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Ion Channel Enzyme in an Oscillating Electric Field

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Summary. To explain the electrical activation of several membrane ATPases, an electroconformational coupling (ECC) model has previously been proposed. The model explained many features of experimental data but failed to reproduce a window of the field intensity for the stimulated activity. It is shown here that if the affinities of the ion for the two conformational states of the transporter (one with binding site on the left side and the other on the right side of the membrane) are dependent on the electric field, the field-dependent transport can exhibit the observed window. The transporter may be described as a channel enzyme which opens to one side of the membrane at a time. It retains the energy-transducing ability of the earlier ECC models. Analysis of the channel enzyme in terms of the Michaelis-Menten kinetics has been done. The model reproduced the amplitude window for the electric field-induced cation pumping by (Na,K)-ATPase.

Key Words ATPase · oscillating electric field · membrane transport · ion pump · electroconformational coupling

Introduction

Alternating electric fields can induce membrane transport ATPases to pump ions up their respective concentration gradients and drive certain biochemical reactions away from chemical equilibrium in the absence of ATP (Witt, Schlodder & Graber, 1976; Serpersu & Tsong, 1983, 1984; Teissie, 1986; Liu, Astumian & Tsong, 1990; Tsong, 1990). To explain these phenomena, an electroconformational coupling (ECC) model has been proposed (Tsong & Astumian, 1986; Astumian et al., 1989; Astumian & Robertson, 1989; Markin et al., 1990, 1991; Robertson & Astumian, 1990*a*; Tsong, 1990; Weaver & Astumian, 1990). The model postulates that a membrane enzyme with several functional states of different charge distributions, or electric moments, will undergo conformational changes in an electric field. If the field is oscillatory, it will enforce the conformational oscillation of the enzyme within its catalytic cycle. This field-enzyme interaction will enable the enzyme to utilize the electrical energy for performing chemical work. Recently, this model has been formulated for the Michaelis-Menten enzyme kinetics, although only electrically neutral substrate was considered (Robertson & Astumian, 1990*b*). The ECC model explains many features of the experimental observations on electrical activation of these ATPases but has failed to reproduce the dependence of enzyme activity on the amplitude of the applied electric field. This dependence is considered to be of primary importance.

Experimentally, this dependence displays a window beyond which the enzyme activity decreases to zero (Liu, Astumian & Tsong, 1990). The ECC model developed so far predicts a sigmoidal increase and saturation of activity with increasing field strength. To overcome this shortcoming, the ECC model is extended from the carrier-like model to a channel-like model. This new model retains all the properties of its predecessor but can demonstrate an amplitude window. Both active pumping and current rectification (Delahay, 1965) of the channel-like ECC model have been investigated.

Membrane Transport by Channel Enzyme

One-Sided Channel

The channel enzyme is not a through pore but rather a pore which can only open to one side at a time (Fig. 1*A*). It will undergo the conformational transitions between different catalytic states. The energy barriers for ion transport in different conformations are presented in Fig. 1*B*. The conformational transitions are influenced by an electric field, but they do not

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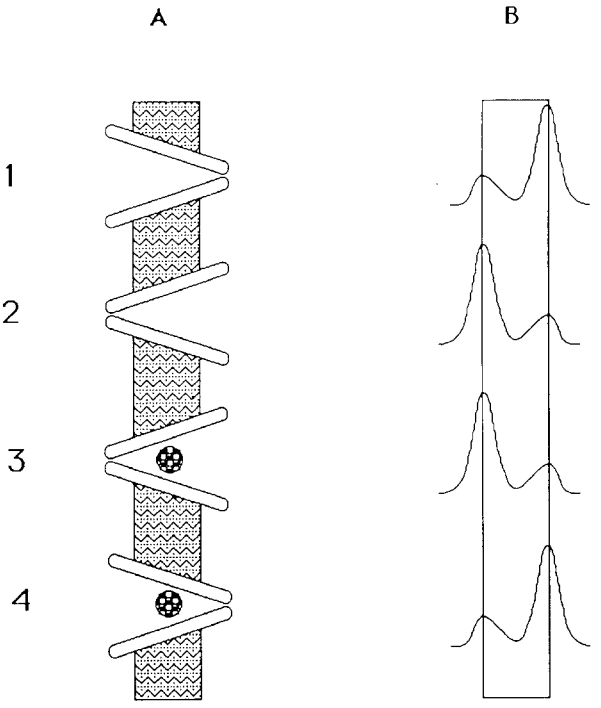
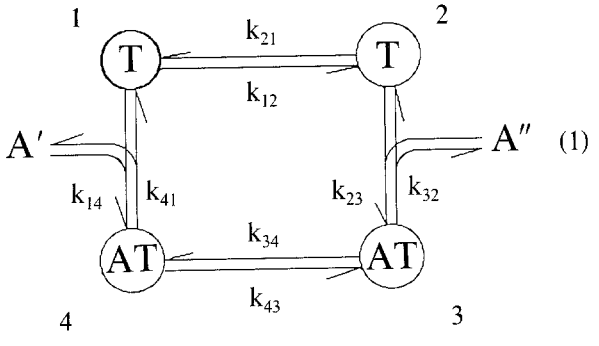


Fig. 1. The model of a one-sided channel in a membrane (*A*) and energy profiles for ion transport (*B*).

carry the ion across the membrane. The ion is driven directly by the electric field. This latter property is characteristic of the channel-like transport mechanisms and is absent in the carrier-like model.

