Michaelis-Menten equation for an enzyme in an oscillating electric field

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ABSTRACT The electric charges on an enzyme may move concomitantly with a conformational change. Such an enzyme will absorb energy from an oscillating electric field. If in addition the enzyme has a larger association constant for substrate than for product, as is often true, it can use this energy to drive the catalyzed reaction away from equilibrium. Approximate analytical expressions are given for the field-driven flux, electrical power absorbed, free-energy produced per unit time, thermodynamic efficiency, and zero-flux concentrations. The field-driven flux is written as a generalized Michaelis-Menten equation.

INTRODUCTION

Recently, a simple paradigm for free-energy transduction by an enzyme has been introduced (1–4). This involves considering an external oscillating field as the energy source and investigating the rate and efficiency with which an enzyme can use this energy to drive a chemical reaction away from equilibrium. The effect will be larger for membrane enzymes because an externally applied electric field is greatly magnified in the membrane. Also, the membrane prevents the enzyme from rotating and thus escaping the effect of the field.

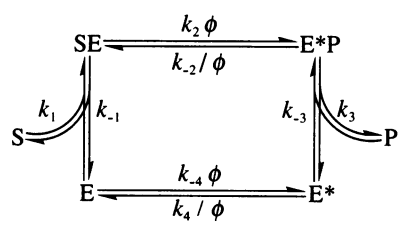
The paradigm developed in an effort to explain experiments with transport enzymes. Tsong and co-workers (5–11) have observed the effect of an oscillating electric field on the transport of Rb^+ (an analogue of K^+) and Na^+ by Na^+-K^+-ATPase in erythrocytes at 3°C. The net movement of ions was independent of ATP concentration but did depend on the frequency and amplitude of the field. The maximum effect on Rb^+ transport occurred at 1 kHz, and the maximum effect on Na^+ transport occurred at 1 MHz. References 6, 7, 10, and 11 compare the predictions of the paradigm with experiment.

In the present paper, we take an analytical approach to a catalyzed chemical reaction that is a generalization of active transport. Approximations suggested by numerical calculations (12) yield simple analytic formulas for the field-driven uphill flux, power absorbed and produced in the process, thermodynamic efficiency, and zero-flux concentrations. The equation derived for the flux is a generalization of the Michaelis-Menten equation in which it is clear that the oscillating field can drive the chemical or transport reaction away from equilibrium. Thus, the usefulness of the Michaelis-Menten equation in designing and understanding experiments is extended to membrane enzymes in an oscillating electric field.

FOUR-STATE MODEL

Consider an enzyme molecule that catalyzes the reaction S ⇄ P. It has two electrically distinct conformational states, E with the binding site for the substrate S exposed, and E* with the binding site for the product P exposed. For simplicity, we assume S and P are constant concentrations and that the reaction S ⇄ P is not electrogenic. The interaction with the electric field occurs only by electroconformational coupling, i.e., the E* forms have a different arrangement of charges than the E forms. Our analysis can easily be generalized to electrogenic reactions.

We assume that all interconversions between states may be treated as either unimolecular or pseudo-first-order transitions. Then, a simple cycle for the enzyme can be described by the four-state kinetic model



(1)

This is a reasonable model for many enzyme-catalyzed processes, including proline racemization (13) and membrane transport (14).

The effect of the electric field is given by ϕ. In zero field, $\phi = 1$. When an electric potential ψ is turned on, the energy of the state E*P is decreased relative to the state SE by $q\psi$, where q is the effective enzyme charge that moves with a conformational change, and ψ is the

potential difference through which the charge moves. Thus, the electric potential causes the equilibrium constant for the reaction SE $\rightleftarrows$ E*P to be multiplied by exp $(q\psi/RT)$. The equilibrium constant equals the ratio of the rate coefficients. We can apportion any fraction of the exponent $q\psi/RT$ to one rate coefficient (with the complementary fraction to the other) and get similar results regardless of the fraction. For simplicity, we apportion half of the exponent to each rate coefficient. Thus, the effect of the electric potential on the transition SE $\rightleftarrows$ E*P in Eqs. 1 is given by the factor

$$\phi = \exp(q\psi/2RT) \tag{2}$$

in the rate coefficients for that transition. Similarly, the energy of the state E* is decreased relative to the state E by $q\psi$, and the effect of the electric potential on this transition is given by the factor ϕ in the rate coefficients.

