VOLUME 115, NUMBER 24
DECEMBER 1, 1993

JOURNAL
OF THE
AMERICAN
CHEMICAL
SOCIETY

Imposed Oscillations of Kinetic Barriers Can Cause an Enzyme To Drive a Chemical Reaction Away from Equilibrium

R. Dean Astumian*,† and Baldwin Robertson‡

Contribution from the Departments of Surgery and of Biochemistry and Molecular Biology, University of Chicago, 5841 South Maryland Avenue, MC 6035, Chicago, Illinois 60637, and Biotechnology Division, National Institute of Standards and Technology, Gaithersburg, Maryland 20899

Received May 17, 1993®

Abstract: The overall Gibbs free energy change (ΔG) of a chemical reaction is often termed the driving force of the reaction. The sign of ΔG defines the direction of spontaneous reaction, and the condition $\Delta G = 0$ defines the point of chemical equilibrium. This is strictly true for elementary reactions—reactions that pass through only one local maximum (the transition state) along the reaction coordinate connecting reactant and product states. However, under many circumstances it is also true for reactions that involve one or more intermediates, particularly if the steady state intermediate concentrations are very small. Here we show that externally imposed oscillations or fluctuations can drive a net chemical reaction away from equilibrium so long as the rate constants of at least one elementary step of the overall reaction depend on the fluctuating parameter. This is true even if the overall ΔG is independent of the perturbation and it is also true even if the concentrations of the intermediate states are very, very small (i.e., experimentally undetectable). The key to understanding this result is to realize that the imposed oscillation does work on the intermediate states of the reaction. Even if the concentrations of the intermediates are very small, this work can accumulate over many cycles of oscillation, leading to a significant shift of the net reaction away from equilibrium. Our results demonstrate that the addition of an enzyme (or any catalyst) to a chemical reaction initially at equilibrium (but exposed to an oscillating field) may cause the reaction to proceed away from equilibrium. This provides an explicit counter example to the adage that the addition of a small amount of catalyst to a chemical reaction at equilibrium cannot cause the reaction to go away from equilibrium.

Introduction

This paper deals with the fundamental thermodynamics and kinetics of chemical reactions in the presence of externally imposed oscillations or fluctuations of a thermodynamic parameter. The theory applies to fluctuation of any thermodynamic parameter, e.g. pressure, acting on any chemical reaction where the thermodynamically conjugate parameter, volume in the case of pressure, changes along the reaction coordinate. However, we expect the principles discussed to have the greatest practical relevance for reactions occurring at membranes and interfaces where large fluctuations of the surface and transmembrane electrical potentials commonly occur.

To illustrate the major point of this paper, consider the transport of an uncharged substance such as glucose across a membrane. The equilibrium ratio of glucose on the two sides of the membrane is unity, irrespective of the electric potential difference across the membrane. Thus, we might imagine that electric energy cannot be used to form a concentration gradient of glucose across the membrane. We show here that if the mechanism of transport involves transient separation of charge, oscillation of the membrane potential *can* drive the reaction uphill to form a gradient. This is equally true if the imposed membrane potential is changed

† University of Chicago.
‡ National Institute of Standards and Technology.
® Abstract published in *Advance ACS Abstracts*, November 1, 1993.

0002-7863/93/1515-11063$04.00/0

randomly. The final steady state gradient, which persists as long as the membrane potential fluctuations are maintained, is proportional to the square of the product of the amplitude of the membrane potential flutuation and the maximum displacement of charge across the membrane along the reaction coordinate. This can be very significant even if the concentrations of all of the intermediates are extremely small compared to the concentrations of reactants and products.