Kinetic Scheme

The formal description of the channel-like mechanism is similar to that of the carrier-like mechanism.



(1)

In this scheme, the transporter is expressed as T, the ligand as A, and the rate constants in the absence of an electric field as k_{ij} (Markin & Chizmadzhev, 1974; Hill, 1989). A' and A'' are expressed in relative concentrations, and all rate constants are reduced to the same dimensions (Markin & Tsong, 1991*a*,*b*).

A special case of Scheme (1) called *the antisymmetric transporter* is defined by assigning the following conditions to the rate constants, k_{ij},

$$k_{14} = k_{32},\ k_{41} = k_{23},\ k_{12} = k_{34},\ k_{21} = k_{43} \tag{2}$$

and to the equilibrium constants, $K_{nm} = k_{mn}/k_{nm}$,

$$K_{14} = K_{12} = K_{32} = K_{34}. \tag{3}$$

This reduces the number of parameters in Scheme (1) to three, namely, k_{12}, k_{14}, and $q = K_{14} = 1/K_{41}$. All other rate and equilibrium constants can be expressed via these three. In what follows, we will consider, for simplicity, the antisymmetric transporter.

The membrane channel is assumed to have a *gating charge*, $z_T e_o$, that moves concomitantly with a conformational change of the protein. Here e_o is the elementary charge and z_T is charge number. The ligand A with charge $z_A e_o$ moves independently of the gating charge. The ratio of these charges will be designated as $s = z_A/z_T$. The change of the electrical energy of the channel in the process of its conformational change is $z_T e_o \varphi$, where φ is the transmembrane potential and e_o is the elementary charge. For brevity we shall use the dimensionless membrane potential $\psi = F\varphi/RT$, where F is the Faraday constant. Therefore, in an applied electrical field the rate constants k_{12} and k_{43} will include the term $\lambda = \exp(z_T\psi/2)$ and the rate constants k_{21} and k_{34} will include the term $1/\lambda$. This assumes that the gating charge moves across the whole distance in which the potential ψ drops and that the energy barrier for the gating charge is located in the middle of the membrane. Ion A has a binding site in the center of the membrane, and its energy barrier is located between the surface of the membrane and the center. Therefore, the rate constants k_{14} and k_{32} will include the term $\zeta = \exp(z_A\psi/4)$, and the rate constants k_{41} and k_{23} will include the term $1/\zeta$.

Kinetic Equations

The kinetic behavior of the transport mechanism (1) is described by a set of four differential equations (Markin & Chizmadzhev, 1974; Hill, 1989).

$$\frac{d(T_1)}{dt} = \frac{1}{\zeta}k_{41}(AT_4) + \frac{1}{\lambda}k_{21}(T_2) - (\zeta k_{14}A' + \lambda k_{12})(T_1) \tag{4}$$

$$\frac{d(AT_4)}{dt} = \zeta k_{14}A'(T_1) + \frac{1}{\lambda}k_{34}(AT_3) - \left(\frac{1}{\zeta}k_{41} + \lambda k_{43}\right)(AT_4). \tag{5}$$

These two equations are for the left side of the membrane. There are two similar equations for the right side.

The overall rate of energy transduction is determined by the rate constants of the system (Markin et al., 1990, 1991). One group is the relaxation rate of the ligand/transporter interaction, k_{chem}, given by $(k_{14}A' + k_{41})$ or $(k_{23}A'' + k_{32})$. The second group is the rate of conformational relaxation of the transporter, k_{conf}, given by $(k_{12} + k_{21})$ or $(k_{43} + k_{34})$ depending on whether or not the protein is loaded with ligand A. In the presence of an electric field, the conformational rate constants will include electrical terms which contain λ. Therefore, the conformational relaxation rates, k_{conf}, in the first half-period of oscillation will be given by $(\lambda k_{12} + k_{21}/\lambda)$ and $(\lambda k_{43} + k_{34}/\lambda)$ and in the second half-period by $(k_{12}/\lambda + \lambda k_{21})$ and $(k_{43}/\lambda + \lambda k_{34})$. Another important parameter, which should be compared with these two groups of rate constants, is the rate of electrical potential change or approximately the frequency of the AC field.

It was shown earlier (Markin et al., 1990, 1991), that the ECC mechanism can work as a pump in an AC field if the rate of the conformational change, k_{conf}, is greater than the rate of the ligand association/dissociation reaction, k_{chem},

$$k_{conf} > k_{chem}. \tag{6}$$

The pump works most effectively if the frequency is confined to an optimum window.