The rate equations for the four enzyme-state concentrations in Eqs. 1 are

$$dE/dt = (k_{-1})SE + (k_4/\phi)E^* - (k_{-4}\phi + k_1 S)E, \tag{3a}$$

$$dSE/dt = (k_1 S)E + (k_{-2}/\phi)E^*P - (k_2\phi + k_{-1})SE, \tag{3b}$$

$$dE^*P/dt = (k_2\phi)SE + (k_{-3}P)E^* - (k_{-2}/\phi + k_3)E^*P, \tag{3c}$$

$$dE^*/dt = (k_{-4}\phi)E + (k_3)E^*P - (k_4/\phi + k_{-3}P)E^*. \tag{3d}$$

Applying an oscillating electric field gives rise to the oscillating potential

$$\psi = \psi_1 \cos \omega t. \tag{4}$$

We do not add a constant potential because the resulting factor that would appear in ϕ in Eq. 2 can be absorbed in the rate coefficients in Eqs. 3. With Eqs. 2 and 4 inserted, the differential Eqs. 3 have periodic coefficients, and hence in the steady state after any transient has decayed, the four enzyme concentrations are also periodic.

Although this problem is much too complicated to solve analytically without approximation, we have solved it numerically (12). Graphs of the rate vs. frequency for eight sets of values of the rate coefficients are given in Figs. 1 and 2. In this paper we will obtain an approximate analytic solution valid on the highest plateau of Fig. 2. On this plateau the rate, power produced, and efficiency of energy transduction are constant and maximum as a function of frequency.

APPROXIMATIONS

Many enzymes, including transport enzymes, have a larger association constant for substrate than for product. A large association constant for substrate implies that the concentrations satisfy E $\ll$ SE, and a small association constant for product implies E*P $\ll$ E*. Furthermore, in order for the catalyzed reaction to go fast, the conformation exchanges, between SE and E*P and between E and E*, should be faster than the association/dissociation exchanges. These conditions follow (5) from the inequalities

$$k_{-4}\phi \gg k_4/\phi, \quad k_{-1} \quad k_1 S, \tag{5a, b, c}$$

$$k_{-2}/\phi \gg k_2\phi, \quad k_{-3}P, \quad k_3. \tag{5d, e, f}$$

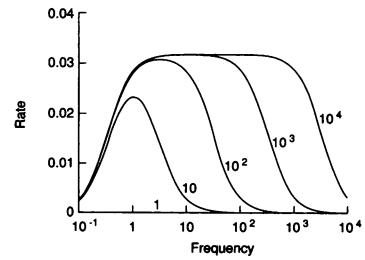


FIGURE 1 Rate of catalyzed reaction vs. frequency (in relative units) for five values of substrate-association and product-dissociation constants. The label for each curve is the value of the following equilibrium constants for the catalytic cycle in Eq. 1: substrate-association constant K_1, product-dissociation constant K_3, and conformation-change equilibrium constants K_2^{-1} and K_4^{-1}, which all equal each other. The substrate dissociation rate coefficient k_{-1}, the product association rate coefficient k_{-3}, and the conformation-change rate coefficients k_2 and k_4 all equal 1. The substrate and product concentrations S and P both equal 1, and the electric interaction energy $q\psi_1/RT$ is 1. This graph shows that increasing the substrate-association and product-dissociation constants, with the conformation-change coefficients k_2 and k_4 unchanged, causes the peak rate of reaction to increase until the rate develops a plateau, which broadens with further increases. The rate is identically zero when the equilibrium constants all equal one.

