On the Efficiency and Reversibility of Active Ligand Transport Induced by Alternating Rectangular Electric Pulses

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ABSTRACT The stationary-state kinetic properties of a simplified two-state electro-conformational coupling model (ECC) in the presence of alternating rectangular electric potential pulses are derived analytically. Analytic expressions for the transport flux, the rate of electric energy dissipation, and the efficiency of the transducing system are obtained as a function of the amplitude and frequency of the oscillation. These formulas clarify some fundamental concept of the ECC model and are directly applicable to the interpretation and design of experiments. Based on these formulas, the reversibility and the degree of coupling of the system can be studied quantitatively. It is found that the oscillation-induced free energy transduction is reversible and tight-coupled only when the amplitude of the oscillating electric field is infinitely large. In general, the coupling is not tight when the amplitude of the electric field is finite. Furthermore, depending on the kinetic parameters of the model, there may exist a "critical" electric field amplitude, below which free energy transduction is not reversible. That is, energy may be transduced from the electric to the chemical, but not from the chemical to the electric.

INTRODUCTION

Recently, theoretical model studies of transporter-mediated ligand transports or enzyme-catalyzed reactions in membranes under the influence of an alternating electric field applied externally across the membrane have attracted considerable attention (Markin et al., 1990; Markin and Tsong, 1991a, b; Tsong, 1992; Tsong and Astumian, 1986, 1987, 1988; Chen, 1987; Astumian et al., 1987, 1989; Westerhoff et al., 1986; Astumian and Robertson, 1989, 1990a, b). The motivation comes from the experimental findings that active transport of Rb^+ or other ions across erythrocyte membranes mediated by Na-K-ATPases could be achieved by applying a regularly oscillating electric field (Liu et al., 1990; Serpersu and Tsong, 1983, 1984; Witt et al., 1976; Teissie, 1986). It is known that, if the ligand is not charged, the direction of the steady-state transport of ligands in a transporter-mediated transport system is determined by the concentration gradient of the ligands (passive transport) and is not affected by a constant "static" membrane potential, whether the transporter is charged or not. However, if the applied electric potential is not static but oscillatory, active transport of uncharged ligands against a concentration gradient can be achieved when the transporters are charged and the kinetic rate constants of the system satisfy certain asymmetry conditions (Chen, 1987; Astumian et al., 1987; Tsong and Astumian, 1986; Westerhoff et al., 1986). That is, the energy of an oscillating electric field can be absorbed by the charged transporters and used to pump uncharged ligands across the membrane against a concentration gradient. This phenomenon has been termed the "electro-conformational coupling" (ECC) (Tsong and Astumian, 1986).

One subject of theoretical interest that has attracted considerable attention recently is the efficiency and reversibility of the free energy transduction of the model. Based on simplifying assumptions and semi-quantitative analyses, Markin et al. (1990) and Markin and Tsong (1991a, b) concluded that the free energy transduction of a simple four-state ECC model with neutral ligands in the presence of an alternating rectangular electric field is completely reversible. That is, when the reverse chemical gradient of the ligand is high, both the transport flux and the rate of absorbed electric energy change sign simultaneously so that the direction of the transduction process is reversed. In this case, the system is considered as *tightly* coupled with a degree of coupling close to one (Caplan and Essig, 1983). However, these studies are based on the assumption that the amplitude of the rectangular electric potential is infinitely large. It is not clear whether the system is still tight-coupled when the amplitude of the rectangular electric field is not infinite. Because the membrane potential used in all experiments is finite and small, we thought it worthwhile to have a detailed analysis on the system without the assumption of an infinite electric amplitude. This is the main purpose of this paper.

In this paper, the kinetic properties of the same ECC model studied by Markin et al. (1990) are studied exactly without any assumption on the magnitude of the electric field amplitude. The kinetic equations describing the time-dependent state probabilities of the model in the presence of an arbitrary rectangular electric wave are solved analytically so that the ligand transport flux and the rate of electrical energy dissipation (and, therefore, the efficiency) of

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the model can be evaluated quantitatively as a function of the amplitude and frequency of the oscillating electric field. In The Model, the model and the solution to its kinetic equations in the presence of an oscillating electric field are discussed. General expressions for the transport flux and the rate of dissipated electric energy are then derived in the Mathematical Analysis and Transport Flux sections. The efficiency and reversibility of the model are discussed and illustrated using a simple numerical example in Electric Energy Dissipation. It is found that, in general, the transport flux and the rate of electric energy dissipation do not change sign simultaneously if the amplitude of the rectangular electric pulses is not infinitely large. That is, when the amplitude of the oscillating electric field is finite, the coupling between the electric field and the ligand transport is not tight. Also, depending on the rate constants of the model, there may exist a "critical" electric field amplitude below which the rate of electric energy dissipation does not change sign no matter how high the reverse ligand concentration gradient is. In this case, chemical energy can never be transduced into electric energy.

THE MODEL

Fig. 1 *a* shows the kinetic mechanisms of a basic four-state model for transporter-mediated ligand transports in membranes. Each transporter can exist in two conformations, I and II. A transporter can bind a ligand from bath 1 only in conformation I and from bath 2 only in conformation II. C_1 and C_2 are the concentrations of the ligand in bath 1 and 2, respectively. The transporter is charged with a valence z, whereas the ligand is assumed to be uncharged. The membrane potential V is defined as the potential in bath 1 relative to that in bath 2. The rate constants of the model in the presence of a constant membrane potential V are shown in Fig. 1 *b* in which k is the rate constant of the transport system in the absence of V, γ and δ are constants, and θ is defined as (Chen, 1987; Astumian et al., 1987; Tsong and Astumian, 1986; Westerhoff et al., 1986)

$$\theta = \exp(-zFV/RT_t) \tag{1}$$

where F, R, and T_t are, respectively, the Faraday constant, the gas constant, and the absolute temperature of the system.

