Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 4734–4738, July 1986
Biophysics

How enzymes can capture and transmit free energy from an oscillating electric field

(free-energy transduction/bioelectrochemistry/ATPase/localized chemiosmotic coupling/active transport)

HANS V. WESTERHOFF*, TIAN YOW TSONG†, P. B. CHOCK‡, YI-DER CHEN*, AND R. D. ASTUMIAN‡

*Section on Theoretical Molecular Biology, Laboratory of Molecular Biology, National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, National Institutes of Health, Building 2, Room 319, Bethesda, MD 20892; †Department of Biological Chemistry, The Johns Hopkins University School of Medicine, Baltimore, MD 21205; and ‡Section on Metabolic Regulation, Laboratory of Biochemistry, National Heart, Lung, and Blood Institute, National Institutes of Health, Building 3, Room 202, Bethesda, MD 20892

Communicated by Terrell L. Hill, March 18, 1986

ABSTRACT **Recently, it has been demonstrated that free energy from an alternating electric field can drive the active transport of Rb^+ by way of the Na^+,K^+-ATPase. In the present work, it is shown why many transmembrane enzymes can be expected to absorb free energy from an oscillating electric field and transduce that to chemical or transport work. In the theoretical analysis it turned out to be sufficient that (*i*) the catalytic process be accompanied by either net or cyclic charge translocation across the membrane and (*ii*) the stability of the enzyme states involved be asymmetric. Calculations based on a four-state model reveal that free-energy transduction occurs with sinusoidal, square-wave, and positive-only oscillating electric fields and for cases that exhibit either linear or exponential field-dependent rate constants. The results suggest that in addition to oscillating electric field-driven transport, the proposed mechanism can also be used to explain, in part, the "missing" free energy term in the cases in which ATP synthesis has been observed with insufficient transmembrane proton electrochemical potential difference.**

In oxidative phosphorylation and in ion transport, cases are found in which the output free energy seems to exceed the input free energy. The most prominent such case in oxidative phosphorylation is that of alkalophilic bacteria (1). Here the transmembrane proton electrochemical potential difference seems too low to account for the observed ATP synthesis. An early explanation of this type of phenomenon was that there existed an additional input term in the free-energy balance sheet for ATP synthesis due to a direct coupling of the conformational energy of the electron transport chain to the F_0F_1-ATPase (2). Alternatively, it was proposed that the protons involved in energy coupling are confined to small domains near the membrane, and thus the equation used for the free-energy balance based on bulk parameters (i.e., spatial averages) would be inappropriate (for review, see ref. 3; see also ref. 4).

Recent results obtained in a different experimental context exhibit a similar deficit in the free-energy balance (for review, see ref. 5). Serpersu and Tsong (6, 7) reported that when an alternating electric field (≈1 kHz) was applied to an erythrocyte suspension, the Na^+,K^+-ATPase catalyzed the active transport of Rb^+ without detectable hydrolysis of ATP even though the time average of the electric field was zero.

The suggested solution was that the Na^+,K^+-ATPase had directly extracted free energy from the oscillations in the field and transduced this to the uphill transport of Rb^+ (5). A crucial role for an oscillating electric field has also been proposed for ATP synthesis driven by a pulsed dc field (5). Since, especially locally, electric fields across biological membranes may well have a large oscillating component, the experimental results of Serpersu and Tsong (6, 7) may also have a more general implication for cases in which input free energy seems to be insufficient to explain output work.

We show here that the properties required to allow for free-energy transduction from an oscillating electric field are in fact common to most proteins and the efficiency and efficacy of such a free-energy transduction can be comparable to those expected and observed for other types of free-energy transduction.

The Model and Calculations

Fig. 1*A* presents a model system considered in discussions of proton pumps (8). The example is an ATPase proton pump that carries a negatively charged basic group, which sticks in the medium bordering the membrane, either outside (states 3–5) or inside (states 1, 2, and 6) the organelle. The conformations of all six states may be different, and the transition from state 1 to state 2 is coupled to ATP synthesis. For a completely coupled pump, direct transitions between states 1 and 4 would not occur. In the ATP synthetic mode, $\Delta\tilde{\mu}_{H^+}$ (in kJ/mol = $-5.7\cdot\Delta pH + 0.096\cdot\Delta\psi$) is the input free energy (where $\Delta\psi$ is the transmembrane electric potential difference in mV) and ΔG_p (the free energy of hydrolysis of ATP) is the output free energy. The $\Delta\tilde{\mu}_{H^+}$, if sufficiently large, can drive the (5, 6, 2, 3, 5, etc.) cycle clockwise, against the opposing ΔG_p, causing net ATP synthesis.