Our results show that the ability of a chemical mechanism to absorb energy from an external energy source depends not only on the molecular properties of the initial and final states but also on the properties of all intermediate states, even those with very small concentrations. Thus, if the above glucose transport mechanism involves intermediates with large dipole moments, the formation of a concentration gradient of glucose across the membrane can be driven by imposed electric fluctuations even though the net reaction does not involve any net charge translocation or separation. This provides one mechanism by which evolution can work to allow enzymes to act as free energy transducers. An enzyme changes the mechanism of any reaction that it catalyzes. If variation of an external parameter can do work on any of the enzyme bound intermediate states, then the overall reaction can be driven away from equilibrium irrespective of whether the reaction is *a priori* coupled to the fluctuating parameter. Thus evolution of a protein to allow for large transient charge displacement in a conformational step provides a way to couple an arbitrary chemical reaction to a source of electrical fluctuation.

Model

The ΔG of a reaction is given by[1]

$$\Delta G = -(\mu_S - \mu_P) = -RT \ln\left(K\frac{[S]}{[P]}\right) = \Delta G^0 - RT \ln\frac{[S]}{[P]} \tag{1}$$

where R is the universal gas constant, T is absolute temperature, K is the equilibrium constant of the reaction, [S] and [P] are substrate and product concentrations and μ_S and μ_P are their chemical potentials, respectively, and ΔG^0 is the standard free energy change for the reaction. The rate times the ΔG for any elementary reaction step in a system of chemical reactions is negative. In a static environment this principle also holds for net reactions that have a well-defined rate.[2] An example of such a reaction is the Michaelis–Menten mechanism for enzyme catalysis

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P \tag{2}$$

so long as the enzyme–substrate complex is a steady-state intermediate with very small concentration relative to free substrate and/or product. The enzyme does not change the equilibrium constant between substrate and product. Thus it would seem that the addition of an enzyme to a reaction at equilibrium cannot cause the reaction to go away from equilibrium. This is not necessarily true in the presence of external oscillations or fluctuations. We show that in the presence of an external oscillation the average rate of such a net reaction can have an opposite sign to the overall affinity even though the product of the rate and ΔG for each elementary step is negative at every instant. Consider for example that $k_1 = k_1^\circ \exp(z)$ and $k_2 = k_2^\circ \exp(-z)$ and that the reverse rate constants do not depend on z, where z is some normalized thermodynamic parameter (e.g., membrane potential multiplied by a displacement charge of activation, divided by RT). The equilibrium constant $K = k_1k_2/(k_{-1}k_{-2})$, and hence the overall ΔG in eq 1 is independent of z. The equilibrium constant is independent of the membrane potential for transport of any uncharged substance across the membrane and, in general, for any chemical reaction where both the reactants and products appear on one side of the membrane only. Nevertheless, as we shall see for $z = z_1 \cos(\omega t)$, the reaction of substrate to product can be driven at large ω in a direction opposite to that predicted by the sign of ΔG. It follows that interactions between an enzyme and its environment can change not only the velocity but also the direction of a reaction, and that interactions between two enzymes can provide a mechanism for energetically coupling two otherwise unrelated chemical reactions. Our results may be particularly important for understanding free energy transduction by enzymes localized in membranes, where large oscillations and fluctuations of the membrane potential are common.

Quantitative Description. In order to calculate the effect of an oscillating field on a chemical reaction, we must take into account the effect of the field on the rate constants and then solve the differential equations for the kinetics with oscillating coefficients. The membrane potential dependence of the rate constants is given by

$$k_i = k_i^0 \exp\left[\frac{z_i\psi(t)}{RT}\right], \quad i = \pm 1, \pm 2 \tag{3}$$

where the subscripts ± 1 and ± 2 refer to the forward and backward reactions 1 and 2 of eq 2, respectively. If ψ is pressure, z_i are volumes of activation; if ψ is an electric field, z_i are dipole moments of activation; if ψ is an electric potential, z_i are displacement charges of activation; etc. A kinetic description of the system is still valid in an oscillating field so long as the frequency of oscillation is small enough that a local equilibrium approximation remains valid (i.e., an adiabatic approximation).[3] This is the case here, since the frequencies of interest, while possibly much greater than the catalytic constant of a typical enzyme (around 10^3/s to 10^5/s), are still much less than a rate constant for a diffusion controlled reaction (around 10^9/s).

