial membrane. The catalytic cycle of the redox enzyme would comprise a cyclic transmembrane transfer of a charged protein group (or a cyclic change of the dipole moment of that enzyme). Also, the catalytic cycle of the ATPase would comprise such a cyclic translocation of charge. The oscillating ("fluctuating") electric field generated by the turnover of the former enzyme could drive the ATPase cycle in reverse, causing the synthesis of ATP. Note that neither enzyme needs to be a proton pump (see Refs. [51] and [54] for cases where they would be).

redox enzyme and use this to drive the synthesis of ATP from ADP and inorganic phosphate.

For those who doubt that this is possible, we have carried out model calculations using the matrix inversion method (see above). Figure 13 shows the rate of ATP synthesis ( $-J_p$ ), the rate of oxidation ( $J_0$ ), the yield (the P/O ratio) and the efficiency of the overall process as a function of the free-energy difference across the redox reaction. Figure 13a shows these quantities at level flow (i.e. a zero phosphate potential) and Fig. 13b at a significant magnitude of the phosphate potential. Both the thermodynamic efficiency and the yield are not incompatible with what has been observed in mitochondrial oxidative phosphorylation [2]. These calculations are for a background transmembrane electric field of zero. As shown in Fig. 13c, the variation of the rate of ATP synthesis with the free-energy difference of the redox reaction, depends on the magnitude of this average  $\Delta\psi$ .

## 5. Conclusions

We have discussed various aspects of the interactions between a transmembrane electric field and membrane proteins, and also the possible electrostatic origins of the coupling of two enzyme functions. In order to do this, we examined the thermodynamics of the effect of the transmembrane electric potential



on a protein conformational equilibrium. It turns out that variations in dipole moment, the position of a charge, or polarizability, provide for a straightforward mechanism of free-energy transduction from electric fields to conformational changes. If such an electrically dependent equilibrium is part of an enzyme catalytic cycle, it turned out that modulation of the electric potential results in a change of the enzyme's catalytic activity. Furthermore, the application of an oscillating electric field [40] or an autonomously fluctuating field [41] can result in the enzyme transducing energy from the field fluctuations

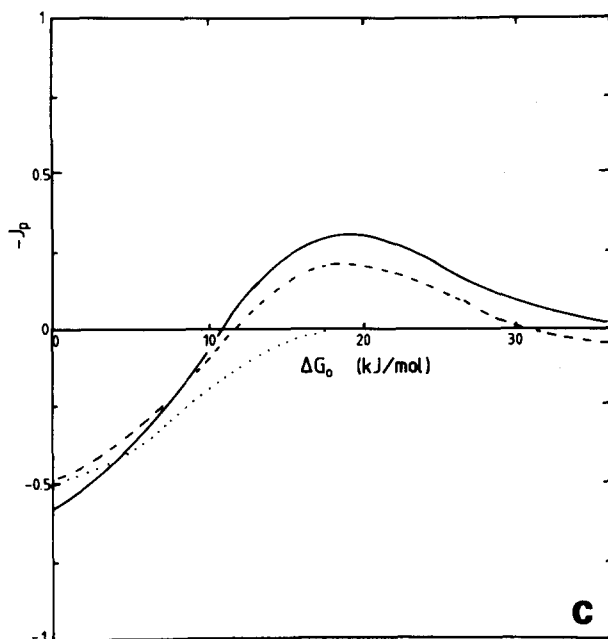


Fig. 13. Free-energy transduction through electrochemical coupling calculated for the system described by Fig. 12, as a function of the free energy difference of the driving, redox, reaction. (a) Level flow, i.e., against zero phosphate potential; (b) against a phosphate potential of 10 kJ/mol. The rate of ATP synthesis (—), the rate of oxidation (····), the P/O ratio (---) and the thermodynamic efficiency (squares, for (b) only, in (a)  $\eta$  is identically zero) are given. (c) Gives the rates of ATP synthesis only, but at (---) +60mV and (····) -60mV time-independent background transmembrane electrical potential. For technical reasons, the back pressure by the phosphate potential was divided equally over the lateral rate constants, through a factor  $\rho$  as in Fig. 9. The same was done for the forward pressure by the redox potential difference. The magnitudes of the free-energy differences are lower than under the physiological conditions, but our values correspond to the actual free-energy differences divided by the charge per enzyme stoichiometries, which are expected to be higher than 1.