In fact, the transition $1 \rightleftarrows 4$ does occur (e.g., ref. 9), causing waste of free energy. Here we will show that the lower cycle in Fig. 1*A* can describe free-energy transduction in and of itself. This cycle (1, 2, 3, 4, 1, etc., of Fig. 1*B*) involves the cyclic translocation of a negative charge across the membrane. In a stationary electric field, it could only catalyze ATP hydrolysis toward equilibrium, which would, for biologically relevant conditions, imply a counterclockwise cyclic flux. If, however, the cyclic charge translocation would occur in phase with an oscillating electric field, a clockwise flux might be enforced and the enzyme would absorb free energy and transduce that to ATP synthesis.

Fig. 1*C*, upon which our calculations shall be based, represents the transport of an uncharged solute molecule, *S*, coupled to a four-state enzyme cycle. Fig. 1*D* is the corresponding general King–Altman–Hill diagram, in which the concentrations of substrate and product are implicit in the pseudounimolecular rate coefficients α_{ij} (8). Note that in the absence and in the presence of a constant transmembrane electrical potential ($\Delta\psi$), the system of Fig. 1*C* would simply catalyze the translocation reaction of *S* toward its equilibrium. We will show that, in the presence of an oscillating $\Delta\psi$, a system like that in Fig. 1*C* can do work corresponding to the transport of *S* against its concentration gradient.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

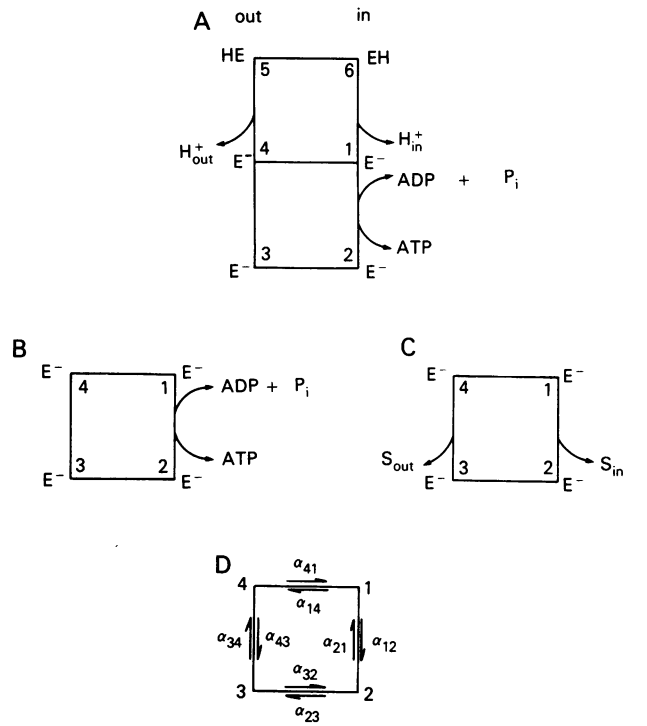

FIG. 1. Diagrams for enzyme cycles considered. (*A*) A proton-translocating ATPase. (*B*) The lower half of *A* as a separate diagram. (*C*) Same as *B* but enzyme cycle coupled to transport of a neutral substance, *S*. (*D*) The King–Altman–Hill diagram of *B* and *C*, defining the pseudo-first-order rate constants. It is implied that states 1–4 may have different conformations.

Since the transitions between states 1 and 4 and between 3 and 2 involve the translocation of a charge through the membrane, the rate coefficients for these transitions will depend on the transmembrane potential (ref. 8, appendix 3). For Fig. 2 the relation between the infield and out of field equilibrium constants is given by:

$$K_{14}(\Delta\psi)/K_{14}(0) = K_{23}(\Delta\psi)/K_{23}(0) = \exp(-F\Delta\psi/RT). \quad [1]$$

Arbitrarily dividing this field dependence equally between the forward and reverse rate constants, we obtain:

