# Spontaneous Onset of Coherence and Energy Storage by Membrane Transporters in an *RLC* Electric Circuit

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Recent experiments have shown that oscillating or fluctuating electric fields can drive thermodynamically uphill transport of ions catalyzed by a molecular ion pump, the Na,K-ATPase. Theory suggests that if the transport reaction is very far from equilibrium the energy flow can be reversed, i.e., power can flow from the downhill transport process into the electric field. Here we show that in an electric circuit with inductance, if the transport reaction is far from equilibrium, small fluctuations in the net polarization of the transporter proteins in the membrane can be spontaneously amplified, resulting in the onset of *coherent behavior*, and *energy transfer* from the chemical gradient to the electric circuit. [S0031-9007(98)06089-X]

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Na,K-ATPase is one of the membrane transport enzymes that can be driven by oscillating or fluctuating electric fields [1–3]. Energy from the field substitutes for the energy normally provided chemically by adenosine triphosphate (ATP). This interpretation was supported by the analysis of a four-state membrane transport model [4,5] based on the theory of electroconformational coupling [6]. The enzyme has several conformational states which interact differently with Na and K on the two sides of the membrane [7]. The different conformations also have different electrical properties [8,9]. Thus, a sufficiently large oscillating or fluctuating electric field can cause such a transporter to alternate its conformation in accordance with the polarity of the field. If the affinity of the transporter for substrate is different in these states the enforced alternation can result in pumping substrate, even against a concentration gradient.

A hypothetical diagram of an experimental setup for investigating the effect of an oscillating electric field on membrane transporters in a plane bilayer is shown in Fig. 1(a). Instead of "in" and "out," the two sides of the membrane are designated "left" and "right." A kinetic diagram of the four-state electroconformational coupling model is seen in Fig. 1(b), where the electrically distinct conformational states are schematically shown as a dipole that can point either to the left (states 1 and 4) or to the right (states 2 and 3). The rate constants $k_{41}$ and $k_{32}$ are second order and are multiplied by the substrate concentrations $[S_{\mathrm{left}}]$ and $[S_{\mathrm{right}}]$, respectively, to get the transition rates from state 4 to state 1 and from state 3 to state 2. The rate constants satisfy detailed balance: $k_{12}k_{23}k_{34}k_{41} = k_{21}k_{32}k_{43}k_{14}$. Since the transporter has a dipole moment $d$, applying an external electric field pointing from the left to the right with magnitude $E$ modifies the transition rates between the two substrate bound states (1 and 2) and between the two substrate free states (3 and 4) in such a way that the equilibrium constants become $K_{\mathrm{B}} = e^{-2\Psi}k_{21}/k_{12}$ and $K_{\mathrm{F}} = e^{2\Psi}k_{43}/k_{34}$, where $\Psi = Ed/kT$. For simplicity, we assumed that the substrate is uncharged, and we checked that the behavior of the system is not qualitatively different when the substrate is charged. Furthermore, supposing rapid equilibration between the two bound and between the two free states (a sufficient condition is $k_{12} + k_{21}, k_{34} + k_{43} \gg k_{23} + k_{32}[S_{\mathrm{right}}] + k_{41}[S_{\mathrm{left}}] + k_{14}$), the kinetic equations for this model reduce to one ordinary first-order differential equation [10] for the time evolution of the probability $P_{\mathrm{B}}$ that the enzyme is in one of the two bound states:

$$\dot{P}_{\mathrm{B}} = k_{41}P_4 + k_{32}P_3 - k_{14}P_1 - k_{23}P_2, \qquad P_{\mathrm{B}} = P_1 + P_2, \qquad P_1/P_2 = K_{\mathrm{B}}, \qquad P_3/P_4 = K_{\mathrm{F}}. \tag{1}$$