In this paper we are interested in those cases where the free energies of substrate and product, and hence also the ΔG of the overall reaction, are independent of the perturbation, but the free energies of some of the intermediate and transition states do depend on the perturbation. This is the case so long as the relation $z_{+1} + z_{+2} - z_{-1} - z_{-2} = 0$ holds. A specific example is given in Figure 1. As seen, in an oscillating field the rate constants will oscillate.

The amplitude of oscillation of the energy levels is obtained as follows. Let E + S be the reference state. The energy of the first transition state is changed by ψz_{+1}. The energy of ES is changed by $\psi(z_{+1} - z_{-1})$, etc. These changes are indicated for an oscillating potential by the arrows in Figure 1.

With constant total enzyme concentration $E_T = [E] + [ES]$, the differential equations for the kinetics of eq 1 are

$$\frac{d[ES]}{dt} + \tau^{-1}[ES] = (k_1[S] + k_{-2}[P])E_T \tag{4}$$

and

$$\frac{d[P]}{dt} = (k_2 + k_{-2}[P])[ES] - k_{-2}E_T[P] \tag{5}$$

where [S] + [P] + [ES] is constant and

$$\tau^{-1} = k_1[S] + k_{-2}[P] + k_{-1} + k_2 \tag{6}$$

is the inverse relaxation time. The effect of an applied field is obtained by inserting the expressions for the rate constants from eq 3 into the above equations. Equations 4 and 5 can be numerically integrated forward in time for an oscillating field,

(1) DeDonder, T.; van Rysselberghe, P. *The Thermodynamic Theory of Affinity*; Stanford University Press: Stanford, CA, 1936.
(2) Keizer, J. *J. Theor. Biol.* **1975**, *49*, 323–335.
(3) Bier, M.; Astumian, R. D. *Phys. Rev. Lett.* **1993**, *71*, 1649–1652.

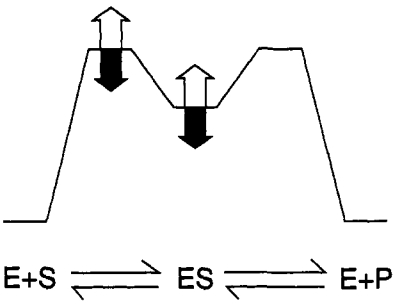

Figure 1. Schematic kinetic barrier diagram[8] illustrating the relative free energies of the states and transition states for the reaction scheme in eq 2. Since charges move in the transitions, a potential ψ causes an interaction energy Δz_i that subtracts from the free energy change ΔG of each transition, where $\Delta z_i = z_{+i} - z_{-i}$ is the displacement charge for the ith transition. The arrows illustrate the effect of oscillating on the energy levels, wtih $z_{+1} = -1$, $z_{+2} = 1$, and $z_{-1} = z_{-2} = 0$ elementary charges. When ψ is positive the energy of ES is decreased relative to E and association of both S and P is favored. Since the activation barrier for association of S is lower during this phase, S initially binds faster. When ψ is negative the energy of ES is increased, and dissociation of both S and P is favored. Since the activation barrier for dissociation of P is lower during this phase, P dissociates faster. Thus the net effect of a cycle is to increase the chemical potential of P at the expense of S. At high frequency, the net left-to-right flux (averaged over a cycle) continues until $[P]/K[S] = 1 + (\psi_1/2RT)^2$.

and typical results are shown in Figure 2a,b. On the time scale of Figure 2b the reaction appears to have a well-defined rate. On a shorter time scale however (Figure 2a) it is seen that the concentrations and rates oscillate, but with very small amplitudes. It is important to note that the instantaneous rates of disappearance of S and appearance of P are different. However, experimentally, one does not measure rate *per se*. Instead, a concentration is measured at two different times, and the concentration difference is divided by the time difference. Even experimental setups in which concentration is measured continuously have finite temporal resolution. In the presence of external oscillations, as $\omega \to \infty$, $\Delta[P]/\Delta t \to -\Delta[S]/\Delta t$ even if $\Delta t \to 0$. What this means in practical terms is that for a perturbation frequency of 10^7 Hz, it would be necessary to make measurements with a time resolution <0.5 μs in order to ascertain that the rate $d[P]/dt$ is not instantaneously identical with $-d[S]/dt$.