As assumed by Markin et al. (1990), the transitions between states 1 and 4 and between states 2 and 3 are extremely fast compared with the other transitions. Thus, the four-state diagram in Fig. 1 *b* can be simplified and reduced to a two-state diagram shown in Fig. 1 *c* by combining states 1 and 4 together as state A and states 2 and 3 together as state B. The four rate constants of this two-state diagram can be obtained from the old four-state diagram as (Hill, 1977)

$$\alpha_{AB} = \frac{k_{41}\theta^{\gamma}}{k_{41}\theta^{\gamma} + k_{14}\theta^{\gamma-1}} k_{12}C_1 = f_A k_{12}C_1 \tag{2}$$

$$\alpha_{BA} = \frac{k_{32}\theta^{\delta}}{k_{32}\theta^{\delta} + k_{23}\theta^{\delta-1}} k_{21} = f_B k_{21} \tag{3}$$

$$\beta_{AB} = \frac{k_{14}\theta^{\gamma-1}}{k_{14}\theta^{\gamma-1} + k_{41}\theta^{\gamma}} k_{43}C_2 = (1 - f_A)k_{43}C_2 \tag{4}$$

$$\beta_{BA} = \frac{k_{23}\theta^{\delta-1}}{k_{23}\theta^{\delta-1} + k_{32}\theta^{\delta}} k_{34} = (1 - f_B)k_{34} \tag{5}$$

$$f_A = \frac{K_{14}\theta}{1 + K_{14}\theta}; \qquad f_B = \frac{K_{23}\theta}{1 + K_{23}\theta}. \tag{6}$$

where K_{14} and K_{23} are the equilibrium constants between states 1 and 4 and states 2 and 3, respectively: $K_{14} = k_{41}/k_{14}$; $K_{23} = k_{32}/k_{23}$. Thus, the basic set of parameters of the two-state model are: θ, K_{14}, K_{23}, k_{12}, k_{21}, k_{43}, and k_{34}. Note that the k values and the K values are connected through the "detailed balance" condition as

$$\frac{k_{12}k_{34}}{k_{21}k_{43}} = \frac{K_{23}}{K_{14}}. \tag{7}$$

Also note that f_A and f_B are simply the fractions of transporters in A and B that are in states 1 and 2, respectively:

$$f_A = \frac{p_1}{p_1 + p_4} = \frac{p_1}{p_A} \tag{8}$$

$$f_B = \frac{p_2}{p_2 + p_3} = \frac{p_2}{p_B} \tag{9}$$

The two-state diagram in Fig. 1 *c* with the rate constants expressed in Eqs. 2–6 constitute the basic ingredients of our model. In the presence of a *constant* membrane potential V, the steady-state transport flux per transporter can be obtained

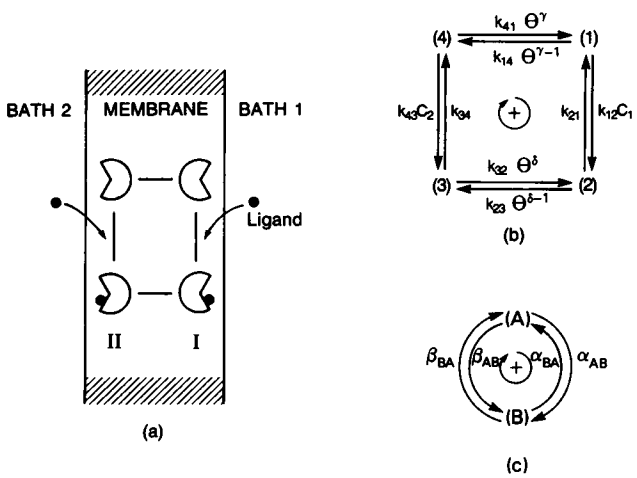

FIGURE 1 (*a*) A four-state transporter-mediated ligand transport system. A charged transporter with a valence of z can exist in two conformations. Conformation I can bind an uncharged ligand only from bath 1 and conformation II only from Bath 2. (*b*) The kinetic diagram of the transport system in (*a*) in the presence of a constant membrane potential V (measured as the potential in Bath 1 relative to that in Bath 2). θ is defined in Eq. 1 and γ and δ are constants. k is the rate constant of the system in the absence of a membrane potential. C_1 and C_2 are the concentrations of the ligand in baths 1 and 2 respectively. (*c*) The reduced kinetic diagram of *b*, when the values of k_{14}, k_{41}, k_{23}, and k_{32} are extremely large compared with the rest of the rate constants.

from Fig. 1 *c* using the diagram method (21):

$$J_{tr} = \frac{1}{\sum}(\alpha_{AB}\beta_{BA} - \beta_{AB}\alpha_{BA}) = \frac{K_{14}k_{12}k_{34}\theta}{\sum(1 + K_{14}\theta)(1 + K_{23}\theta)}(C_1 - C_2), \quad (10)$$

where

$$\sum = \alpha_{AB} + \alpha_{BA} + \beta_{AB} + \beta_{BA}. \quad (11)$$

Because the quantity in front of $(C_1 - C_2)$ in Eq. 10 is always positive, the sign of J_{tr} (or the direction of the transport) is completely determined by the concentration gradient $(C_2 - C_1)$ of the ligand, independent of the membrane potential V. That is, in the presence of a constant membrane potential the flow of the ligand is always passive. In contrast, active transport of ligand against a concentration gradient can be achieved by applying an oscillating electric potential across the membrane. In this paper, we will show how to derive the transport flux and the electric energy dissipated (and, therefore, the efficiency) for the model when the membrane potential is oscillating alternatively with square pulses.