The variables $P_i$ $(1 \le i \le 4)$ denote the probability that the enzyme is in the $i$th state with the normalization condition $\sum_{i=1}^{4} P_i = 1$. The expectation of the polarization of the enzyme can be expressed as $pd$, where

$$p = P_2 + P_3 - P_1 - P_4 = \frac{K_{\mathrm{F}} - 1}{K_{\mathrm{F}} + 1} + 2\,\frac{1 - K_{\mathrm{B}}K_{\mathrm{F}}}{(1 + K_{\mathrm{B}})(1 + K_{\mathrm{F}})}\,P_{\mathrm{B}}. \tag{2}$$

By numerically solving the kinetic equation with an alternating sinusoidal electric field $\Psi(t) = \Psi_0 \sin(\omega t)$ we determined the electric power $P_{\mathrm{el}} = kT\langle\Psi\dot{p}\rangle$ exerted by the field on the transporter and the chemical power $P_{\mathrm{ch}} = kT\log(S_{\mathrm{right}}/S_{\mathrm{left}})\langle k_{32}P_3 - k_{23}P_2\rangle$ exerted by the concentration gradient of the substrate, averaged over one period of the time. For a realistic set of the parameters, Fig. 1(c) shows these powers as a function of $\Psi_0$ for two different concentration gradients. It can be seen that for a small gradient (dashed lines) the electric field loses energy, and if its amplitude is large enough part of this energy can be converted to do work on the concentration gradient. The transporter acts as a "Brownian ratchet" [11], with the required anisotropy introduced by having different dissociation constants for substrate on the left

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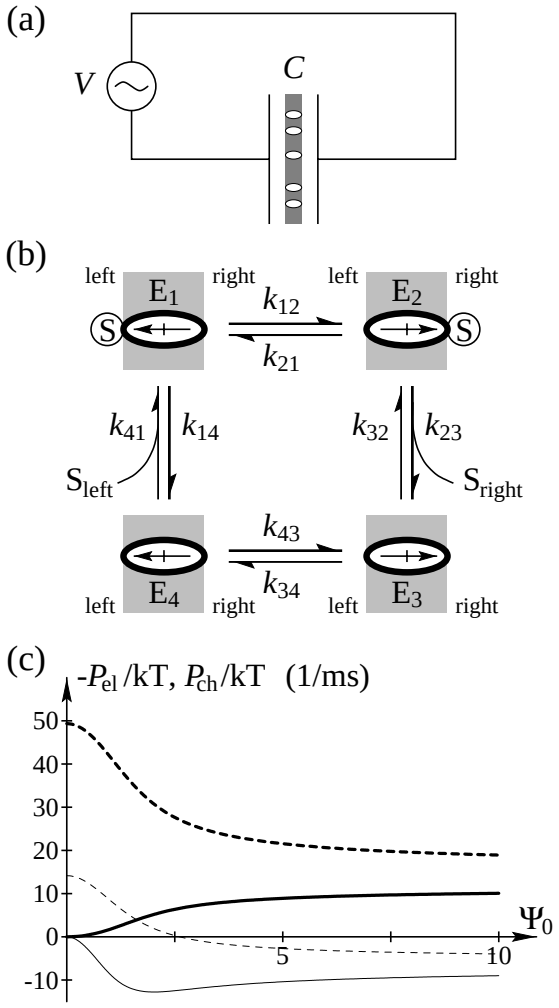