Membrane Flux in an AC Field

Frequency Dependence

The membrane flux J has been derived by a method outlined in (Markin & Tsong, 1991*b*). It consists of solving equations for the frequency in the region $f < k_{conf}$ and in the region $f > k_{chem}$. These two solutions are joined together in the region where they overlap. At the very low frequency the flux is due to rectification J_{rect} (Markin & Tsong, 1991*a*). It linearly increases with frequency, displays an optimum window with boundaries f_{low} and f_{high}, and decreases as f^{-2} beyond the frequency f_{high}. Therefore, the flux can be approximated by

$$J = J_{rect} + \frac{(J^{plateau} - J_{rect})ff_{high}^2}{(f + f_{low})(f^2 + f_{high}^2)} \tag{7}$$

where $J^{plateau}$ is the maximum flux attainable in the frequency window if this window is broad (Markin & Tsong, 1991*b*). The total flux consists of rectification flux, J_{rect}, and pump flux, J_{pump}.

The maximum flux in the frequency window for an antisymmetric transporter was calculated to be

$$J^{plateau} = \frac{\text{numerator}}{\text{denominator}} \tag{8}$$

where

$$\begin{aligned}\text{numerator} = {} & k_{41}\{A'[q^2(\lambda^S + 1)^2 \\ & + q(\lambda^2 + \lambda^{-2})(\lambda^S + 1)^2 + (\lambda^{S+4} + 1)(\lambda^{S-4} + 1)] \\ & - A''[(\lambda^S + 1)^2 + q(\lambda^2 + \lambda^{-2})(\lambda^S + 1)^2 \\ & + q^2(\lambda^{S+4} + 1)(\lambda^{S-4} + 1)]\}\end{aligned} \tag{9}$$

$$\begin{aligned}\text{denominator} = {} & 2\lambda^{S/2}(1 + q\lambda^2)(1 + q\lambda^{-2}) \\ & \times \{A'[q(\lambda^S + 1) + \lambda^{S-2} + \lambda^2] \\ & + A''[\lambda^S + 1 + q(\lambda^{S-2} + \lambda^2)] \\ & + (1 + q)(\lambda^{-2} + 1)(\lambda^{S+2} + 1)\}.\end{aligned} \tag{10}$$

Recall that $s = z_A/z_T$.

The boundaries of the frequency window at $A' = A'' = A$ are

$$f_{low} = \frac{k_{14}(A + q\lambda^S)(Aq + \lambda^S)}{2\lambda^{S/2}(A + \lambda^S)} \text{ and}$$

$$f_{high} = \frac{\lambda}{4}\sqrt{\frac{k_{12}k_{21}}{3}}. \tag{11}$$

For simplicity, we assume that A is close to one.

Voltage Dependence

Figure 2 presents the dependence of the flux in the frequency window $J^{plateau}$ for different charges of ligand. All curves pass through a maximum and diminish to zero at another side of it. The only exception is the neutral ligand. Thus, this model reproduces the experimental observation on the voltage dependence of the activity of ATPases for the charged ligands (Serpersu & Tsong, 1983, 1984; Liu, Astumian & Tsong, 1990).

Both the rectification and the pumping components of the flux depend on the amplitude of the potential. The rectification flux at $A' = A'' = A$ was calculated to be

$$\begin{aligned}J_{rect} = {} & 0.5\, k_{14}A(1 - q)\lambda^{S/2}(\lambda^S - \lambda^{-S})(\lambda^2 - 1) \\ & \times [A(\lambda^2 + \lambda^S) - \lambda^{2+S} - 1] \\ & \times [A(\lambda^2 + \lambda^S)(q\lambda + 1/\lambda) \\ & + (\lambda^{2+S} + 1)(\lambda + q/\lambda)]^{-1} \\ & \times [A(\lambda^2 + \lambda^S)(\lambda + q/\lambda) \\ & + (\lambda^{2+S} + 1)(q\lambda + 1/\lambda)]^{-1}.\end{aligned} \tag{12}$$

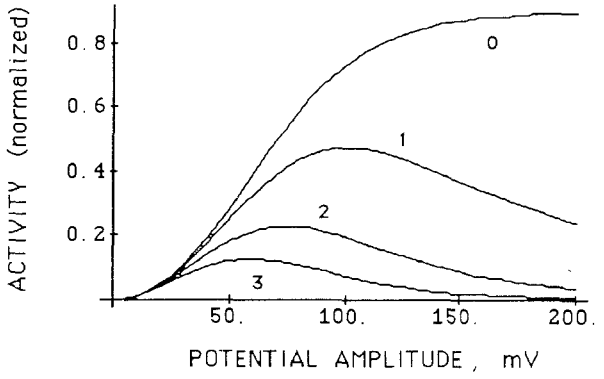


Fig. 2. Enzyme activity (normalized by $k_{14}/4$) as a function of potential amplitude for channel gating charge $z_T = 1$ and different charges of ligand indicated on the plot. The curves are drawn from Eq. (8) for $A' = A'' = 1$ and $q = 0.1$. The charge of the ligand is indicated on the curves. All curves start at zero and are indistinguishable at small potentials. The increase is determined by gating charge only and does not depend on ligand charge. All curves display a maximum except the curve for $z_A = 0$. This latter curve is sigmoidal and reaches a maximum at high potentials.