MATHEMATICAL ANALYSIS

In this section, the dynamics of the model in the presence of an alternating square membrane potential will be analyzed. This analysis is essential for deriving the transport flux and the energy dissipation rate of the system. As shown in Fig. 2, a regular rectangular electric wave consists of a train of alternating positive and negative rectangular electric pulses of the same amplitude (Δ) and pulse duration (lifetime, T). A "cycle" of the oscillation consists of one positive pulse followed by a negative one. Thus, the period of a cycle is equal to $2T$. The switching between two pulses is assumed to be very fast in the time scale of the kinetics of Fig. 1 *c*. That is, no transition occurs between states A and B when the pulse is switched from the positive to the negative or vice versa. When a membrane is subjected to a regular rectangular electric wave, the kinetic properties of the charged transporters in the membrane will also show periodic oscillations when the system reaches a "stationary" state. Define $p^+_{A,(n)}(t)$ and $p^-_{A,(n)}(t)$ as the time-dependent probabilities of finding a transporter in state A in the positive $(+\Delta)$ and negative $(-\Delta)$ pulses at time t in the nth cycle of the oscillating wave. Then, they obey the differential equations

$$\frac{dp^+_{A,(n)}(t)}{dt} = -(\alpha^+_{AB} + \beta^+_{AB})p^+_{A,(n)}(t) + (\alpha^+_{BA} + \beta^+_{BA})(1 - p^+_{A,(n)}(t)) \quad (12)$$

and

$$\frac{dp^-_{A,(n)}(t)}{dt} = -(\alpha^-_{AB} + \beta^-_{AB})p^-_{A,(n)}(t) + (\alpha^-_{BA} + \beta^-_{BA})(1 - p^-_{A,(n)}(t)) \quad (13)$$

$$n = 1, 2, 3, \cdots \qquad 0 \le t \le T,$$

where α^+_{AB}, β^-_{AB}, etc., are the same as those in Eqs. 2–5, except that the θ values are replaced with corresponding θ^+ (at $V = +\Delta$) and θ^- (at $V = -\Delta$) defined as

$$\theta^+ = \exp(-z\varphi), \qquad \theta^- = \exp(z\varphi), \quad (14)$$

and

$$\varphi = \frac{F\Delta}{RT_t}. \quad (15)$$

Note that φ is always positive. Because no transition occurs between states A and B during the pulse-switching process, the probability of finding state A at the end of a pulse is equal to that at the start of the following pulse. Thus,

$$p^+_{A,(n)}(0) = p^-_{A,(n-1)}(T); \qquad p^-_{A,(n)}(0) = p^+_{A,(n)}(T). \quad (16)$$

With these boundary conditions, the solutions of Eqs. 12 and 13 can be obtained as

$$p^+_{A,(n)}(t) = p^+_\infty + (p^-_{A,(n-1)}(T) - p^+_\infty)\exp(-\textstyle\sum^+ t) \quad (17)$$

$$p^-_{A,(n)}(t) = p^-_\infty + (p^+_{A,(n)}(T) - p^-_\infty)\exp(-\textstyle\sum^- t) \quad (18)$$

where

$$\textstyle\sum^\pm = \alpha^\pm_{AB} + \alpha^\pm_{BA} + \beta^\pm_{AB} + \beta^\pm_{BA} \quad (19)$$

and p^+_∞ and p^-_∞ are the "equilibrium" probabilities of a transporter being in state A when the membrane potential

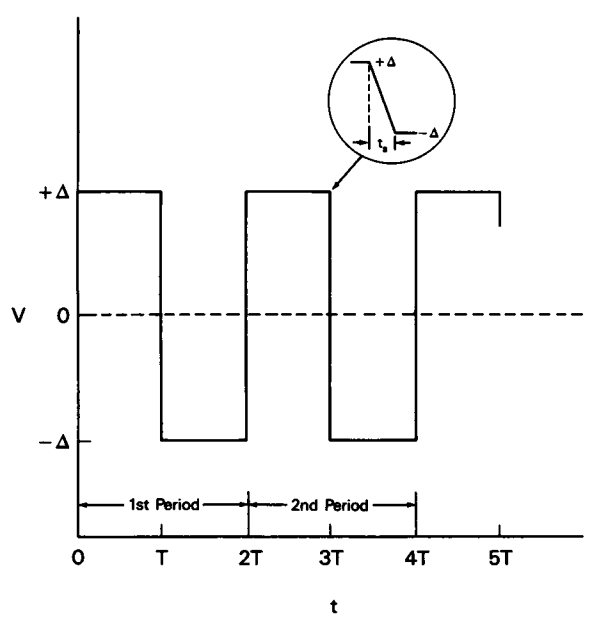

FIGURE 2 Potential profile of the oscillation. The time to switch the potential from the positive to the negative pulse or vice versa is denoted as t_s. The value of t_s is very small compared to the lifetime of a pulse, T. Each cycle consists a positive rectangular pulse followed by a negative pulse. The period of a cycle is thus equal to $2T$.

is held *fixed* at $+\Delta$ and $-\Delta$, respectively:

$$p_\infty^+ = \frac{\alpha_{BA}^+ + \beta_{BA}^+}{\sum^+}; \qquad p_\infty^- = \frac{\alpha_{BA}^- + \beta_{BA}^-}{\sum^-}. \tag{20}$$