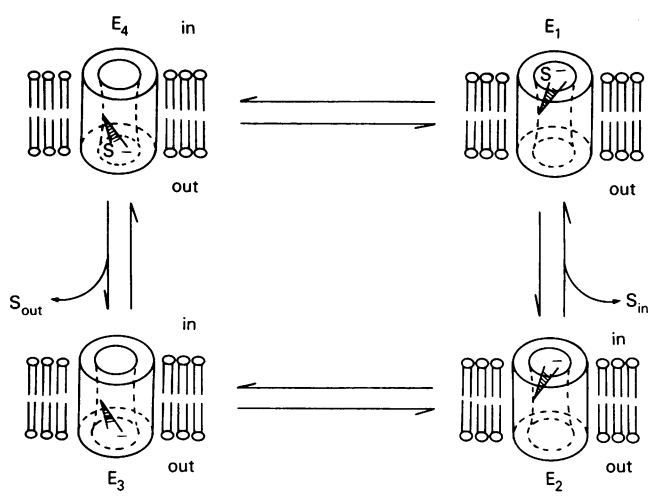

FIG. 2. Physical conceptualization of an enzyme cycle that would be influenced by an electric field. The only conformation differences between states 4 and 1 (and between 3 and 2) is the location of the protein arm carrying the −1 charge. The charge is displaced by exactly the thickness of the membrane, *d*, between each pair of states.

$$\alpha_{14}(\Delta\psi)/\alpha_{14}(0) = \alpha_{23}(\Delta\psi)/\alpha_{23}(0) = \exp(-F\Delta\psi/2RT). \quad [2]$$

$$\alpha_{41}(\Delta\psi)/\alpha_{41}(0) = \alpha_{32}(\Delta\psi)/\alpha_{32}(0) = \exp(+F\Delta\psi/2RT). \quad [3]$$

We take α_{12}, α_{21}, α_{34}, and α_{43} to be field independent.

When the transmembrane potential of the system is changed, the relative enzyme state populations will relax toward new equilibrium values. Under certain circumstances (see below), this induced flux will occur in a net clockwise fashion. If reversal of the potential similarly causes a net clockwise flux, it is obvious that an alternating field will cause the enzyme to cycle ("turn over") and the possibility to do work exists. Our subsequent calculations quantify and give substance to these ideas by a concrete analysis of the properties of Fig. 2 in an alternating field. For simplicity, we have chosen the system to be symmetric with respect to a point in the middle of the diagram (Fig. 1*D*) when $S_{in} = S_{out}$ and $\Delta\psi = 0$. Then we defined three parameters, the "bias factor" b, the "output factor" ρ, and the "potential factor" ϕ:

$$b \equiv ([E_4]/[E_3])_{eq} = ([E_2]/[E_1])_{eq} = (\alpha_{34}/\alpha_{43})_{\rho=1} = (\alpha_{12}/\alpha_{21})_{\rho=1} \quad [4]$$

$$\Delta G_{out} = \Delta\mu_S \equiv -2\cdot R\cdot T\cdot\ln(\rho) \quad [5]$$

and

$$\phi = \exp(F\Delta\psi/2RT). \quad [6]$$

ΔG_{out} is the free energy of the output reaction and $\Delta\mu_S$ is the chemical potential gradient of S across the membrane. ρ will be considered to be buffered to a time-independent value. The equilibrium condition of microscopic reversibility implies that (8):

$$\alpha_{14}\cdot\alpha_{43}\cdot\alpha_{32}\cdot\alpha_{21} = \alpha_{41}\cdot\alpha_{34}\cdot\alpha_{23}\cdot\alpha_{12}/\rho^2. \quad [7]$$

Arbitrarily setting α_{43} to unity, we can write all of the rate coefficients in terms of these three parameters:

$$\alpha_{12} = b;\ \alpha_{21} = 1/\rho;\ \alpha_{23} = b/\phi;\ \alpha_{32} = b^2\cdot\phi;$$

$$\alpha_{34} = b\cdot\rho;\ \alpha_{43} = 1;\ \alpha_{14} = b^2/\phi;\ \alpha_{41} = b\cdot\phi. \quad [8]$$

In the actual calculations we used two methods. The first was numerical integration in which the net formation rate of every enzyme state was written as a function of the concentration of all enzyme states:

$$d\mathbf{e}/dt = -\mathbf{M}\cdot\mathbf{e} + \mathbf{p}. \quad [9]$$

$\mathbf{e}(t)$ is the column vector $(E_1, E_2, E_3)^T$, which is the transpose of (E_1, E_2, E_3). E_x stands for the probability of the enzyme to be in state x. $\mathbf{M}$ is a matrix of rate constants:

$$\mathbf{M} \equiv \begin{bmatrix} \alpha_{14}+\alpha_{12}+\alpha_{41} & \alpha_{41}-\alpha_{21} & \alpha_{41} \\ -\alpha_{12} & \alpha_{21}+\alpha_{23} & -\alpha_{32} \\ \alpha_{43} & \alpha_{43}-\alpha_{23} & \alpha_{32}+\alpha_{34}+\alpha_{43} \end{bmatrix} \quad [10]$$

and

$$\mathbf{p} \equiv (\alpha_{41}, 0, \alpha_{43})^T = \mathbf{M}^{-1}\cdot\mathbf{e}(\infty). \quad [11]$$