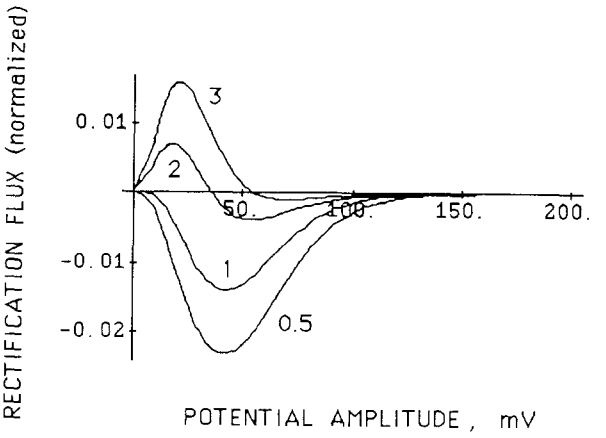


Fig. 3. Rectification component of flux (normalized by $k_{14}/4$) as a function of potential amplitude for different concentrations $A' = A'' = A$. The curves are drawn from Eq. (12) with $q = 0.1$, $z_A = 1$ and $z_T = 3$. Dimensionless concentrations are indicated on the curves. Note that with variation of potential the rectification flux changes its sign. The amplitude of this flux remains small.

Figure 3 presents the rectification flux as a function of the voltage. Both the magnitude of this flux and the sign vary with voltage. The magnitude of the rectification flux remains small compared to the pumping flux. Therefore, the activity of this model enzyme in an AC field is mainly attributed to active pumping.

Concentration Dependence and the Static Head

The flux depends on the concentration of the transported ligand on both sides of the membrane. Its dependence on A'' for a fixed concentration A' is presented in Fig. 4*A*. With increasing A'' the flux from left to right decreases. The same would happen to a passive flux without oscillating potential. But in contrast to the passive flux, the flux driven by an oscillating potential is not zero at the point $A'' = A'$. In the broad frequency window, it is

$$J = \frac{Ak_{41}(1-q)\lambda^{S/2}(\lambda^2-1)^2(\lambda^2+1)}{2(1+q\lambda^2)(\lambda^2+q)[A(\lambda^S+\lambda^2)+\lambda^{S+2}+1]}. \tag{13}$$

The point where J decreases to zero is called *static head* (Markin et al., 1990, 1991). As can be found from Eqs. (8) and (9), the zero flux in an AC field is achieved when the concentration ratio is

$$\left(\frac{A''}{A'}\right)_{sh} = \frac{q^2(\lambda^S+1)^2 + q(\lambda^2+\lambda^{-2})(\lambda^S+1)^2 + (\lambda^{S+4}+1)(\lambda^{S-4}+1)}{(\lambda^S+1)^2 + q(\lambda^2+\lambda^{-2})(\lambda^S+1)^2 + q^2(\lambda^{S+4}+1)(\lambda^{S-4}+1)}. \tag{14}$$

The dependence of the static head on the transmembrane electric potential is presented in Fig. 5. If the charge ratio, s, is less than 2, the curves rise from 1 (no static head) to $(1/q)^2$. When $s = 2$, the curve is sigmoidal with the limiting level equal to $1/q$. If $s < 2$, the curves pass through the maximum and then drop to 1.

If A'' exceeds the static head value, A''_{sh}, the flux changes its sign and drives the pump in the opposite direction (Fig. 4*A*). As was shown previously for the case of neutral ligands (Markin et al., 1990, 1991), the carrier-like transporter was transformed into a generator of electrical oscillations in this region. The same would also happen to the channel-like transporter with neutral ligand. But if the ligand is charged, the situation is more complicated.

Michaelis-Menten Kinetics

The activity of this channel enzyme is formulated for the Michaelis-Menten enzyme with the rate given by the expression