A recursive relation between $p_{A,(n)}^-(T)$ and $p_{A,(n-1)}^-(T)$ can be obtained easily from Eqs. 17 and 18:

$$p_{A,(n)}^-(T) = p_\infty^- + (p_\infty^+ - p_\infty^-)E^- + [p_{A,(n-1)}^-(T) - p_\infty^+]E^+E^- \tag{21}$$

where

$$E^\pm = \exp(-\textstyle\sum^\pm T). \tag{22}$$

Thus, the general expression for $p_{A,(n)}^-(T)$ can be obtained from this recursive equation as

$$p_{A,(n)}^-(T) = p_\infty^- + \frac{(p_\infty^+ - p_\infty^-)E^-}{(1 - E^+E^-)} \cdot [1 - (E^+E^-)^{n-1} - E^+ + E^+(E^+E)^{n-2}] + (p_0 - p_\infty^+)(E^+E^-)^n \tag{23}$$

where p_0 is the initial probability of a transporter in state A before the onset of the oscillating electric wave. Evaluating the right side of Eq. 23 at $n \to \infty$ (the stationary-state) and recognizing that both E^+ and E^- are less than 1, we obtain the stationary-state probability of state A at the end of a negative pulse as

$$p_A^-(T) = p_\infty^- + (p_\infty^+ - p_\infty^-)\frac{E^-(1 - E^+)}{1 - E^+E^-}. \tag{24}$$

Similar procedure can be used to derive $p_A^+(T)$. The final result is

$$p_A^+(T) = p_\infty^+ - (p_\infty^+ - p_\infty^-)\frac{E^+(1 - E^-)}{(1 - E^+E^-)}. \tag{25}$$

Because $p_A^+(0) = p_A^-(T)$ and $p_A^-(0) = p_A^+(T)$, Eqs. 24 and 25 give the probabilities at both ends of a pulse. The stationary-state probabilities of being in state A at time t in the positive and the negative pulses can be obtained from Eqs. 17 and 18 as

$$p_A^+(t) = p_\infty^+ - \frac{(p_\infty^+ - p_\infty^-)(1 - E^-)}{1 - E^+E^-}\exp(-\textstyle\sum^+ t), \tag{26}$$

$$p_A^-(t) = p_\infty^- + \frac{(p_\infty^+ - p_\infty^-)(1 - E^+)}{1 - E^+E^-}\exp(-\textstyle\sum^- t), \qquad 0 < t < T. \tag{27}$$

Note that p_0 does not appear in Eqs. 24–27, because the memory of the initial distribution is lost when the system reaches the stationary state. The average transport flux and the average electric energy dissipation rate at stationary state can be evaluated from Eqs. 26 and 27.

TRANSPORT FLUX

The mean transport flux of ligands from bath 1 to bath 2 averaged over one cycle of oscillation at stationary state can be obtained by integrating the flux in the α reaction path (see Fig. 1 *c*) over one period of time:

$$\overline{J_{tr}} = (2T)^{-1}\int_0^T [\alpha_{BA}^+ p_A^+(t) - \alpha_{BA}^+(1 - p_A^+(t)) + \alpha_{BA}^- p_A^-(t) - \alpha_{BA}^-(1 - p_A^-(t))]\,dt. \tag{28}$$

Alternatively, it can be evaluated from the β reaction path. Both should give the same result. Note that the $\overline{J_{tr}}$ defined in Eq. 28 is positive when there is a net transport of ligands from bath 1 to bath 2 and is negative for a net transport from bath 2 to bath 1. Substituting Eqs. 26 and 27 into Eq. 28, we have

$$\overline{J_{tr}} = \frac{1}{2}[(\alpha_{AB}^+ + \alpha_{BA}^+)\overline{p_A^+} - \alpha_{BA}^+ + (\alpha_{AB}^- + \alpha_{BA}^-)\overline{p_A^-} - \alpha_{BA}^-] \tag{29}$$

where

$$\overline{p_A^+} = T^{-1}\int_0^T p_A^+(t)\,dt = p_\infty^+ - \frac{(p_\infty^+ - p_\infty^-)(1 - E^+)(1 - E^-)}{T(1 - E^+E^-)\sum^+} \tag{30}$$

$$\overline{p_A^-} = T^{-1}\int_0^T p_A^-(t)\,dt = p_\infty^- + \frac{(p_\infty^+ - p_\infty^-)(1 - E^+)(1 - E^-)}{T(1 - E^+E^-)\sum^-}. \tag{31}$$

Eq. 29 is the basic equation to calculate the ligand transport flux when the equilibrium and rate constants of the model are given. The asymmetric conditions necessary to make the flux in Eq. 29 positive (net transport from bath 1 to bath 2) at $C_1 = C_2$ are: $k_{12} > k_{43}$ and $k_{34} > k_{21}$ (Chen, 1987). From Eq. 7, these conditions imply that $K_{23} > K_{14}$ (1–3). When the sign of the charge on the transporter is changed, the θ^+ and θ^- in Eq. 14 exchange values. As a result, Eq. 29 remains unchanged. This means that $\overline{J_{tr}}$ depends only on the absolute value, not the sign, of the charge on the transporter. That is, as long as $K_{23} > K_{14}$, ligands will always be transported from bath 1 to bath 2 at $C_1 = C_2$, whether the transporter is positively or negatively charged. As will be discussed below, the rate of electric energy dissipated in the system is also independent of the sign of the charge on the transporter. Thus, for convenience, we may take z as positive.