The time constant for reaching the steady state for [ES] is τ, while the time constant for reaching the new steady-state ratio [S]/[P] is $[\tau^{-1}/k_{-1}k_{-2}E_T]$. Thus, the ratio of [S] to [P] varies slowly compared to [ES] for the rate constants used in Figure 2, and we can solve eq 4 under steady oscillating conditions using a method described elsewhere.[4] The average rate of conversion of S to P is then given by inserting the value of [ES] into eq 5 and averaging over a cycle of the field. A plot of average rate vs frequency is shown in Figure 3. The average rate depends on the frequency $\omega/2\pi$ and changes sign in going from low to high frequency. In the low-frequency limit, the direction of the reaction is the same as predicted from the sign of the affinity (i.e., from right to left). For frequencies much greater than $1/\tau$ however, the reaction proceeds from left to right even though the affinity $(-\Delta G)$ is negative. Hence, the product of catalytic rate and overall affinity is negative at high frequency. Even so, the product of rate and affinity for each elementary reaction step is positive at every instant, in agreement with ref 2.

Low- and High-Frequency Limits. We can obtain analytic expressions for the average rate in the low- and high-frequency limits. At low frequency, the flux is an average over steady-state fluxes and is

(4) Robertson, B.; Astumian, R. D. *Biophys. J.* **1990**, *57*, 689–696.