For every point in time ($\Delta\psi$ may be time dependent) and every choice of kinetic parameters (i.e., b, ρ, and ϕ), all α's are known and hence $\mathbf{M}$ can be evaluated. We also note that:

$$dv/dt = \mathbf{V}\cdot\mathbf{e} + \mathbf{q}, \quad [12]$$

where v is the column vector $(v_{top}, v_{bottom}, v_{left}, v_{right})^T$. Any v stands for the number of net transitions along a branch in the cycle of Fig. 1*D*. V is the 4 × 3 matrix:

$$\mathbf{V} \equiv \begin{bmatrix} -\alpha_{14} - \alpha_{41} & -\alpha_{41} & -\alpha_{41} \\ 0 & -\alpha_{23} & \alpha_{32} \\ -\alpha_{43} & -\alpha_{43} & -\alpha_{34} - \alpha_{43} \\ \alpha_{12} & -\alpha_{21} & 0 \end{bmatrix} \quad [13]$$

and

$$\mathbf{q} \equiv (\alpha_{41}, 0, \alpha_{43}, 0)^T. \quad [14]$$

The program MLAB (10), running on the National Institutes of Health DEC10 computer, was used to solve the above two differential equations numerically.

For cases in which the electric field was a square wave, we solved Eq. **9** analytically for each period of constant field:

$$\mathbf{e}(t) - \mathbf{e}(\infty) = \mathbf{A}\cdot\mathbf{L}(t)\cdot\mathbf{c}. \quad [15]$$

A is a 3 × 3 matrix, the columns of which correspond to the eigenvectors of matrix M, and L(t) is the 3 × 3 matrix given by:

$$\mathbf{L}(t) \equiv \begin{bmatrix} \exp(-\lambda_1 t) & 0 & 0 \\ 0 & \exp(-\lambda_2 t) & 0 \\ 0 & 0 & \exp(-\lambda_3 t) \end{bmatrix}. \quad [16]$$

$\lambda_1, \lambda_2, \lambda_3$ are the eigenvalues of M, and c is obtained from the initial condition,

$$\mathbf{c} \equiv \mathbf{A}^{-1}\cdot[\mathbf{e}(0) - \mathbf{e}(\infty)]. \quad [17]$$

Integrating Eq. **12** from $t = 0$ to t using Eq. **7** and the fact that only L(t) depends on time, we find:

$$\mathbf{v}(t) = \mathbf{V}\cdot\mathbf{A}\cdot\mathbf{K}(t)\cdot\mathbf{c} + t\cdot(\mathbf{V}\cdot\mathbf{M}^{-1}\cdot\mathbf{p} + \mathbf{q}) \quad [18]$$

with

$$\mathbf{K}(t) \equiv \begin{bmatrix} (1 - e^{-\lambda_1 t})/\lambda_1 & 0 & 0 \\ 0 & (1 - e^{-\lambda_2 t})/\lambda_2 & 0 \\ 0 & 0 & (1 - e^{-\lambda_3 t})/\lambda_3 \end{bmatrix}. \quad [19]$$

Using Eq. **18** for consecutive time periods of different, but constant, potentials we could calculate the net number of transitions through any of the branches of the cycle of Fig. 1*D*. Yields and efficiencies reported are for the cycles after the oscillations in enzyme states had become stationary.

The calculations in this paper were centered around a "standard case" with $b = 500$, $\phi = 16$ (i.e., $\Delta\psi = 142$ mV), $\rho = 0.5$ (i.e., $\Delta G_{out} = 36$ meV), frequency = 7.4. These values were found to yield optimal efficiency for a square-wave field at a b value of 500.