The concentration ratio (C_2/C_1) at which $\overline{J_{tr}} = 0$ is called the "static head." One question we are interested in is to determine whether the static head occurs at the same place where the rate of electric energy dissipation disappears. In

general, an explicit expression for static head is difficult to derive from Eq. 29 for an arbitrary value of Δ. Thus, most of our discussions will be based on numerical calculations. However, the static head can be derived easily from Eq. 29 when the amplitude of each rectangular pulse is infinitely large. It is easy to show that when $\Delta = \infty$, the static head occurs at

$$\left(\frac{C_2}{C_1}\right)^* = \frac{K_{23}}{K_{14}}. \tag{32}$$

This result agrees with that obtained by Markin et al. (1990).

ELECTRIC ENERGY DISSIPATION

Electric energy is dissipated when charged transporters are translocated from one side of the membrane to the other in the presence of a membrane potential. The dissipated energy is positive when a positively charged transporter is translocated down-hill in the electric field and is negative when it is translocated up-hill against the electric field. The reverse is true for negatively charged transporters. A negative dissipation means the energy is given back to the electric field. It is easy to see that charge translocations occur both during the lifetime of a pulse (T) and during the lifetime of a pulse switch (t_s, see Fig. 2). As shown in the Appendix, the average rate of total electric energy dissipation per one cycle is derived as

$$\overline{W} = \frac{(p_\infty^+ - p_\infty^-)(1 - E^+)(1 - E^-)}{2T(1 - E^+E^-)} \cdot \ln\left[\frac{(K_{14} + e^{z\varphi})(K_{23} + e^{-z\varphi})}{(K_{14} + e^{-z\varphi})(K_{23} + e^{z\varphi})}\right]. \tag{33}$$

The expression for $\overline{W}$ in Eq. 33 remains the same when z is replaced with $-z$. That is, the rate of electric energy dissipation is also independent of the sign of the charge on the transporter. Because $K_{23} \neq K_{14}$, the logarithm term in Eq. 33 is always non-zero. Thus, $\overline{W}$ becomes zero only when $p_\infty^+ - p_\infty^- = 0$. That is, the "critical" point at which the value of $\overline{W}$ changes sign can be obtained from the equation $p_\infty^+ - p_\infty^- = 0$. From Eq. 20 and Eqs. 2–5, the critical ligand concentration gradient is thus obtained as

$$\left(\frac{C_2}{C_1}\right)^{**} = \left(\frac{K_{14}k_{12}}{k_{43}}\right)\left\{\frac{k_{21}K_{23}(K_{23} - K_{14}) + k_{34}\Psi}{k_{21}K_{23}\Psi_{II} - k_{34}(K_{23} - K_{14})}\right\}, \tag{34}$$

where

$$\Psi_I = 1 + K_{14}K_{23} + K_{23}(e^{z\varphi} + e^{-z\varphi}),$$

$$\Psi_{II} = 1 + K_{14}K_{23} + K_{14}(e^{z\varphi} + e^{-z\varphi}).$$

Because T is absent in Eq. 34, $(C_2/C_1)^{**}$ does not depend on the period ($2T$) or frequency ($1/2T$) of the oscillation.

When $z\varphi = \infty$, the critical value in Eq. 34 reduces to

$$\left(\frac{C_2}{C_1}\right)^{**} = \frac{K_{23}}{K_{14}}, \tag{35}$$

which is identical to that in Eq. 32. This means that both the transport flux and the rate of energy dissipation change sign simultaneously at K_{23}/K_{14}, when the amplitude of the electric field is infinitely large. This is exactly the conclusion of Markin et al. (1990).

Because $K_{23} > K_{14}$, the denominator inside the {.} in Eq. 34 decreases faster than the numerator when the value of $z\varphi$ decreases. Thus, $(C_2/C_1)^{**}$ will increase as $z\varphi$ decreases and will become negative when the denominator becomes negative. A negative $(C_2/C_1)^{**}$ means that it is no longer a critical point. Thus, so that $\overline{W}$ can change sign, the value of $z\varphi$ must satisfy the inequality

$$e^{z\varphi} + e^{-z\varphi} \geq \frac{k_{34}(K_{23} - K_{14}) - k_{21}K_{23}(1 + K_{14}K_{23})}{k_{21}K_{23}K_{14}}. \tag{36}$$

Eq. 36 defines the critical "interaction parameter" ($z\varphi$) of the model that determines whether the rate of electric energy dissipation will change sign or not at high C_2/C_1 values.

SAMPLE CALCULATIONS

In this section, the coupling between oscillating electric field and ligand transport is studied quantitatively for the model with the following equilibrium and rate constants: $K_{14} = 0.1$, $K_{23} = 10$, $k_{12} = 10$, $k_{21} = 1$, $k_{43} = 1$, $k_{34} = 10$. The purpose is to examine the effect of the amplitude of the electric field (or specifically $z\varphi$) on the efficiency and reversibility of the model. The "critical interaction parameter" of this model can be calculated from Eq. 36 and is found to be 4.3693 for this set of parameters. Thus, the dissipated electric energy will always be positive, and the energy transduction is not reversible for this model, if $z\varphi < 4.3693$.