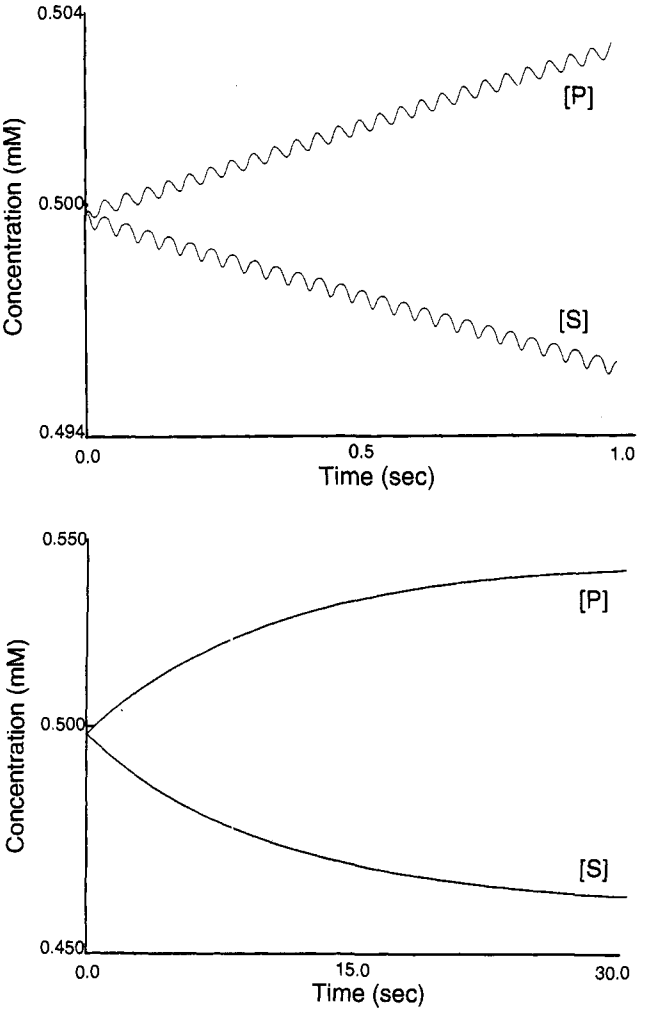

Figure 2. (a, top) Plot of the change in [S] and [P] in a closed system exposed to an oscillating field $\psi = \psi_1 \cos(\omega t)$, where $\omega = 2\pi f$ is the angular frequency. The results were calculated by numerical integration of eqs 4 and 5. The parameters used in the calculation are $f = 25$ Hz, $\psi_1 = 25$ mV, and $k_1 = k_{-2} = 10^4$ L/(mol s), $k_{-1} = k_2 = 10^2$/s, and $z_{+1} = -1$, $z_{+2} = 1$, and $z_{-1} = z_{-2} = 0$ elementary charges. The starting concentrations were [S] = [P] = 0.5 mM and $E_T = 10$ μM. Here ES is a steady state intermediate with a concentration <1% of [S] or [P]. This graph shows that an oscillating field causes product to accumulate on average. The oscillations in S and P can be seen clearly on this time scale. (b, bottom) Same as plot a but on a longer time scale (30 s). This graph shows that the accumulation continues until the ratio of product to substrate is just large enough to overcome the intrinsic differences in rates of association and dissociation during the positive and negative phases of the perturbation, respectively. The limiting ratio here is not as large as predicted from eq 10 because 25 Hz is not much larger than $\tau^{-1}/(2\pi)$. The time scale for the relaxation of S and P to their new steady-state values is given by the coefficient of [P] in eq 9, which for our case is about 10 s. On this time scale, the charge in S and P appears smooth and the reaction has an apparently-well-defined rate.

$$\left\{\frac{d[P]}{dt}\right\}\Big|_{hf} = \frac{\overline{k_1k_2[S] - k_{-1}k_{-2}[P]}}{\overline{\tau^{-1}}}E_T \qquad (7)$$

At high frequency, [ES] is approximately independent of time. To compute the value of this constant, average eq 4 over a cycle of the field, set $d[ES]/dt = 0$, and solve for $[\overline{ES}]$. The result is inserted into eq 5 which is averaged over a cycle of the field to obtain the average rate at high frequency

$$\left\{\frac{d[P]}{dt}\right\}\Big|_{hf} = \frac{\overline{k_1k_2}[S] - \overline{k_{-1}k_{-2}}[P]}{\overline{\tau^{-1}}}E_T \qquad (8)$$

Displacement from Equilibrium. For the specific case used in the figures (i.e., with $z_{+1} = -1$, $z_{+2} = 1$, and $z_{-1} = z_{-2} = 0$) the

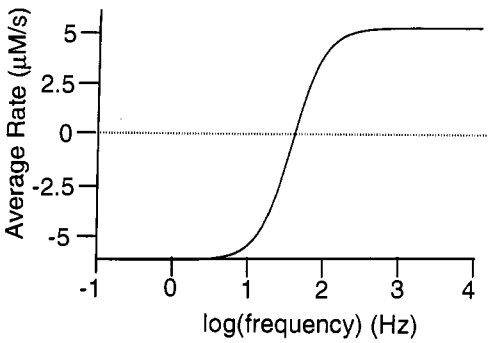

Figure 3. Plot of the average rate versus frequency, which we calculated for stationary oscillation using a previously published method.[4] The parameters are the same as in Figure 2, with [S] = 0.44 mM and [P] = 0.56 mM, so the affinity is $0.24RT$. This plot shows that the sign as well as magnitude of the rate of reaction eq 1 depends on the frequency of an applied field even though the affinity is independent of the field. At low frequency the rate is negative, as predicted by the sign of the affinity. At high frequency, the rate is positive even though the overall affinity is negative. The enzyme works as a "switch" that can be turned on or off by varying the frequency. The midpoint of the transition is at $1/(2\pi\tau) \approx 30$ Hz.