Results

The application of an oscillating electric field to a system such as that shown in Fig. 1*C* will result in free-energy absorption if an oscillation of the enzyme between states on the left and right is induced. Part of this absorbed free energy can be transduced if the oscillation results in net cycling, and at least one of the transitions of the cycle is coupled to an endergonic physical or chemical process (e.g., solute transport in Fig. 1*C* or ATP synthesis in Fig. 1*B*). A scenario is as follows. Suppose that in the absence of an electrical potential there is a bias in the equilibrium constants such that $[E_4]_{eq} >> [E_3]_{eq}$ and $[E_2]_{eq} >> [E_1]_{eq}$. Since the transitions 4 ⇄ 3 and 1 ⇄ 2 are independent of the field, such a bias in concentrations would also be in effect after a long time in a constant electric field, say positive on the left. A sudden inversion of the electric field would have the system move to the right. Since, however, less enzyme is in state 3 than there is in state 4, state 3 gets depleted more rapidly and more enzyme moves from 4 to 1 than from 3 to 2. If such a movement is followed by a reequilibration between the enzyme states 1 and 2, $[E_2]$ would again become higher than $[E_1]$ and sudden inversion of the field would cause more flux from 2 to 3 than from 1 to 4. The net consequence of one complete cycle of the oscillating field would be flow from 4 to 1 and flow from 2 to 3, with subsequent flows from 1 to 2 and from 3 to 4, respectively—or, in other words, a cyclic turnover of the enzymes.

Oscillating Electric Field Can Do Work. Fig. 3 gives a case in which it has been calculated that the system in Fig. 2 does work in an oscillating electric field as anticipated above. At $t = 0$ the system starts at its zero field equilibrium, which, owing to the choice $b = 500$, implies that $[E_4]$ (the full line in Fig. 3*A*) and $[E_2]$ (the upper dashed line) are slightly less than 0.5, whereas $[E_3]$ (the dotted line) and $[E_1]$ (the lower dashed line) are approximately equal to 0.1% of total enzyme concentration. As the electric field begins to increase, $[E_4]$ and $[E_3]$ begin to decrease at the same rate. However, because $[E_4] >> [E_3]$ the flux from E_4 to E_1 is sustained longer. The flux from E_4 to E_1 gives rise to a (transient) increase in E_1, which leads to a flux to E_2. Indeed, E_2 is seen to increase and reaches a maximum near 1 when the electric field is approximately maximal. Upon decrease and reversal

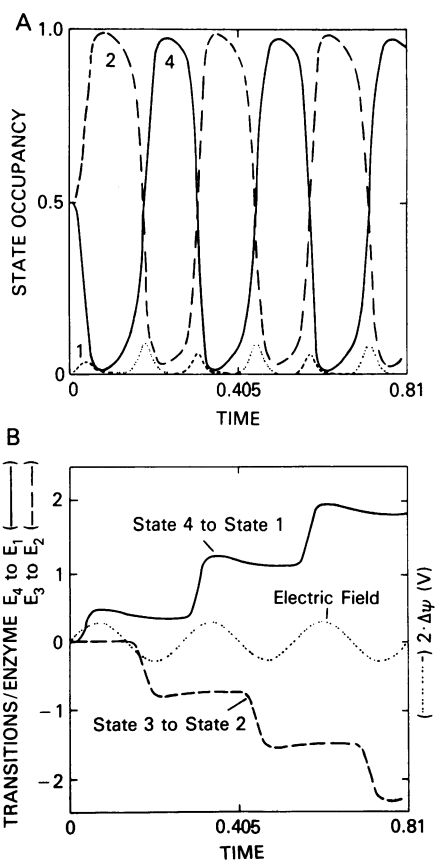

FIG. 3. How an oscillating electric field can cause an enzyme to cycle. Calculation for the model in Fig. 1*D* with parameter values: $b = 500$, $\Delta\psi = 142$ mV, $\rho = 0.5$, frequency = 7.4. (*A*) The normalized concentrations of E_1 (– – –), E_2 (— — —), E_3 (·····), and E_4 (—) as functions of time. (*B*) The electric field (·····), the integrated net number of transitions from E_4 to E_1 (—), and the integrated net number of transitions from E_3 to E_2 (— — —) as functions of time.

of the field, E_2 decreases, but this now leads to a transient increase in E_3 rather than E_1, suggesting that now the flux from E_2 to E_4 is predominantly through E_3, which is corroborated in Fig. 3*B*. Thus, after one cycle, there are 0.3 net transitions per enzyme from E_4 to E_1 and 0.8 net transitions from E_2 to E_3 (Fig. 3*B*). Not shown are the excess transitions from E_1 to E_2 and from E_3 to E_4, which similarly exceed zero. Thus, in addition to some redistribution of the enzyme among the conformational states, there is net clockwise flux of the enzyme through its states. In subsequent periods of the oscillating field, the cyclic flux persists (see Fig. 3*B*) while the redistribution fades away—i.e., the concentrations of enzyme states return to the same magnitude after each complete (360°) oscillation of the field (see Fig. 3*A*). Thus, after a few cycles, the system reaches a stationary, oscillating state, with a constant yield of cyclic flux per cycle of the electric field.