The average ligand transport flux ($\overline{J}_{tr}$) and electric energy dissipation rate ($\overline{W}$) as calculated from Eqs. 29 and 34 for the model with $C_1 = 1$ and $T = 1$ are shown in Fig. 3. As shown in the figure, $\overline{J}_{tr}$ is more sensitive to the value of $z\varphi$ than $\overline{W}$ and, when $z\varphi = 12$, both $\overline{J}_{tr}$ and $\overline{W}$ are already very close to those for the infinite amplitude case. As expected, $\overline{W}$ calculated for $z\varphi = 4$ is always positive independent of the ligand gradient. In this case, the energy transduction is not reversible. The critical gradients where $\overline{J}_{tr}$ and $\overline{W}$ change sign are difficult to see from the curves in Fig. 3. Therefore, we plot them as a function of $z\varphi$ in Fig. 4. As discussed before, both $\overline{J}_{tr}$ and $\overline{W}$ change sign at $C_2/C_1 = 100$ ($=K_{23}/K_{14}$, see Eqs. 32 and 35), when $z\varphi$ is infinitely large. As $z\varphi$ decreases, the critical concentration gradient for $\overline{J}_{tr}$ decreases and increases for $\overline{W}$. That is, for a finite $z\varphi$ value, $\overline{J}_{tr}$ always changes sign at a smaller C_2/C_1 value than $\overline{W}$. Also, as discussed before, the critical gradient is a function of the frequency of the oscillation for $\overline{J}_{tr}$ but not for $\overline{W}$.

When both $\overline{W}$ and $\overline{J}_{tr}$ are positive at low C_2, the energy of the electric field is driving the transport of ligand from bath 1 to bath 2 and the efficiency of the transduction is equal to

$$\eta = \frac{\overline{J}_{tr}\ln(C_2/C_1)}{\overline{W}}.$$

As C_2 increases beyond the critical gradient (static head)

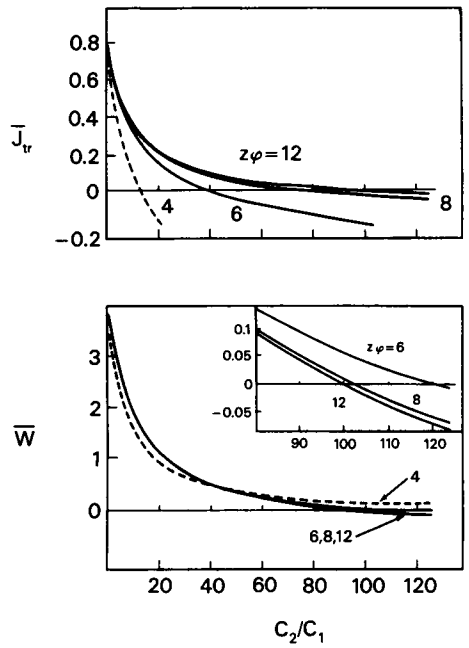

FIGURE 3 $\overline{J}_{tr}$ and $\overline{W}$ as a function of C_2/C_1 calculated for the model with $K_{14} = 0.1$, $K_{23} = 10$, $k_{12} = 10$, $k_{21} = 1$, $k_{43} = 1$, $k_{34} = 10$ at $C_1 = 1$ and $T = 1$. The value of $z\varphi$ is indicated beside each curve.

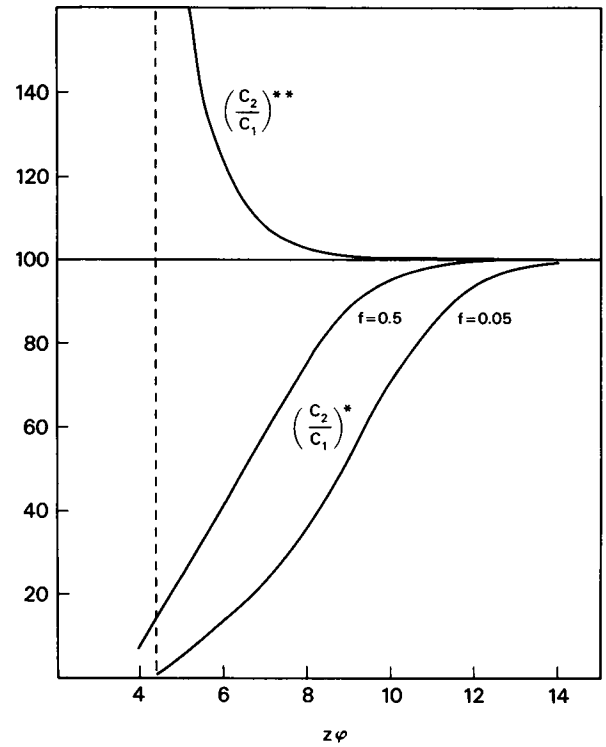

FIGURE 4 The critical C_2/C_1 values at which $\overline{J}_{tr} = 0$ (*lower* panel) and $\overline{W} = 0$ (*upper* panel) are plotted as a function of $z\varphi$. $(C_2/C_1)^{**} = \infty$, when $z\varphi$ equals the critical value of 4.3693.

value, $\overline{J}_{tr}$ becomes negative. If $\overline{W}$ also becomes negative when C_2 is increased further (for $z\varphi > 4.3693$ case), then the chemical energy is transduced into electric energy and the efficiency is defined as

$$\eta = \frac{\overline{W}_{tr}}{\overline{J}_{tr} \ln(C_2/C_1)}.$$

Calculated efficiency curves at different $z\varphi$ values are shown in Fig. 5. As expected, when the interaction parameter, $z\varphi$, is equal to 4, the system is not reversible. In case the interaction parameter is larger than the critical value of 4.3693, efficiency increases as the amplitude of the electric field increases, whether the energy transduction is from electric to chemical (the left panel) or vice versa (the right panel). The gap in each efficiency curve in Fig. 5 represents the region where both the chemical and the electric energies are dissipated (no energy transduction). The larger the gap, the weaker the coupling is. In principle, complete coupling occurs only when the electric amplitude is infinitely large. However, as shown in Fig. 5, the coupling is already very tight, even for $z\varphi = 12$. This value corresponds to a membrane potential of 310 mV when $z = 1$.