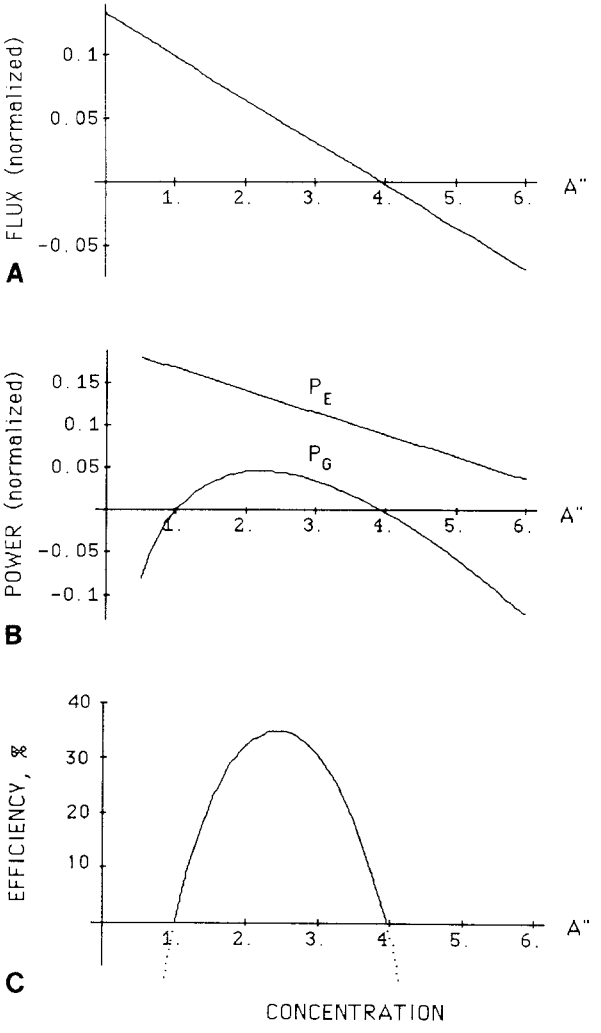


Fig. 4. The characteristics of energy transduction with $A' = 1$, ion charge $z_A = 1$, gating charge $z_T = 3$, and $q = 0.5$ as the function of concentration A''. (*A*) Ion flux (normalized by $k_{14}/4$) drawn from Eq. (7). The curve crosses the abscissa at the static head point close to 4. (*B*) Power absorbed from electric field P_E and power exerted on concentration gradient P_G normalized by $RTk_{14}/4$. (*C*) Efficiency of energy transduction from electric field to concentration gradient. Transduction occurs in one direction only. The efficiency reduces to zero for equal concentrations and at the static head. The maximum efficiency of about 35% is achieved between these two points.

$$V = V_{\max}\frac{[S]}{[S] + K_M}. \tag{15}$$

The flux of the channel enzyme can also be presented in the same form. Let the concentration of A' be equivalent to the substrate concentration $[S]$ and $A'' = 0$. Then from Eqs. (8)–(10) one obtains the maximum rate in an AC field

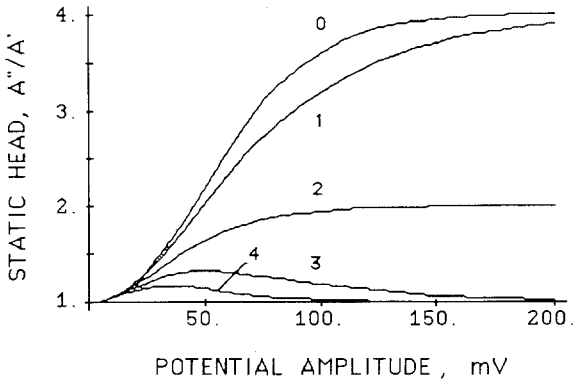


Fig. 5. Static head as a function of potential amplitude for different ion charges (charge ratios $s = z_A/z_T$ for $z_T = 1$) indicated on the plot. The curves are drawn from Eq. (14) with $q = 0.5$ and $z_T = 1$. With increasing potential the curves with $z_A < 2$ reach the maximum value $(1/q)^2 = 4$, the curve with $z_A = 2$ reaches the level $(1/q) = 2$, and the curves with $z_A > 2$ pass through the maximum and finally decrease to zero.

$$V_{\max} = \frac{k_{41}[q(q + \lambda^2 + \lambda^{-2})(\lambda^S + 1)^2 + (\lambda^{S+4} + 1)(\lambda^{S-4} + 1)]}{2\lambda^{S/2}(1 + q\lambda^2)(1 + q\lambda^{-2})[q(\lambda^S + 1) + \lambda^{S-2} + \lambda^2]} \tag{16}$$

and the Michaelis constant

$$K_M = \frac{(1 + q)(1 + \lambda^{-2})(\lambda^{S+2} + 1)}{q(\lambda^S + 1) + \lambda^{S-2} + \lambda^2}. \tag{17}$$

For the channel enzyme in the absence of an electric field, the same constants can be derived as

$$V_{\max}^{\text{ns}} = \frac{k_{41}}{(1 + q)} \quad \text{and} \quad K_M^{\text{ns}} = 2 \tag{18}$$

where the superscript, ns, specifies that the enzyme is not stimulated by an AC field.