FIG. 1. (a) Hypothetical diagram of an experimental setup for investigating the effect of an oscillating electric field on membrane transporters embedded in a plane bilayer. The bilayer is placed between the plates of a capacitor, and the electric field is supplied by an alternating voltage generator. (b) Kinetic diagram of the four-state electroconformational coupling model of Na,K-ATPase. The kinetic constants used in the calculations are $k_{23} = 10 \times 10^3\ \mathrm{s}^{-1}$, $k_{32} = 2 \times 10^3\ \mathrm{mM}^{-1}\,\mathrm{s}^{-1}$, $k_{41} = 250 \times 10^3\ \mathrm{mM}^{-1}\,\mathrm{s}^{-1}$, $k_{14} = 50 \times 10^3\ \mathrm{s}^{-1}$. Since we suppose rapid equilibration between the two bound and between the two free states, it is not necessary to determine the forward and backward rates concerned, but only their ratios: $k_{21}/k_{12} = k_{43}/k_{34} = 5$. (c) The electric power $P_{\mathrm{el}}$ (solid lines) exerted by the field on the transporter, and the chemical power $P_{\mathrm{ch}}$ (dashed lines) exerted by the concentration gradient of the substrate as a function of the amplitude of the alternating field (with frequency $10^5\ \mathrm{s}^{-1}$) for small ($[S_{\mathrm{left}}] = 1/10$ mM, $[S_{\mathrm{right}}] = 10$ mM, thin lines) and large ($[S_{\mathrm{left}}] = 1$ mM, $[S_{\mathrm{right}}] = 10$ mM, thick lines) concentration gradient.

and right ($K_{\mathrm{d,left}} = k_{14}/k_{41} < K_{\mathrm{d,right}} = k_{23}/k_{32}$). What is more surprising is that for a large concentration gradient (solid lines) energy can flow back from the gradient to the field. This can be understood by tracing the changes of the probabilities of the states during one period of the oscillating field.

Consider the case when the concentration gradient is small ($[S_{\mathrm{right}}]/[S_{\mathrm{left}}] < K_{\mathrm{d,right}}/K_{\mathrm{d,left}}$) and where $[S_{\mathrm{left}}] > K_{\mathrm{d,left}}$ and $[S_{\mathrm{right}}] < K_{\mathrm{d,right}}$ (Fig. 2). When the field points to the right ($\Psi > 0$), states 2 and 3 are favored, and since $[S_{\mathrm{right}}]$ is small compared to the dissociation constant $K_{\mathrm{d,right}}$, the unbound state 3 predominates. When the polarity of the field is reversed (favoring states 1 and 4), a large part of the probability flows directly from state 3 to 4. Note, that when the field goes through zero the occupancy of state 3 is still greater than that of state 4 because $k_{34}/k_{43}$ was chosen to be less than unity. Thus the change of the transporter polarization lags the change of the field. If $\Psi$ remains negative for some time probability flows from state 4 to state 1 (substrate associates on the left side) because $[S_{\mathrm{out}}]$ is large compared to the dissociation constant $K_{\mathrm{d,left}}$. Then, when the field reverses polarity again, probability flows from state 1 to 2 and the transporter polarization lags the field again because $k_{12}/k_{21}$ is less than one. As seen in Fig. 2, the preferred direction of cycling is in the order $1 \rightarrow 2 \rightarrow 3 \rightarrow 4 \rightarrow 1$. Thus, we conclude that substrate flows from the side with low concentration (left) to the side with higher concentration (right) because the transporter goes through the cycle in the clockwise direction and energy flows from the electric field, because the polarization of the transporter lags the change of the field [Fig. 2(c)].