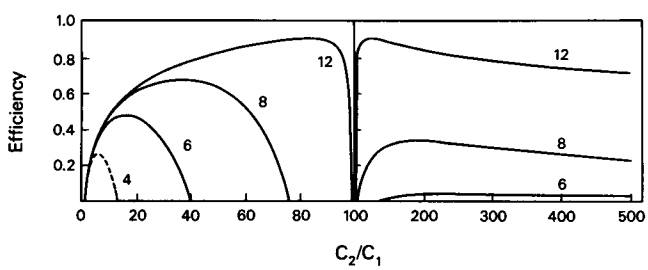

FIGURE 5 Efficiency calculated from the $\overline{J}_{tr}$ and $\overline{W}$ in Fig. 3. Energy flows from the electric to the chemical in the left panel and vice versa in the right panel.

DISCUSSION

In the presence of an oscillating electric field across a membrane, charged transporters inside the membrane can absorb energy from the field and use it to pump ligand across the membrane against a concentration gradient (the ECC model). Previously, we have shown that the stationary-state kinetic behavior of an arbitrary ECC model can be determined analytically, if the oscillation is of the "random telegraph" type (alternating rectangular pulses with random lifetimes) (Chen, 1987; Astumian et al., 1987). As a result, theoretical questions such as the asymmetry conditions for the model to work, or the flux as a function of the amplitude and frequency of the oscillations, etc. (Chen, 1987) can be answered easily for the model. Here, we show that analytical solutions for the dynamics of the model are also obtainable when the membrane potential is not oscillating randomly, but regularly with rectangular pulses of uniform lifetimes. In this paper, we consider only the two-state model. In another report, we show that analytical solutions can also be obtained for models with an arbitrary number of states. Thus, some of the results and conclusions obtained here are rather general.

With analytical formulas for the flux and the rate of electric energy dissipation given, the reversibility and efficiency of ECC models can be readily studied. As discussed by Markin et al. (1990), a simplified two-state ECC model in the presence of alternating rectangular electric pulses with uniform lifetimes is reversible and tightly coupled, if the amplitude of the electric field is infinitely large. That is, the electric and the chemical energies can be transduced from one to the other and vice versa, and the efficiency of energy transduction can be as high as one-hundred percent. In ordinary cotransport systems in which two substrates are carried on a single transporter, the free-energy transduction between the two substrates is always reversible. But, to reach one-hundred percent efficiency, the kinetic diagram underlying the mechanism of the system must contain only one single cycle (Caplan and Essig, 1983). Thus, one might liken the ECC model to an ordinary cotransport system containing only one kinetic cycle. In this paper, we show that, in general, this is not the case. As discussed in previous sections, reversible and tight coupling occur in this system only when the amplitude of the oscillating electric field is infinitely large. For finite and small amplitudes, the coupling is not tight and in some cases may not be reversible at all. In general, the coupling between oscillating electric field and ligand transport in ECC models decreases when the amplitude of the oscillating electric field is decreased. Depending on the charge and the kinetic parameters of the transporter, there may exist a critical amplitude below which the electric energy of the oscillating electric field can be transduced into the chemical energy, but not the reverse process.

Although our main goal in this study is to investigate the efficiency and reversibility of ECC models, the formulas obtained here are also very useful in deducing the rate constants of general membrane transport models. Recently, harmonic analyses have been proposed and used to study the kinetic properties of transporter-mediated membrane transport systems (Robertson and Astumian, 1992; Horn, 1993; Astumian, 1993). In this method, the frequency-dependent steady-state transport flux measured in the presence of small sinusoidal oscillations is used to elucidate the kinetic parameters of the transport system. With an analytic formula available for the transport flux, the use of oscillating electric fields with regular rectangular pulses appears very promising for probing the kinetic parameters of membrane transport systems. The advantage of using alternating rectangular pulses over sinusoidal oscillations is that no limitation on the amplitude of the electric field used in the experiment is required.

In conclusion, we have solved analytically the dynamics of a two-state ECC model in the presence an oscillation containing regular alternating rectangular pulses. The formalism can be extended easily to models with an arbitrary number of states or models with ligands that are charged. It is found that energy transduction in an ECC model is reversible and tightly coupled only when the amplitude of the oscillation is infinitely large. When the amplitude is small, the coupling is not tight and the transduction may not be reversible.

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APPENDIX

Derivation of the rate of electric energy dissipation of the model

In this Appendix, the rate of electric energy dissipation per cycle at stationary state as expressed in Eq. 33 is derived. As mentioned before, energy dissipation occurs in both the pulse and the switching phases of the oscillation. We first consider the pulse phase and then the switching.

Consider the kinetic diagram in Fig. 1 *b* and define $J_{ij}^{\pm}(t)$ as the net transport flux from state i to state j at time $t(0 \le t \le T)$ in the positive $(V = +\Delta)$ and the negative $(V = -\Delta)$ pulse, respectively. Then, the *mean rate* of electric energy dissipated (in unit of RT_r) for one period of oscillation $(2T)$ is equal to

$$\overline{W_{\text{pulse}}} = \frac{1}{2}[z\varphi(\overline{J_{14}^{+}} + \overline{J_{23}^{+}}) - z\varphi(\overline{J_{14}^{-}} + \overline{J_{23}^{-}})], \tag{A1}$$

where the horizontal bar means integration over t for the lifetime of one pulse (T). From Fig. 1 *b*, it is easy to see that