It may be noted that the oscillations in the enzyme concentrations are not in phase with each other and that there is a difference in amplitude between the oscillation in E_4 and that in E_2. This asymmetry is related to the fact that a constant non-zero output force is present in the calculations, which reduces the net transition probabilities from E_3 to E_4 and that from E_1 to E_2.

The yield (in enzyme cycles per field cycle) and the thermodynamic efficiency of the free-energy transduction, obtained in the stationary state, are given in line 2 of Table 1. The efficiency, η, is defined to be the output work divided by the free energy absorbed by the enzyme from the electric field. Output work was obtained by multiplying the yield with the transmembrane free-energy difference of the translocated substrate. The absorbed free energy was obtained for every point in time by summing the net transitions from E_4 to E_1 and those from E_3 to E_2, multiplying the result by $F\cdot\Delta\psi$ and integrating over an entire field cycle. For the case depicted in Fig. 3, the output free-energy difference was taken to equal 3.6 kJ/mol, corresponding to a concentration ratio across the membrane of 4. Partly because of this low output force, the thermodynamic efficiency was only ≈15%. On the other hand, the yield of 0.77 is quite high.

Table 1. A number of cases of work driven by oscillating electric fields

Case	Form of field	$(\Delta\psi_{max} - \Delta\psi_{min})/2$, mV	ΔG_o, meV	Dependence	Yield	η, %
1	Sinusoidal	142	0	Exp	0.88	0
2	Sinusoidal	142	36	Exp	0.77	15
3	Sinusoidal	142	105	Exp	0.25	21
4	Square	142	0	Exp	0.98	0
5	Square	142	36	Exp	0.96	12
6	Square	142	105	Exp	0.70	28
7	Sinusoidal, + only	71	0	Exp	0.09	0
8	Sinusoidal, + only	71	11	Exp	0.07	2
9	Sinusoidal, + only	71	36	Exp	0.02	1
10	Sinusoidal, + only	71	105	Exp	−0.09	−9
11	Sinusoidal	50	0	Lin	0.23	0
12	Sinusoidal	50	36	Lin	0.20	33
13	Sinusoidal	50	105	Lin	−0.002	−1.7
14	Square	50	0	Lin	0.35	0
15	Square	50	36	Lin	0.32	27
16	Square	50	105	Lin	0.16	55
17	Sinusoidal	142	0	Exp	0.20	0
18	Sinusoidal	142	11	Exp	0.15	3
19	Sinusoidal	142	36	Exp	0.07	4
20	Sinusoidal	142	105	Exp	−0.14	−22

Parameter values: b = 500, frequency = 7.4. "Exp" means exponential field dependence in which ϕ is determined according to Eq. 6. "Lin" means linear field dependence in which rate constants α_{41} and α_{32} contain the factor $(1 + F\Delta\psi/RT)$ and rate constants α_{14} and α_{23} contain the factor $(1 - F\Delta\psi/RT)$. Cases 17–20 are from calculations in which α_{32} and α_{23} were taken to be field independent. Cases 2 and 5 correspond to the standard case.

Rows 4–6 of Table 1 demonstrate that the same enzyme system, with the same rate constants, also carries out free-energy transduction when the field is a square wave. This suggests that the phenomenon is not overly dependent on the wave form of the field.

Up to this point, we have only considered oscillating electric fields with zero time average. In some experimental systems, halfwave, or pulsed fields are used. Both of these wave forms have an average non-zero field strength. Rows 8 and 9 of Table 1 show that with a field oscillating between a positive value and zero, the model enzyme can also transduce free energy, although with low yields and efficiencies. It may be argued that this phenomenon is not special because there now is an average electric field, but we stress that there is no way in which the enzyme modeled here could steadily harvest free energy from a constant electric field. The net charge movement in the electric field in an entire cycle is always zero (see Figs. 1 *B* and *C* and 2).