to do chemical or transport work. These phenomena were discussed in terms of standard enzyme parameters. In order to make these ideas concrete, we discussed a new, “electrodynamical”, model for membrane-linked free-energy transduction based on the combination of two transport proteins to yield a single “functionally coupled” unit. Although the only interactions taken into consideration were coulombic, the efficiency and efficacy at static head were seen to be comparable to experimentally observed values. The model can account for ATP synthesis under conditions where the time-averaged  $\Delta\tilde{\mu}_{H^+}$  is equal to zero and can thus account for the experimental evidence against the most simple, “delocalized”, form of chemiosmotic coupling.

## Acknowledgements

We wish to express our gratitude to Drs. Tsong, Kamp, Chen, Chock, and Welch for very meaningful discussions. DBK thanks the Science and Engineering Research Council for financial support.

## References

- 1 M.C. Kohn and D. Garfinkel, Computer simulation of metabolism in palmitate-perfused rat heart. I. Palmitate oxidation, *Ann. Biomed. Eng.*, 11 (1983) 361-384.
- 2 H.V. Westerhoff and K. van Dam, *Thermodynamics and Control of Biological Free-Energy Transduction*, Elsevier, Amsterdam, 1987.
- 3 H. Kacser and J. A. Burns, in: D.D. Davies (Ed.), *Rate Control of Biological Processes*, Cambridge University Press, 1973, pp. 65-104.
- 4 R. Heinrich, S.M. Rapoport and T.A. Rapoport, Metabolic regulation and mathematical models, *Prog. Biophys. Mol. Biol.*, 32 (1977) 1-83.
- 5 H.V. Westerhoff, A.K. Groen and R.J.A. Wanders, Modern theories of metabolic control and their applications, *Biosci. Rep.*, 4 (1984) 1-22.
- 6 G.R. Welch and F.H. Gaertner, Influence of an aggregated multienzyme system on transient time: Kinetic evidence for compartmentation by an aromatic-amino-acid synthesizing complex of *Neurospora crassa*, *Proc. Natl. Acad. Sci. U.S.A.*, 72 (1975) 4218-4222.
- 7 G.R. Welch and J.S. Clegg (Eds.), *Organization of Cell Metabolism*, Plenum Press, New York, NY, 1986.
- 8 D.K. Srivastava and S.A. Bernhard, Biophysical chemistry of metabolic reaction sequences in concentrated enzyme solution and in the cell, *Annu. Rev. Biophys. Chem.*, 16 (1987) 175-204.
- 9 G.N. Ling, *In Search of the Physical Basis of Life*, Plenum Press, New York, NY, 1984.
- 10 A.L. Hodgkin and B. Katz, The effect of sodium ions on the electrical activity of the giant axon of the squid, *J. Physiol.*, 108 (1949) 37-77.
- 11 P. Mitchell, Coupling of phosphorylation to electron and hydrogen transfer by a chemi-osmotic type of mechanism, *Nature*, 191 (1961) 144-148.
- 12 C.A. Coulson, *Electricity*, Oliver and Boyd, Edinburgh, 1956.
- 13 F. Kamp, Y. Chen and H.V. Westerhoff, Energization-induced redistribution of charge carriers near membranes, *Biophys. Chem.*, in press (1988).
- 14 D.G. Nicholls, The influence of respiration and ATP hydrolysis on the proton-electrochemical gradient across the inner membrane of rat liver mitochondria as determined by ion distribution, *Eur. J. Biochem.*, 50 (1974) 305-315.
- 15 R.L. Poste, C.R. Merritt, C.R. Kinsolving and C.D. Albright, Membrane adenosine triphosphatase as a participant in the active transport of sodium and potassium in the human erythrocyte, *J. Biol. Chem.*, 235 (1960) 1796-1802.
- 16 M.C. Trachtenberg, D.J. Packey and T. Sweeney, *In vivo* functioning of the  $\text{Na}^+, \text{K}^+$ -ATPase, *Curr. Top. Cell. Regul.*, 19 (1981) 159-217.
- 17 P. Mitchell, *Chemiosmotic coupling and energy transduction*, Glynn Research Ltd., Bodmin, U.K., 1968.
- 18 T.Y. Tsong, Voltage modulation of membrane permeability and energy utilization in cells, *Biosci. Rep.*, 3 (1983) 487-505.
- 19 H.V. Westerhoff and Zs. Dancshazy, Keeping a light-driven proton pump under control, *Trends Biochem. Sci.*, 9 (1984) 112-116.