The parameters $V_{\max}$ and K_M vary with potential amplitude. These functions are presented in Fig. 6 for the parameters $z_T = 1$, $q = 0.1$ and $s = 2$. In the absence of an AC field, which corresponds to zero amplitude in this figure, $V_{\max}/k_{41} = 0.909$ and $K_M = 2$. With increasing potential, both $V_{\max}$ and K_M increase but in different manners. As a result, the enzyme activity, V, changes nonmonotonously as presented in Fig. 6*C*. However, the Michaelis constant, K_M, increases faster than $V_{\max}$ does. The net result is that V decreases beyond the potential threshold. So at zero amplitude, the activity is equal to 0.4545. At approximately 70 mV it passes through a maximum of 1.3635. This means that the AC field

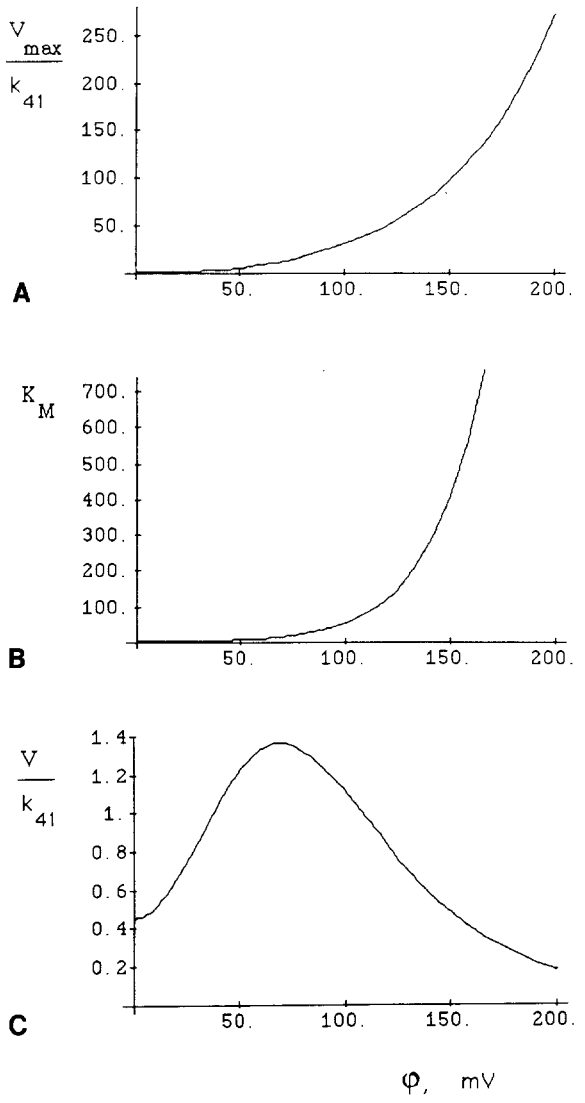


Fig. 6. Dependence of Michaelis-Meten parameters on membrane potential amplitude: (A) maximum activity, (B) Michaelis-Meten constant, (C) enzyme activity. The curves are drawn from Eqs. (15)–(17) with the parameters $z_{T_l} = 1$, $q = 0.1$, $s = 2$, and $[S] = 2$. At zero amplitude, $V_{\max}/k_{41} = 0.909$ and $K_M = 2$. With increasing potential amplitude both $V_{\max}$ and K_M increase but in a different manner. Therefore, the enzyme activity, V, changes nonmonotonously. At zero amplitude it is 0.4545, and at about 70 mV it passes through the maximum of 1.3635. It means that AC field can increase the activity of this particular model enzyme threefold.

can enhance the activity of this model enzyme by threefold.

An AC field can stimulate but can also inhibit the activity. It was demonstrated in a similar plot with the same parameters, except that q was changed to 10. In that case, the curve of Fig. 6*C* decreased from the very beginning (*data not shown*).

Efficiency of Energy Transduction

The general expression for the average power absorbed by the transport protein from an external electric field is

$$P_E = f \int \psi i \, dt \tag{19}$$

where i is the transmembrane electric current and the integral is done over one period of oscillation. This power will be expressed in RT per second. We shall consider the energy transduction at the optimum frequency. The first, P_{switch}, is the work done when the electric field switches from one value to another. The second, P^T_{relax}, is due to the redistribution of the concentrations of different forms of the transporter in the constant field. The third, P^A_{relax}, is due to rectification. The sum is

$$P_E = P_{\text{switch}} + P^T_{\text{relax}} + P^A_{\text{relax}}. \tag{20}$$

Integration of Eq. (19) gives

$$P_{\text{switch}} = \frac{k_{14} q^2 (\lambda^2 - \lambda^{-2}) w_o (\zeta\lambda + 1/\zeta\lambda)}{(\lambda + q/\lambda)^2 (q\lambda + 1/\lambda)^2} \times \frac{(A'\lambda/\zeta + A''\zeta/\lambda)(\lambda + q/\lambda)^2 - (A'\zeta/\lambda + A''\zeta/\lambda)(q\lambda + 1/\lambda)^2}{(A'\lambda/\zeta + A''\zeta/\lambda)(\lambda + q/\lambda)(q\lambda + 1/\lambda) + (A'\zeta/\lambda + A''\lambda/\zeta)(q\lambda + 1/\lambda) + (\zeta\lambda + 1/\zeta\lambda)(1 + q)(\lambda + 1/\lambda)} \tag{21}$$