Dependence of Rate Constants on Electric Fields. In our calculations we formulated the effect of the electric field on the rate constants as an exponential term. In this way each potential-sensitive unidirectional rate had rectification properties: at high positive field, the unidirectional rate would go to plus infinity, but at high negative field, the unidirectional rate would only go to zero. Though this is clearly the correct form for high fields, the influence of small fields on the rate constants is almost linear. It seemed possible that nonlinear dependence on the field was essential for the free-energy transduction. However, lines 11–16 in Table 1 show that when the field dependence of the rate constants is taken to be linear [e.g., $\alpha_{ij}(0)\cdot(1 - F\Delta\psi/RT)$], free-energy transduction still occurs, both with sinusoidal and square-wave fields.

Free-energy transduction from an oscillating electric field does not strictly depend on both transmembrane transitions (i.e., $4 \leftrightarrows 1$ and $3 \leftrightarrows 2$ in Fig. 1) being $\Delta\psi$ dependent. Lines 17–20 in Table 1 document such free-energy transduction taking place when α_{32} and α_{23} were taken to be independent of $\Delta\psi$. This suggests that an enzyme that solely exhibits transitions of the 5, 6, 2, 3 cycle in Fig. 1*A* (i.e., a strictly coupled proton pump) can also transduce free energy from oscillations in the electric field.

Our results show that the conditions necessary for an enzyme to harvest free energy from an external electric field are easily met, and, in fact, are general properties of many transmembrane proteins.

Discussion

We have demonstrated that an enzyme, the transitions of which involve a cyclic translocation of a charge, can absorb free energy from an oscillating electric field and use this energy to do work with it. In our calculations this work was the transport of a neutral substance across the membrane, but it could equally well have been an uphill chemical reaction (like the phosphorylation of ADP). To give rise to this type of free-energy transduction, it is sufficient that the equilibrium ratios $[E_4]/[E_1]$ and $[E_3]/[E_2]$ be affected by the electric field. An alternative to having states 4 and 1 differ by the position of a charge in the electric field is to have the two states differ in dipole moment. Proteins generally have large dipole moments, partly arising from their α-helices *per se* (11) and such dipole moments vary with the conformation. As

stressed recently by Tsong and Astumian (5), this provides a general link between conformational and electrical energy.

In a general sense, the electric field is a thermodynamic quantity that may affect the equilibrium between enzyme states (here between states 4 and 1 and between 3 and 2). An oscillation of such a thermodynamic parameter may then make the enzyme cycle. That oscillations in temperature might allow biological systems to absorb free energy from their environment has indeed been postulated (12).

The free-energy transduction between the oscillating electric field and transport (or chemical work) reported in this paper is distinct from "parametric pumping" (13) and from "parametric excitation" (14). Parametric pumping involves the coherent, externally imposed oscillation of *two* parameters. In our calculations only one parameter (i.e., the electric field) was oscillated from the outside. Only if it would have been necessary that S be electrically charged, our model could have been considered a special case of parametric pumping. In our calculations, however, S was electrically neutral. Parametric excitation requires the coupling of three systems that are by themselves oscillatory. Left to itself, our enzyme would have no tendency to oscillate nor would our "third system," the transmembrane concentration difference of S.

The results presented here account for the observation that the Na^+,K^+-ATPase mediates Rb^+ uptake into erythrocytes, driven by an oscillating electric field (5–7). They also suggest an explanation for ATP synthesis by proton-translocating ATPases, apparently in the absence of a sufficiently high electrochemical potential difference for protons (reviewed in refs. 3 and 15): the time average of $\Delta\tilde{\mu}_{H^+}$ may indeed be low, as observed, but there may be a large oscillating electric component (see also ref. 16). It should, perhaps, be stressed that for such oscillations to be significant, either the turnover of the electron-transfer chains has to occur coherently (such as would occur in light-flash-driven photophosphorylation) or the effective unit of energy coupling has to be small (see also ref. 17). The importance of proton concentration fluctuations in small systems has been discussed (3, 4). The question may be raised as to whether noise in the electric field could also be transduced to work by our model enzyme. Indeed, we have calculated that externally defined random noise in the electric field was transduced to work. However, real equilibrium noise around our enzyme system would not be random if considered for the enzyme being in any of its states. When we took this correlation between enzyme state and noise in the electric field into account, the calculations no longer turned up work (R.D.A., Y.-d.C., and H.V.W., unpublished).