- 20 T.L. Hill, *An Introduction to Statistical Thermodynamics*, Addison-Wesley, Reading, MA, 1960.
- 21 S.W. Thorne and J.T. Duniec, The physical principles of energy transduction in chloroplast thylakoid membranes, *Q. Rev. Biophys.*, 16 (1983) 197-278.
- 22 W.G.J. Hol, L.M. Halie and C. Sander, Dipoles of the  $\alpha$ -helix and  $\beta$ -sheet: their role in protein folding, *Nature*, 294 (1981) 532-536.
- 23 T.Y. Tsong and R.D. Astumian, Absorption and conversion of electric field energy by membrane-bound ATPases, *Bioelectrochem. Bioenerg.*, 15 (1986) 457-476.
- 24 D. Eisenberg, Three-dimensional structure of membrane and surface proteins, *Annu. Rev. Biochem.*, 53 (1984) 595-623.
- 25 Y. Kimura, M. Fujiwara and A. Ikegami, Anisotropic electric properties of purple membrane and their change during the photoreaction cycle, *Biophys. J.*, 45 (1984) 615-625.
- 26 E. Papp, G. Fricovszky and G. Meszena, Electrochromism of purple membrane. Ionic Strength dependence, *Biophys. J.*, 49 (1986) 1089-1100.
- 27 Z.A. Schelly and R.D. Astumian, A theory for the apparent "negative second Wien effect" observed in electric-field-jump studies of suspensions, *J. Phys. Chem.*, 88 (1984) 1152-1156.
- 28 T.L. Hill, *Free Energy Transduction in Biology*, Academic Press, New York, NY, 1977.
- 29 H. Fröhlich, The biological effects of microwaves and related questions, *Adv. Electr. Electron Phys.*, 53 (1980) 85-182.
- 30 D.B. Kell and C.M. Harris, On the dielectrically observable consequences of the diffusional motions of lipids and proteins in membranes, *Eur. Biophys. J.*, 12 (1985) 181-197.
- 31 E.L. King and C. Altman, A schematic method of deriving the rate laws for enzyme-catalyzed reactions, *J. Phys. Chem.*, 60 (1956) 1375-1378.
- 32 T.L. Hill, Studies in irreversible thermodynamics. IV. Diagrammatic representation of steady state fluxes for unimolecular systems, *J. Theor. Biol.*, 10 (1966) 442.
- 33 T.L. Hill and E. Eisenberg, Can free energy transduction be localized at some crucial part of the enzymatic cycle? *Q. Rev. Biophys.*, 14 (1981) 463-511.
- 34 D. Pietrobon and S.R. Caplan, Flow-force relationships for a six-state proton pump model: Intrinsic uncoupling, kinetic inequivalence of input and output forces, and domain of approximate linearity, *Biochemistry*, 24 (1985) 5764-5776.
- 35 B.G. Malmström, Cytochrome c oxidase as a proton pump. A transition state mechanism, *Biochim. Biophys. Acta*, 811 (1985) 1-12.
- 36 R.W. Hendler and H.V. Westerhoff, Cooperativity in redox driven proton pumps. Theory and practice, *Q. Rev. Biophys.* (in preparation).
- 37 D. Walz, Thermodynamics of oxidation-reduction reactions and its application to bioenergetics, *Biochim. Biophys. Acta*, 505 (1979) 279-353.
- 38 T. Bakker-Grünwald, The energy-linked conformational equilibrium in chloroplast ATPase, *Biochim. Biophys. Acta*, 368 (1974) 386-392.
- 39 P. Gräber, U. Junesch and G.H. Schatz, Kinetics of proton-transport-coupled ATP synthesis in chloroplasts. Activation of the ATPase by an artificially generated  $\Delta pH$  and  $\Delta\psi$ , *Ber. Bunsenges. Phys. Chem.*, 88 (1984) 599-608.
- 40 H.V. Westerhoff, T.Y. Tsong, P.B. Chock, Y. Chen and R.D. Astumian, How enzymes can capture and transmit free energy from an oscillating electric field, *Proc. Natl. Acad. Sci. U.S.A.*, 83 (1986) 4734-4738.
- 41 R.D. Astumian, P.B. Chock, T.Y. Tsong, Y. Chen and H.V. Westerhoff, Can free energy be transduced from electrical noise? *Proc. Natl. Acad. Sci. U.S.A.*, 84 (1987) 434-438.
- 42 Y. Chen, Asymmetry and external noise-induced free-energy transduction in cyclic kinetic systems, *Proc. Natl. Acad. Sci. U.S.A.*, 84 (1987) 729-733.
- 43 W.R. Adey and A.F. Lawrence (Eds.), *Nonlinear electrodynamics in biological systems*, Plenum Press, New York, NY, 1984.