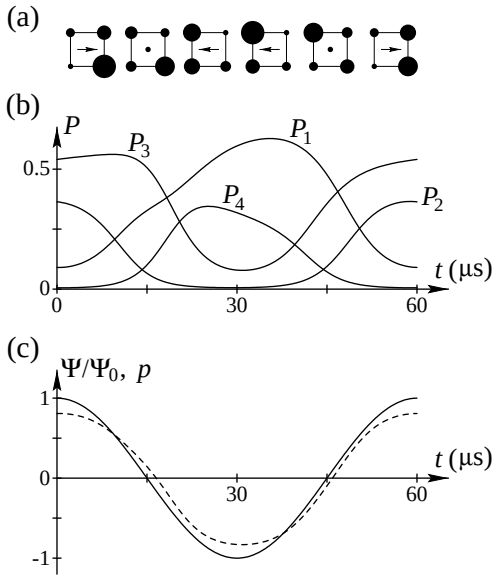


FIG. 2. Schematic illustration of the operation of the membrane transporter model in an alternating electric field (with frequency $1.66 \times 10^4\ \mathrm{s}^{-1}$ and amplitude $\Psi = 1.5$) for small concentration gradient. (The actual values of the substrate concentrations are $[S_{\mathrm{left}}] = 1/2$ nM, $[S_{\mathrm{right}}] = 4$ nM.) (b) This shows the probability of the four states as a function of time, and (a) illustrates it with circles, the areas of which are proportional to the corresponding probabilities. The arrows represent the electric field. (c) This shows the field and the quantity $p$ (which is proportional to the average polarization of the transporters) as a function of time. It is seen clearly that the polarization lags the field, indicating that the electric field loses energy.

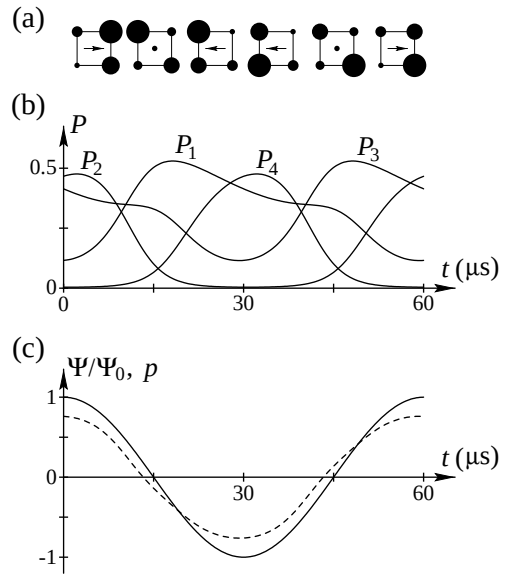


FIG. 3. Same as Fig. 2, but for large concentration gradient. (Now the actual values of the substrate concentrations are $[S_{\text{left}}] = 1/25$ mM, $[S_{\text{right}}] = 25$ mM.) Here the polarization of the transporter precedes the field, indicating the electric field gains energy.

Figure 3 describes the situation when the concentration gradient is much larger ($[S_{\text{right}}]/[S_{\text{left}}] \gg K_{\text{d,right}}/K_{\text{d,left}}$) and where $[S_{\text{left}}] \ll K_{\text{d,left}}$ and $[S_{\text{right}}] \gg K_{\text{d,right}}$, but nothing else is changed. Now, when the field points to the right ($\Psi > 0$), states 2 and 3 are favored, but since $[S_{\text{right}}]$ is very large compared to the dissociation constant $K_{\text{d,right}}$, the bound state 2 predominates. When the polarity of the field is reversed, a large part of the probability flows directly from state 2 to 1. Note, that when the field goes through zero the occupancy of state 1 is already greater than the that of state 2 because $k_{21}/k_{12}$ was chosen to be larger than unity. Thus the change of the transporter polarization precedes the change of the field. If $\Psi$ remains negative for some time, probability flows from state 1 to 4 because $[S_{\text{left}}]$ is small compared to the dissociation constant $K_{\text{d,left}}$. Then, when the field reverses polarity, probability flows from state 4 to 3 in such a way that the polarization again precedes the field because $k_{43}/k_{34}$ is larger than one. Thus, we can conclude that the system loses chemical energy (i.e., the substrate flows from the side with high concentration to the side with low concentration) because the transporter goes through the cycle predominately in the counterclockwise direction [Fig. 3(b)], and that energy flows to the electric field, because the polarization of the transporter precedes the change of the field [Fig. 3(c)].

Energy flow from oscillating and fluctuating electric fields to a concentration gradient has been experimentally observed [1–3]. Kamp *et al.* [12] proposed a model for coupling two enzymes purely electrostatically, in which energy can flow from one enzyme to the other through the electric field acting between the two enzymes, but direct observation of the energy flow from a chemical gradient to the electric field is a big challenge, and has never been performed. Here we propose a simple setup—"a biological electric generator"—to carry out this experiment.