$$\frac{dp_1^{\pm}(t)}{dt} = -J_{14}^{\pm}(t) - J_{12}^{\pm}(t); \tag{A2}$$

$$\frac{dp_2^{\pm}(t)}{dt} = J_{12}^{\pm}(t) - J_{23}^{\pm}(t). \tag{A3}$$

Thus, we have

$$\begin{aligned} J_{14}^{\pm}(t) + J_{23}^{\pm}(t) &= -\left(\frac{dp_1^{\pm}(t)}{dt} + \frac{dp_2^{\pm}(t)}{dt}\right) \\ &= -\left(f_A^{\pm}\frac{dp_A^{\pm}(t)}{dt} + f_B^{\pm}\frac{dp_B^{\pm}(t)}{dt}\right) = (f_B^{\pm} - f_A^{\pm})\frac{dp_A^{\pm}(t)}{dt}, \end{aligned} \tag{A4}$$

where Eqs. 8 and 9 have been used. Integrating the last expression in Eq. A4 over t for the positive and the negative pulse, respectively, and substituting the results into Eq. A1, we obtain the mean rate of electric energy dissipation per one cycle as

$$\overline{W_{\text{pulse}}} = z\varphi(2T)^{-1}(f_B^{+} - f_A^{+} + f_B^{-} - f_A^{-})(p_A^{+}(T) - p_A^{-}(T)). \tag{A5}$$

where $p_A^{-}(T)$ and $p_A^{+}(T)$ are given in Eqs. 24 and 25, respectively.

As discussed before, the switching rate is fast compared with the reaction rates between states A and B. This implies that the sum of transporters in states 1 and 4 (state A) remains unchanged during the switching process, although redistribution between them may take place. So are those in states 2 and 3. As proposed by Markin et al. (1990), equilibrium distribution is assumed to hold between states 1 and 4 and between states 2 and 3 during the lifetime (t_s) of the switching process (the transitions between states 1 and 4 and between states 2 and 3 are much faster than the rate of change of membrane potential). This implies that Eqs. 6–9 are still applicable, except that θ (and, therefore, f_A and f_B) is now time-dependent, because the membrane potential V in Eq. 1 changes as a function of time. Therefore, the fluxes from states 1 to 4 and from states 2 to 3 at time t after the start of a pulse switch can be expressed as

$$J_{14}(t) = -\frac{dp_1(t)}{dt} = -p_A(T)\frac{df_A}{dt}, \tag{A6}$$

$$J_{23}(t) = -\frac{dp_2(t)}{dt} = [p_A(T) - 1]\frac{df_B}{dt}, \qquad 0 \le t \le t_s, \tag{A7}$$

where t_s is the duration of the switching (which is extremely small compared to T) and $p_A(T)$ is the probability of a transporter being in state A immediately before the start of a pulse switch. Define W_{14}^{-+} as the total electric energy dissipated for the 1 to 4 reaction when the membrane potential is switched from $V = -\Delta$ at $t = 0$ to $V = +\Delta$ at $t = t_s$. Then,

$$W_{14}^{-+} = \frac{zF}{RT_t}\int_0^{t_s} V(t)J_{14}(t)\,dt = -\frac{zF}{RT_t}\int_{-\Delta}^{\Delta} V(t)p_A^-(T)\left(\frac{\partial f_A}{\partial V}\right)dV, \quad \text{(A8)}$$

where Eq. A6 has been used. Integrating Eq. A8 by parts and using the first equation in Eq. 6 we obtain

$$W_{14}^{-+} = p_A^-(T)\left[-z\varphi(f_A^+ + f_A^-) + 2z\varphi - \ln\left(\frac{K_{14} + e^{z\varphi}}{K_{14} + e^{-z\varphi}}\right)\right]. \quad \text{(A9)}$$

Expressions for W_{14}^{+-}, W_{23}^{-+}, and W_{23}^{+-} can be derived similarly. They are

$$W_{14}^{+-} = p_A^+(T)\left[z\varphi(f_A^+ + f_A^-) - 2z\varphi + \ln\left(\frac{K_{14} + e^{z\varphi}}{K_{14} + e^{-z\varphi}}\right)\right] \quad \text{(A10)}$$

$$W_{23}^{-+} = [p_A^-(T) - 1]\left[z\varphi(f_B^+ + f_B^-) - 2z\varphi + \ln\left(\frac{K_{23} + e^{z\varphi}}{K_{23} + e^{-z\varphi}}\right)\right] \quad \text{(A11)}$$

$$W_{23}^{+-} = [p_A^+(T) - 1]\left[-z\varphi(f_B^+ + f_B^-) + 2z\varphi - \ln\left(\frac{K_{23} + e^{z\varphi}}{K_{23} + e^{-z\varphi}}\right)\right] \quad \text{(A12)}$$

Thus, the mean rate of energy dissipation for the pulse switchings averaged over one period of time is equal to the sum of Eqs. A9–A12 divided by $2T$:

$$\overline{W_{\text{switch}}} = (2T)^{-1}(p_A^+(T) - p_A^-(T))\left\{ z\varphi(f_A^+ - f_B^+ + f_A^- - f_B^-) + \ln\left[\frac{(K_{14} + e^{z\varphi})(K_{23} + e^{-z\varphi})}{(K_{14} + e^{-z\varphi})(K_{23} + e^{z\varphi})}\right]\right\}. \quad \text{(A13)}$$

Sum of Eqs. A5 and A13 gives the total rate of electric energy dissipation in one cycle as

$$\overline{W} = (2T)^{-1}(p_A^+(T) - p_A^-(T))\ln\left[\frac{(K_{14} + e^{z\varphi})(K_{23} + e^{-z\varphi z})}{(K_{14} + e^{-z\varphi})(K_{23} + e^{z\varphi})}\right]. \quad \text{(A14)}$$

Thus, Eq. 33 is obtained when $p_A^+(T)$ and $p_A^-(T)$ in Eq. A14 are substituted with Eqs. 24 and 25.

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