Oscillating electric fields have been observed around cells (reviewed in ref. 18), and, in fact, it has been shown recently that Na^+,K^+-ATPase itself can give rise to oscillating electric fields (19) (as "reverse" operation of our model system would do). Conversely, oscillating electric fields applied to intact cells have been shown to affect their metabolism (reviewed in ref. 20). The amplitudes of the electric fields used to produce such effects are often much lower than the transmembrane field strength. However, because of the focusing effect (21), an overall field of 100 V/cm would imply a transmembrane electric potential of 30 mV for a cell of 5 μm in diameter, sufficient to cause substantial free-energy transduction in our model.

It would be interesting to explore the possibility that a constant membrane potential could be locally modulated by the system itself, say, for example, by the opening and closing of an ion channel correlated to the state of the transducing protein. Such a mechanism was proposed by Tsong and Astumian (5) for F_0F_1-ATP synthase. Yet, such channel modulation may not be necessary and one of the ramifications of the present work is that, theoretically, electron transfer and recombination alone could cause an oscillating electric field and thus give rise to free-energy transduction without need of any additional "high-energy" intermediate. This possibility is of course contingent upon the electron-transport protein being close to the ATPase during the reaction, as proposed by Boyer (16), Westerhoff *et al.* (3), Rottenberg (22), and Slater *et al.* (23).

We thank Merry Peters for typing this manuscript. This work was supported, in part, by National Institutes of Health Grant GM28795 to T.Y.T.

1. Guffanti, A. A., Fuchs, R. T. & Krulwich, T. A. (1983) *J. Biol. Chem.* **258,** 35–37.
2. Boyer, P. D. (1965) in *Oxidases and Related Redox Systems*, eds. King, T. E., Mason, H. S. & Morrison, M. (Wiley, New York), Vol. 2, pp. 994–1008.
3. Westerhoff, H. V., Melandri, B. A., Venturoli, G., Azzone, G. F. & Kell, D. B. (1984) *Biochim. Biophys. Acta* **768,** 257–292.
4. Westerhoff, H. V. & Chen, Y. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 3222–3226.
5. Tsong, T. Y. & Astumian, R. D. (1986) *J. Electroanal. Chem.*, in press.
6. Serpersu, E. H. & Tsong, T. Y. (1983) *J. Membr. Biol.* **74,** 191–201.
7. Serpersu, E. H. & Tsong, T. Y. (1984) *J. Biol. Chem.* **259,** 7155–7162.
8. Hill, T. L. (1977) *Free Energy Transduction in Biology* (Academic, New York).
9. Pietrobon, D., Zoratti, M. & Azzone, G. F. (1983) *Biochim. Biophys. Acta* **723,** 317–321.
10. Knott, G. D. & Shrager, R. I. (1972) *Comput. Graph.* **6,** 138–151.
11. Hol, W. G. J., Halie, L. M. & Sander, C. (1981) *Nature (London)* **294,** 532–536.
12. Muller, A. W. J. (1983) *Phys. Lett. A* **96,** 319–321.
13. Wilhelm, R. H. & Sweed, N. H. (1968) *Science* **159,** 522–524.
14. Bartlett, T. W. (1982) *J. Theor. Biol.* **99,** 293–307.
15. Ferguson, S. J. (1985) *Biochim. Biophys. Acta* **866,** 47–95.
16. Boyer, P. D. (1984) in *H^+-ATPsynthase: Structure, Function, Biogenesis*, eds. Papa, S., Altendorf, K., Ernster, L. & Packer, L. (Adriatica Editrice, Bari, Italy), pp. 329–338.
17. Skulachev, V. P. (1982) *FEBS Lett.* **146,** 1–6.
18. Pohl, H. A. (1984) in *Nonlinear Electrodynamics in Biological Systems*, eds. Adey, W. R. & Laurence, A. F. (Plenum, New York), pp. 87–103.
19. Yoshikawa, K., Sakabe, I., Matsubara, Y. & Ota, T., (1984) *Biophys. Chem.* **20,** 107–109.
20. Adey, W. R. (1984) in *Nonlinear Electrodynamics in Biological Systems*, eds. Adey, W. R. & Laurence, A. F. (Plenum, New York), pp. 3–22.
21. Tsong, T. Y. (1983) *Biosci. Rep.* **3,** 487–505.
22. Rottenberg, H. (1985) *Mod. Cell Biol.* **4,** 47–83.
23. Slater, E. C., Berden, J. A. & Herweijer, M. A. (1985) *Biochim. Biophys. Acta* **811,** 217–231.