Let us take a piece of membrane with a large number of transporters and place it between the plates of a capacitor in an *RLC* electric circuit as shown in Fig. 4(a). Since the medium that contains the membrane is a good conductor, the capacitor plates are effectively almost as close to each other as the thickness of the membrane, and the resistance of the medium is added to the resistance of the circuit. The membrane resistance is large but finite and is parallel to the effective membrane capacitance. The equivalent circuit for the proposed experimental system containing an inductance $L$, an effective capacitance $C$, and series and parallel resistances $R_{\text{c}}$ and $R_{\text{m}}$, respectively, is shown in Fig. 4(a). The electrical properties are described by the following system of differential equations:

$$\begin{aligned} L\dot{J}_{\text{c}} + R_{\text{c}}J_{\text{c}} + U_{\text{m}} &= 0, \qquad \dot{Q} = J_{\text{c}} + J_{\text{m}}, \\ R_{\text{m}}J_{\text{m}} + U_{\text{m}} &= 0, \qquad U_{\text{m}} = \frac{Q}{C} - \frac{Npd}{\varepsilon_{\text{m}}\varepsilon_0 A}, \end{aligned} \tag{3}$$

where the variables $J_{\text{c}}$ and $J_{\text{m}}$ are the currents in the circuit and through the membrane, respectively, $U_{\text{m}}$ is the voltage drop on the capacitor (on the membrane), $Q$ is the charge stored by the capacitor, and the parameters $l$ and $A$ denote the width and surface area of the membrane, respectively.

These equations are nonlinearly coupled to the kinetic equation (1) via the electric field $E = U_{\text{m}}/l$ that alters the equilibrium constants $K_{\text{B}}$ and $K_{\text{F}}$. The instantaneous state of the transporter molecules in turn enters into the differential equations (3) of the circuit via the average polarization $pd$.

Numerically solving the equations of the *RLC* circuit, together with the kinetic equation, we have found the following results: If the concentration gradient of the substrate is large enough and an electric current $J_{\text{c}}$ oscillates in the circuit (with an angular frequency $\omega \approx 1/\sqrt{LC}$), the transporters between the capacitor plates feel an oscillating electric field and convert chemical energy to electric energy of the circuit by increasing the amplitude of the oscillation of the current if the electric dissipation is small enough. The most striking result is that without any initial electric oscillation an oscillating electric current $J_{\text{c}}$ starts from thermal noise, with exponentially increasing amplitude, and reaches a plateau $J_{\text{c}}^*$, where the energy losses compensate the energy gain [Fig. 4(c)]. During this process, the initially independently and stochastically working transporters begin to work more and more coherently. The reason for this kind of behavior is that for small electric oscillation both the energy loss in the circuit and the energy transferred from the chemical gradient to the circuit have a parabolic dependence on the amplitude of the oscillation [5] [see Fig. 4(b)].

The calculations were done using realistic values of kinetic constants and circuit elements, so the approach may form the basis for a technique to study the kinetic and