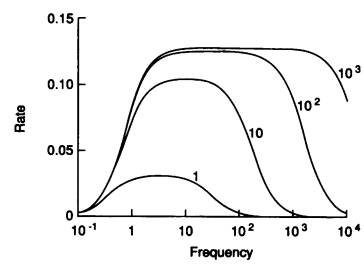


FIGURE 2 Rate of catalyzed reaction vs. frequency (in relative units) for four values of conformation-change rate. The label for each curve is the value of the conformation-change rate coefficients k_2 and k_4. The substrate dissociation rate coefficient k_{-1} and the product association rate coefficient k_{-3} both equal 1. The equilibrium constants are the same as for the 10^2 curve of Fig. 1, and so the curve labeled 1 in this figure is the same as the 10^2 curve of Fig. 1. This graph shows that increasing the conformation-change coefficients, with the equilibrium constants unchanged, causes the peak rate of reaction to increase until the rate develops a plateau, which broadens with further increases. The calculations of rate and efficiency in this paper are valid on the highest plateau of this graph.

The inequalities 5a and 5b make E small, and the inequalities 5d and 5e make E*P small, and so the derivatives of these quantities in Eqs. 3a and 3c are negligible. This is the steady-state approximation. We can use these equations to eliminate E and E*P in the rate Eq. 3d for E* and use the total enzyme concentration, $E_T = SE + E^*$, to eliminate SE. Then E* is the only unknown, and it satisfies

$$dE^*/dt = \sigma - \tau^{-1}E^*, \tag{6}$$

where, after simplification using the inequalities 5c and 5f,

$$\sigma = (k_{-1} + K_2k_3\phi^2)\,E_T, \tag{7a}$$

$$\tau^{-1} = k_{-1} + K_2k_3\phi^2 + k_{-3}P + k_1K_4S\phi^{-2}. \tag{7b}$$

Here K_2 is the equilibrium constant k_2/k_{-2}, etc.

The reduction from four unknowns to one considerably simplifies the problem. However, τ^{-1} and σ involve ϕ, which is a periodic function of time. So, although Eq. 6 can be solved formally, the solution still cannot be written in a useful form without approximation.

An approximate solution to Eq. 6 for E* is obtained as follows. At intermediate frequencies, on the highest plateau of Fig. 2, numerical calculations show that E* is very nearly independent of time. To compute the value of this constant, average Eq. 6 over one cycle and solve for E* to get

$$E^* = \bar{\sigma}/\overline{\tau^{-1}},$$

where $\bar{\sigma}$ and $\overline{\tau^{-1}}$ are averages of Eqs. 7 over one cycle. This corresponds to the exact infinite-frequency solution obtained previously (4). The average of ϕ^2 and ϕ^{-2} in these expressions equals the function I_0 $(q\psi_1/RT)$. Let $z = q\psi_1/RT$. The function I_0 (z) equals 1 when $z \ll 1$ and approaches $e^z/(2\pi z)^{1/2}$ when $z \gg 1$. More precise values for this function can easily be computed or looked up in a table (15).

RATE OF REACTION

The instantaneous net rate of association of substrate is

$$V_1 = k_1K_4S\phi^{-2}E^* - k_{-1}(E_T - E^*), \tag{9}$$

where a term has been neglected by using the inequality 5a. The time average of this equals the average net rate of each of the other three transitions. These equal the average rate of clockwise cycling of the enzyme and the average rate $\overline{V}$ of the catalyzed reaction. To compute $\overline{V}$ observe that at intermediate frequencies, on the plateau, E* is constant. Insert Eq. 8 into Eq. 9 and average over a cycle to get the generalized Michaelis-Menten equation

$$\overline{V} = A\,E_T(KSI_0^2 - P)/(1 + P/K_M^- + S/K_M^+), \tag{10}$$

where $K = K_1K_2K_3K_4$ is the equilibrium constant of the reaction $S \rightleftarrows P$,