$$P^T_{\text{relax}} = \frac{z_T \psi q k_{14} (1 - q^2)}{2(\lambda + q/\lambda)^2 (q\lambda + 1/\lambda)^2} \times \{(1 - T_o)(1 - q)(\zeta\lambda + 1/\zeta\lambda)(\lambda - 1/\lambda) + T_o[(\lambda^2/\zeta - \zeta/\lambda^2)(A' - qA'') - (\zeta - 1/\zeta)(qA' - A'')]\} \tag{22}$$

$$P^A_{\text{relax}} = \frac{z_A \psi q k_{14} (1 - q^2)}{4(\lambda + q/\lambda)(q\lambda + 1/\lambda)} \times \{(1 - T_o)(1 + q)(\zeta\lambda - 1/\zeta\lambda)(\lambda + 1/\lambda) - T_o[(\lambda^2/\zeta - \zeta/\lambda^2)(A' + qA'') - (\zeta - 1/\zeta)(qA' + A'')]\} \tag{23}$$

where w_o is electrical work done on the channel during the conformational change

$$w_o = \ln \frac{\lambda + q/\lambda}{q\lambda + 1/\lambda} - \frac{z_T \psi (1 - q^2)}{(\lambda + q/\lambda)(q\lambda + 1/\lambda)} \tag{24}$$

and T_o is total population of the unloaded states

$$T_o = \frac{(1 + q)(\zeta\lambda + 1/\zeta\lambda)(\lambda + 1/\lambda)}{(A'\lambda/\zeta + A''\zeta/\lambda)(\lambda + q/\lambda) + (A'\zeta/\lambda + A''\lambda/\zeta)(q\lambda + 1/\lambda) + (\zeta\lambda + 1/\zeta\lambda)(1 + q)(\lambda + 1/\lambda)}. \tag{25}$$

The power exerted by the transporter on the concentration gradient is

$$P_G = J\Delta G = J \ln(A''/A'). \tag{26}$$

This equation does not contain membrane potential because the oscillations are symmetrical and the average potential is zero. If a bias potential is used, this expression will contain an electrical term. When P_E and P_G are positive, energy flows from electric potential to the concentration gradient. The efficiency of this energy transduction is

$$\eta_{EG} = \frac{P_G}{P_E} = \frac{\ln(A''/A')}{P_E}. \tag{27}$$

If both P_E and P_G are negative, energy would flow from the concentration gradient to electric potential. The efficiency in this case η_{GE} would be the reciprocal of Eq. (27).

This reverse in energy transduction occurs when the ligand is neutral. If the ligand is charged, the situation is more complicated. Figure 4 presents the characteristics of energy transduction with ion charge 1 and gating charge 3. There is a unidirectional transduction of energy from the electric field to the concentration gradient. The efficiency reduces to zero when $A' = A''$ and at the static head. The maximum efficiency achieved between these two points is about 35%.

Discussion

The ECC channel enzyme can generate active transport, build up and maintain a concentration difference between two solutions separated by a membrane. There is a frequency window for the applied AC field at which the pumping occurs most effectively. In contrast to the carrier-like mechanism, it also displays a potential optimum. The reason for this behavior is that the ion entrance into and exit from the channel are potential-controlled processes. Therefore, if z_T and z_A have the same sign, the channel will remain empty at high potentials. If the charges are of opposite sign, the channel will be filled with ions but cannot conduct current. The enzyme activity decreases when the voltage exceeds an optimum value.

Similar models were considered by Lauger (1980, 1987) and by Lauger, Stephen and Frehland (1980). This type of channel enzyme is more realistic than the carrier-like transporter investigated previously in connection with the electroconformational coupling. It has many attractive features including the electrically silent exchange of anions