- 44 E.H. Serpersu and T.Y. Tsong, Stimulation of a ouabain-sensitive  $\text{Rb}^+$  uptake in human erythrocytes with an external electric field, *J. Membr. Biol.*, 74 (1983) 191–201.
- 45 E.H. Serpersu and T.Y. Tsong, Activation of electrogenic  $\text{Rb}^+$  transport of  $(\text{Na},\text{K})$ -ATPase by an electric field, *J. Biol. Chem.*, 259 (1984) 7155–7162.
- 46 S.J. Ferguson, Fully delocalised chemiosmotic or localised proton flow pathways in energy coupling? A scrutiny of experimental evidence, *Biochim. Biophys. Acta*, 811 (1985) 47–95.
- 47 P.D. Boyer, T.E. King, H.S. Mason and M. Morrison (Eds.), in: *Oxidases and Related Redox Systems*, Wiley, New York, NY, 1965, Vol. 2, pp. 994–1008.
- 48 H.V. Westerhoff, B.A. Melandri, G. Venturoli, G.F. Azzone and D.B. Kell, A minimal hypothesis for membrane-linked free-energy transduction. The role of independent, small coupling units, *Biochim. Biophys. Acta*, 768 (1984) 257–292.
- 49 H. Rottenberg, Proton-coupled energy conversion: Chemiosmotic and intramembrane coupling, *Mod. Cell Biol.*, 4 (1985) 47–83.
- 50 D.B. Kell and H.V. Westerhoff, in: G.R. Welch (Ed.), *Organized Multienzyme Systems. Catalytic Properties*, Academic Press, New York, NY, 1985, pp. 63–139.
- 51 P.D. Boyer, Correlations of the binding change mechanism with a new concept for proton translocation and energy transmission, in: S. Papa, K. Altendorf, L. Ernster and L. Packer (Eds.),  $\text{H}^+$ -ATPase (ATP synthase): Structure, Function, Biogenesis, Adriatica Editrice, Bari, Italy, 1985, pp. 329–338.
- 52 V.P. Skulachev, The localized  $\Delta\mu_{\text{H}}$  problem. The possible role of the local electric field in ATP synthesis, *FEBS Lett.*, 146 (1982) 1–4.
- 53 H.V. Westerhoff and Y. Chen, Stochastic free-energy transduction, *Proc. Natl. Acad. Sci. U.S.A.*, 82 (1985) 3222–3226.
- 54 H.V. Westerhoff, The organization and control of protonic coupling, *EBEC Rep.*, 4 (1986) 8–9.
- 55 D.B. Kell, Protonmotive energy-transducing systems: some physical principles and experimental approaches, in: C.J. Anthony (Ed.), *Bacterial Energy Transduction*, Academic Press, London, pp. 429–490.
- 56 E.C. Slater, J.A. Berden and M.A. Herweijer, A hypothesis for the mechanism of respiratory-chain phosphorylation not involving the electrochemical gradient of protons as obligatory intermediate, *Biochim. Biophys. Acta*, 811 (1985) 217–231.
- 57 W. Junge and A. Polle, Theory of proton flow along appressed thylakoid membranes under both non-stationary and stationary conditions, *Biochim. Biophys. Acta*, 848 (1986) 265–273.