$$K_M^+ = (k_{cat}^+ + k_{cat}^-)/k_1K_4I_0, \tag{11a}$$

$$K_M^- = (k_{cat}^+ + k_{cat}^-)/k_{-3}, \tag{11b}$$

are the Michaelis constants for substrate and product, respectively,

$$k_{cat}^+ = K_2k_3I_0, \tag{12a}$$

$$k_{cat}^- = k_{-1}, \tag{12b}$$

are the maximum catalytic rate coefficients in the forward and reverse directions, respectively, and

$$A = k_{cat}^+/K_M^+KI_0^2 = k_{cat}^-/K_M^-, \tag{13a, b}$$

is a coefficient. These equations have a form that is familiar from steady-state enzyme kinetics (16–18). Eq. 10 reduces to the usual Michaelis-Menten equation for $S \rightarrow P$ when $P = 0$ and Eq. 13a is used. It reduces to the Michaelis-Menten equation for $P \rightarrow S$ when $S = 0$ and Eq. 13b is used. The effect of the oscillating electric field is given by the function I_0, which appears in the numerator of Eq. 10, in the Michaelis constants, in k_{cat}^+, and in the coefficient A. Eq. 13b is a generalization of the Haldane equation (16, 19), to which it reduces in zero field ($I_0 = 1$). When k_{cat}^+ is determined in the usual way both in zero field and in nonzero field, the ratio of these two values gives a value for I_0, which in turn can be used to determine $q\psi_1/RT$ and, thus, q.

The oscillating field has several interesting effects. It shifts the zero-reaction-rate condition in Eq. 10 to $P = KSI_0^2$. Also, even if $k_{cat}^+ = k_{cat}^-$ in zero field, we have $k_{cat}^+ \gg k_{cat}^-$ when the field is very large. A very large field makes the enzyme kinetically irreversible even if it is not irreversible in zero field. Furthermore, as the field increases, the Michaelis constant for substrate decreases, and the Michaelis constant for product increases. These effects on the Michaelis-Menten parameters of the enzyme are manifested in an ac-field-induced dramatic increase of the downhill rate when ΔG is slightly negative. This may be important in understanding the effect of very weak electric fields on biological systems (20).

However, the most striking conclusion from Eq. 10 is that, in the interval $1 < P/KS < I_0^2$, the oscillating field makes $\overline{V} > 0$, which means that it drives the reaction $S \rightleftarrows P$ away from equilibrium. This is shown in Fig. 3, in which the reaction rate is plotted vs. $\Delta G = \ln(P/KS)$. A qualitative explanation of how the oscillating field drives the reaction from equilibrium is given in Fig. 4. This

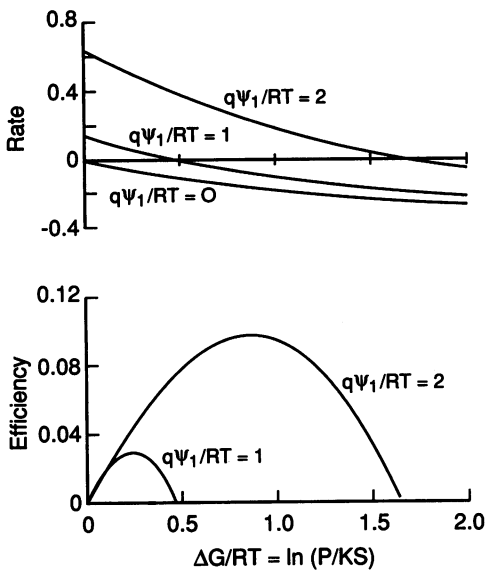


FIGURE 3 Reaction rate (*upper graph*) and efficiency of energy transduction (*lower graph*) vs. $\Delta G/RT = \ln(P/KS)$ for three values of electric interaction energy $q\psi_1/RT$, where K is the equilibrium constant between substrate S and product P. The parameters used to draw these curves are: Zero-field maximum-catalytic-rate coefficients k_{cat}^+ and k_{cat}^- both equal to one in relative units, and Michaelis constants for substrate K_M^+ and product K_M^- both equal to two in relative units. These values correspond to those for the highest plateau of Fig. 2. The upper graph shows that when $q\psi_1/RT$ is nonzero there is an interval of ΔG over which the rate is positive even though ΔG is positive. This says that the oscillating electric field causes the enzyme to drive the reaction away from equilibrium in that interval. The interval, found to be $1 \ll P/KS \ll I_0^2$ in the text, increases with increasing $q\psi_1/RT$. The lower graph shows that the efficiency of energy transduciton from the oscillating field is positive in the same interval.