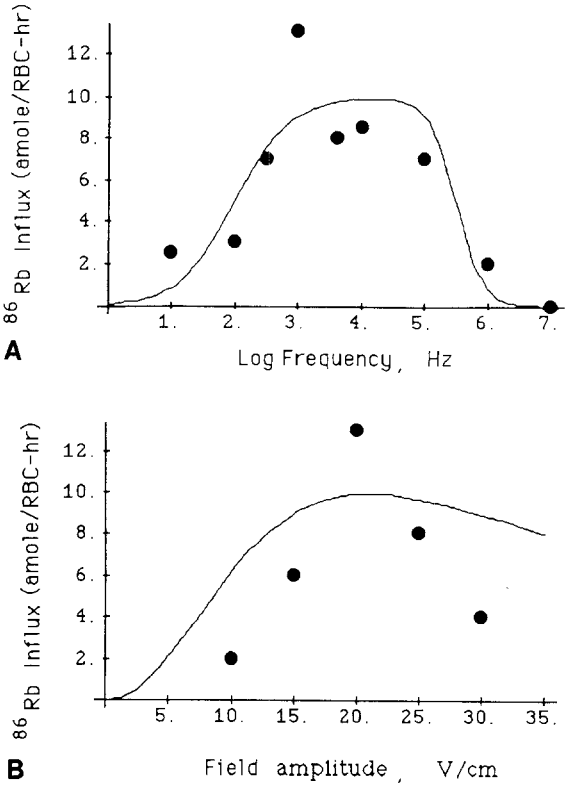


Fig. 7. Experimental frequency (*A*) and potential dependence (*B*) of Rb^+ pumping activities of (Na,K)-ATPase of human erythrocytes stimulated by the optimal AC field of 20 V/cm (Liu, Astumian & Tsong, 1990) and the theoretical curves drawn from Eqs. (7) and (8). The curve fitting showed that the low boundary of frequency window f_{low} is 100 Hz and high boundary f_{high} is 300 kHz; $r = 0$, $q = 0.3$, $s = 0.6$, and $Nk_{41}(1 - q)/2 = 10$ amol/RBC-hr. The characteristic length, a, was determined by $z_A a = 30$ μm.

observed in erythrocytes (Grygorczyk, Schwarz & Passow, 1987).

This channel enzyme has been formulated for the Michaelis-Menten kinetics, as was done by Robertson and Astumian for the carrier-like transporter (Robertson & Astumian, 1990*b*). The behavior of the V_{max} and the Michaelis constant, K_M, of the channel enzyme under an AC field have been examined.

Experimental Example

We applied this model to the description of the Rb^+ pumping by (Na,K)-ATPase of human erythrocytes in an AC field (Liu, Astumian & Tsong, 1990). Equations (8) and (13) give the current per channel. To compare calculated current with the data for the red blood cells, we multiply these values by the number of channels per cell, N. This will be the case only if the transport is by ion pumping and there is no ion leakage. If there is ion leakage, our results will give an underestimate of N.

The curve fitting for the frequency (Fig. 7*A*) and voltage (Fig. 7*B*) dependencies of ATPase activity demonstrate a reasonable description of the experimental data and permit kinetic parameters of this enzyme to be found. From the frequency dependence of Rb^+ pumping (Fig. 7) we found $NJ^{\text{plateau}} = 10$ amol/RBC-hr and $r = 0$. The window boundaries are $f_{\text{low}} = 100$ Hz and $f_{\text{high}} = 300$ kHz.

Experimentally, the amplitude dependence of Rb^+ pumping was obtained at the optimum frequency of 1 kHz (Liu, Astumian & Tsong, 1990)(Fig. 7*B*). To analyze this dependence, we simplified Eq. (8) for the flux by assuming $A' = A'' = 1$. The flux per cell was found to be

$$NJ = \frac{Nk_{41}(1 - q)(\lambda^2 - 1)^2}{2(1 + q\lambda^2)(q + \lambda^2)(\lambda^{S/2} + \lambda^{-S/2})}. \tag{28}$$

At the next step we converted the amplitude of the external electrical field, E, to the amplitude of the membrane potential, $\varphi = aE$, where a is a characteristic length which should be of the order of the cell size if the membrane confuctance is low and frequency is not too high. Results of curve fitting are presented in Fig. 7*B* for Rb^+ pumping in AC field with frequency $f = 1$ kHz. The best fit gave the parameters $r = 0$, $q = 0.3$, $s = 0.6$, and $Nk_{41}(1 - q)/2 = 10$ amol/RBC-hr. Hence, $Nk_{41} = 4.5\ 10^3$ sec^{-1} and for the optimum amplitude of $E = 20$ V/cm we have $\lambda = 7$.

Characteristic length, a, was found to satisfy the equation $z_A a = 30\ \mu$m. If $z_A = 1$, then $a = 30\ \mu$m, which seems a little high. But if $z_A = 3$, then $a = 10\ \mu$m, which is close to the size of human erythrocyte. Taking into consideration the approximate character of the theory, the agreement is considered good.

Now let us return to the frequency dependence and find the rate constants of the transporter. For the values $\lambda = 7$ and $s = 0.6$, we can calculate from Eq. (11) that $f_{\text{low}} = k_{14}$. Hence, $k_{14} = 100$ sec^{-1}, $k_{41} = 30$ sec^{-1}, and we find $N = 150$ per erythrocyte, which is close to a generally accepted number of 200 ATPase per cell. From the position of high boundary $f_{\text{high}} = 300$ kHz and Eq. (11), we obtain $k_{12} = 6 \times 10^5$ sec^{-1}, and $k_{21} = 1.8 \times 10^5$ s^{-1}. Therefore, this enzyme has frequency and potential windows for ion transport.

The theory can be applied to other types of enzymes, and its predictions may be verified. The rate constants of an enzyme and the equilibrium constants can be determined by measuring the position of the frequency, concentration and amplitude windows, as well as, fluxes and static head, by varying experimental conditions.

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