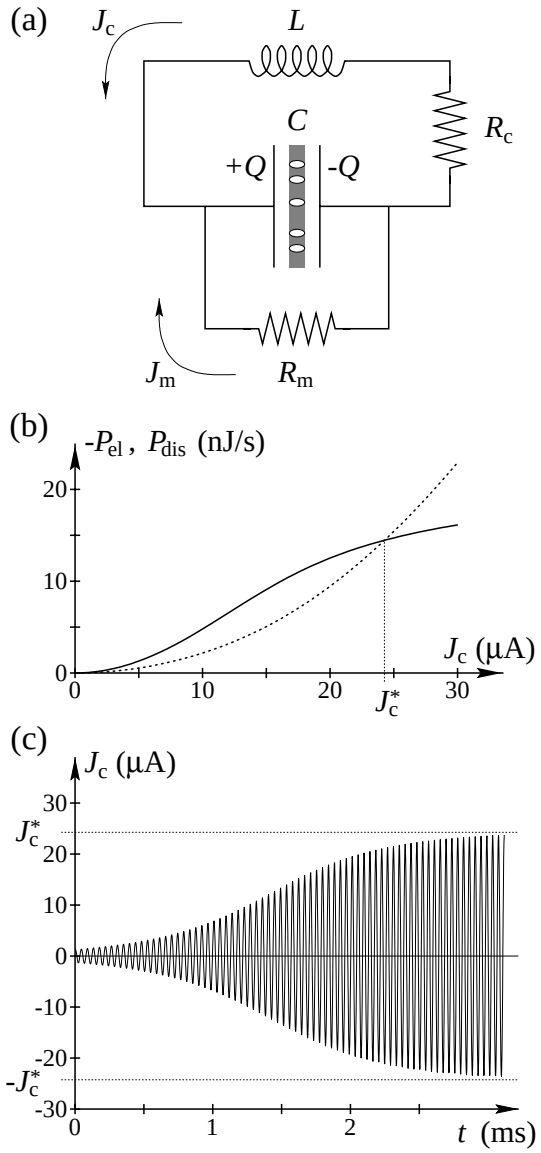


FIG. 4. (a) Schematic diagram of an *RLC* electric circuit designed for the investigation of how chemical energy can be transferred to electric energy by coherently operating membrane transporters. In the numerical calculation we have used the following realistic set of parameters: $\varepsilon_0 = 8.9 \times 10^{-12}\ \mathrm{C^2\,N^{-1}\,m^{-2}}$ is the dielectric constant of the vacuum, $\varepsilon_m = 2$ is the relative dielectric constant of the membrane, $A = 1\ \mathrm{mm^2}$ is the surface area of both the capacitor plates and the membrane, $l = 10$ nm is the width of the membrane, $C = \varepsilon_m \varepsilon_0 A/l \approx 1.8\ \mu\mathrm{F}$ is the effective capacitance, $L = 20$ MH, $R_m = 500\ \mathrm{k\Omega}$ $(= 5 \times 10^7\ \Omega\,\mathrm{m} \times 10\ \mathrm{nm}/1\ \mathrm{mm^2})$, $R_c = 30\ \Omega$ $(= 0.3\ \Omega\,\mathrm{m} \times 0.1\ \mathrm{mm}/1\ \mathrm{mm^2}$ if the width of the cell that contains the medium is 0.1 mm). $N = 2 \times 10^8$ is the number of the transporters in the membrane, $d = 1.6 \times 10^{-27}$ Cm $(1.6 \times 10^{-19}\ \mathrm{C} \times 10$ nm) is the dipole momentum of a transporter. (b) The electric power $(-P_{el})$ transferred to the circuit by the membrane transporters and the power $(P_{dis})$ dissipated in the circuit as a function of the amplitude of the electric current. (c) The electric current as a function of time. The amplitude of the current increases exponentially until reaching plateau $(J_c^*)$, where the energy losses compensate the energy gain.

dynamic behavior of membrane enzymes and the role of their interaction with the electric field. More importantly, by splitting the system into a transport reaction with very simple linear kinetics, and a passive electric circuit, the results offer insight into mechanisms by which feedback allows for spontaneous oscillatory behavior when a system is far from equilibrium. If the energy losses could not be reduced sufficiently, extra amplification could be applied that alone is insufficient to increase the oscillation, or by simply measuring the rate of damping of an initial oscillation in the circuit started externally, it should be smaller for larger concentration gradient.

The analogy between electrical circuits and chemical networks has a long history [13], and recent studies [14] have attempted to extend the correspondence to include far-from-equilibrium nonlinear effects. Here we have taken this analogy a step further and shown that a nonequilibrium chemical reaction catalyzed by a membrane enzyme can act as an active element in an external electric circuit and provide the energy for driving electrical oscillations. The realization that membrane transporters can act as reversible chemoelectrical converters provides new insights into mechanisms of free energy transduction by proteins. Although we have focused on a specific case of membrane transporters, the concept applies more generally to any system that is coupled both to an external field and to a chemical reaction.

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