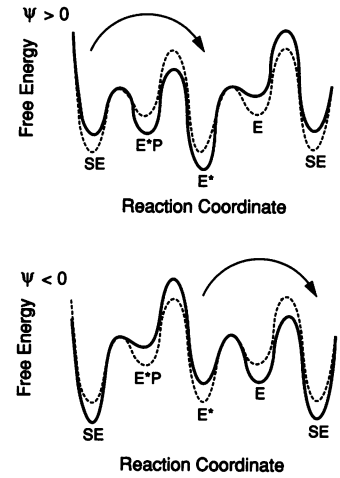


FIGURE 4 Free energy of the enzyme states of Eq. 1 vs. reaction coordinate for zero, positive, and negative electric potential ψ. These curves show qualitatively how an oscillating electric potential can cause an enzyme to catalyze a nonelectrogenic reaction away from equilibrium. The dotted curves in both graphs are for zero potential. For simplicity we have chosen $K_1 = K_2^{-1} = K_3 = K_4^{-1} \gg 1$ and $S = P = 1$ as in Figs. 1 and 2. Thus at zero potential the energy levels of states SE and E* are equal and lower than those of states E and E*P. So at equilibrium the concentrations of states SE and E* are equal and much larger than those of states E and E*P. When the potential is positive, the energies shift from the dotted to the solid curve in the upper graph. The changes in state energies and activation-barrier heights follow from Eqs. 1 and 2. Because E* is more stable than SE, the system must relax toward a new equilibrium, and so SE must convert to E*. Because the activation barrier for the transition via E*P is now lower than for the transition via E, the relaxation goes mostly through E*P, thus releasing more P than S. When the potential is negative, the energies shift from the dotted (*zero potential*) curve to the solid curve in the lower graph, where SE is more stable than E*, and so E* must convert to SE. Because the activation barrier for the transition via E this time is lower than for the transition via E*P, the relaxation goes mostly through E, binding more S than P. This process repeats with a net conversion of S to P.

explanation applies at all frequencies, even on the plateau where the concentrations E* and SE are very nearly constant since there the fluxes still cycle as described in Fig. 4.

These effects do not occur in a constant electric field. A derivation similar to that of Eqs. 10–13 can be made for a constant potential ψ. The result is the same except that I_0^2 in the numerator of Eq. 10 is replaced by $\phi^2\phi^{-2} = 1$, the I_0 in k_{cat}^+ is replaced by ϕ^2, and the I_0 in K_M^+ by ϕ^{-2}. We see that, in a constant electric field, the zero-flux condition, $P = KS$, does not depend on $q\psi/RT$. As expected, the reaction always proceeds toward equilibrium. This is in contrast to the prediction of Eq. 10 for an oscillating field.

EFFICIENCY OF ENERGY TRANSDUCTION

The power exerted by the enzyme when it cycles is

$$P_{out} = \overline{V}\Delta G, \qquad (14)$$

where $\overline{V}$ is given by Eq. 10, and ΔG is the free-energy change associated with the reaction $S \rightleftarrows P$. The power supplied by an oscillating electric field is the time average of the oscillating potential $\psi_1 \cos(\omega t)$ times the electric current $q\, d(E^*P + E^*)/dt \simeq q\, dE^*/dt$. This average is easily computed by multiplying Eq. 6 by $q\psi_1 \cos(\omega t)$, using Eq. 8, and averaging over one cycle to get

$$P_{in} = k_{cat}^+ E_T q\psi_1 \frac{I_1}{I_0} \times \frac{P/K_M^- + (2 + K_M^-/K_M^+ KI_0^2)S/K_M^+}{1 + P/K_M^- + S/K_M^+}. \qquad (15)$$

Here the function $I_1(q_{\psi_1}/RT)$ is the average of $\phi^2 \cos(\omega t)$ or $-\phi^{-2} \cos(\omega t)$ over a cycle. $I_1(z)$ equals z when $z \ll 1$ and increases to $I_0(z)$ when $z \gg 1$. More precise values for this function can also easily be computed or looked up in a table (15).

The efficiency of energy transduction from the ac field is the ratio P_{out}/P_{in}. Because P_{in} is always positive, the efficiency is positive when P_{out} is positive, which, because $\Delta G = RT \ln (P/KS)$, occurs in the interval $1 < P/KS < I_0^2$. This is the same interval over which an oscillating electric field drives the reaction $S \rightleftarrows P$ away from equilibrium, where without the oscillating field the reaction would of course proceed toward equilibrium. This is shown in Fig. 3, which compares graphs of rate and efficiency vs. ΔG.

Numerical calculations show that the frequency range over which these results are valid is approximately

$$\overline{\tau^{-1}} \ll \omega \ll (k_{-4} + k_{-2})/(2I_0), \qquad (16a, b)$$

where

$$\overline{\tau^{-1}} = (k_{cat}^- + k_{cat}^+)(1 + P/K_M^- + S/K_M^+) \qquad (17)$$

is the average of Eq. 7b over one cycle. Previously (4) we found that $\overline{\tau^{-1}}/2\pi$ is the characteristic frequency of the dispersion predicted by Eq. 6 for weak fields. This frequency is the half-amplitude point just below the plateau. It is also approximately the lowest nonzero eigenvalue of Eqs. 3. The expression on the right of the inequality 16b in a weak field very nearly equals the two largest eigenvalues for Eqs. 3. This is true because the sum of the four eigenvalues of Eqs. 3 equals the trace of the matrix of Eqs. 3, and because one eigenvalue is zero and the remaining one is very small. The right side of the inequality 16b has been simplified using the inequalities 5. We have guessed the dependence on field strength in the expressions for the frequency range and very roughly confirmed the range numerically.

The ranges of concentrations S and P and of interaction energy $q\psi_1$ over which the results are valid are also limited by the inequalities 5. Provided the inequalities 5 and 16 are satisfied, our approximate analytical formulas for rate and efficiency agree within <1% with numerical calculations of these quantities for the original four-state problem.

SUMMARY

If an enzyme has electric charges that move when it changes conformation, it will absorb energy from an oscillating electric field. When the enzyme also has a large association constant for substrate and a small association constant for product, it can use this energy to drive a chemical or transport reaction away from equilibrium. This difference in binding energy for substrate and product is the interaction energy discussed by Jencks (21). The more asymmetric the enzyme, the larger the rate of reaction and the broader the frequency range for the large rate, as shown in Fig. 1. Also, the larger the conformation–change rate coefficients of the enzyme, the larger the reaction rate and frequency range, as shown in Fig. 2.

We have obtained analytic expressions for the reaction rate and the efficiency of energy transduction from the oscillating–electric field. These expressions are valid on the highest plateau of Fig. 2.

The rate is described by a Michaelis-Menten equation that has been generalized to include the effect of the oscillating–electric field. The zero-reaction-rate condition on the substrate and product concentrations is shifted from thermodynamic equilibrium by an amount that increases with increasing strength of the oscillating–electric field. This shift and the corresponding increase of the interval over which the oscillating field drives the reaction away from equilibrium is shown in Fig. 3. A qualitative explanation of the mechanism is given in Fig. 4.

The effect is synergistic. Without the enzyme, the reaction proceeds toward equilibrium, although perhaps very slowly. Adding an oscillating–electric field does nothing, provided the reaction is nonelectrogenic. Adding the enzyme without the field causes the reaction to proceed more rapidly towards equilibrium. Adding both the enzyme and an oscillating field of suitable frequency and amplitude causes the reaction, in the interval of Fig. 3, to proceed away from equilibrium.

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