numerator in eq 7 is independent of ψ, so at low frequency the sign of the flux is the same as the sign of the affinity. At high frequency however, the flux is

$$\left\{\frac{d[P]}{dt}\right\}\Big|_{hf} = \frac{k_{-1}^0 k_{-2}^0 [P] E_T}{\overline{\tau^{-1}}}\left\{K\frac{[S]}{[P]}\left[\overline{\exp\left(\frac{eF\psi}{RT}\right)}\right]^2 - 1\right\} \quad (9)$$

where e is the electronic charge and F is the Faraday constant. Interestingly, eq 9 is of the same form as the equation for the average current through a diode rectifier subject to an ac potential. A major difference is that here, the rectified current is the flux of an uncharged substance, or equivalently, a nonelectrogenic chemical conversion between substrate and product. Another important difference is that the present situation applies at frequencies that are large compared to $1/\tau$, the characteristic time constant of the enzyme. Classic rectification in a chemical system, where the affinity is caused to oscillate and the flux responds asymmetrically, is a low-frequency phenomenon.[5] The general behavior described here may provide a mechanism by which enzymes can average, or rectify, an oscillating signal, and may therefore be important in understanding the response of biological systems to weak ac electric fields.[6]

To find the displacement from equilibrium caused by the oscillating potential for the parameters used in the figures, set $d[P]/dt = 0$ in eq 9 and solve to find

$$\frac{[P]}{[S]} = K\left[\overline{\exp\left(\frac{eF\psi}{RT}\right)}\right]^2 = K\left[1 + \frac{1}{2}\left(\frac{eF\psi_1}{RT}\right)^2\right] \quad (10)$$

where the approximation holds for $\psi = \psi_1 \cos(\omega t)$ with small ψ_1. This equation shows that [P]/[S] is shifted from its equilibrium value by approximately $K(e\psi_1/RT)^2/2$. Equation 10 is valid irrespective of the relative magnitudes of any of the rate constants. If $k_1[S] + k_{-2}[P]/k_{-2}[P]/k_{-1} + k_2 \ll 1$, the concentration of ES will be very very small, and in the limit that the ratio goes to zero, [ES] also approaches zero. Nevertheless, the final steady-state ratio of [P]/[S] does not change. What does change in this limit is the rate at which this final steady-state ratio is approached, as can be seen from eq 9.

These points are important because Keizer[2] has suggested that reactions where the concentrations of all intermediates are very small (he uses the term negligible, but this seems to beg the question) should be termed "elementary-complex" reactions and that the product of the rate and affinity of such net reactions must be positive. In the presence of external oscillation or fluctuations this is not true. We have shown that it is possible to have a chemical reaction in which all of the intermediates have concentrations that are so small that they could not be detected by any known experimental technique, and for which the rates $\Delta[P]/\Delta t$ and $-\Delta[S]/\Delta t$ are identical on any experimentally accessible time scale, in other words, a chemical reaction that by any experimental criterion appears to involve no intermediate and to have an instantaneously well-defined rate (i.e., it appears to be an elementary reaction step) and nevertheless to have the product of rate times affinity be negative.

Power and Dissipation. The power for driving the chemical reaction against its affinity comes from the oscillating perturbation. If, e.g., the perturbation is an ac electric field, the interconversion between the various chemical species gives rise to an electric displacement current, and the input power is the time average of the product of current and voltage, $P_{in} = \overline{\psi d[ES]/dt}$. As usual, the output chemical power is the affinity times the average rate, $P_{out} = -\Delta G\, \overline{d[P]/dt}$. In all cases, output power is less than input power. Thus, $P_{in} - P_{out}$, which is the dissipation, is positive as required by thermodynamics. For very large perturbations the efficiency (P_{out}/P_{in}) can be near 100%.[7] An interesting point to note is that P_{in} becomes very small as [ES] becomes small. Nevertheless, the ratio [S]/[P] that causes all the average rates to be zero does not approach the equilibrium distribution as P_{in} goes to zero. This has to do with the natural separation of time scales in any chemical reaction that involves more than one elementary step. In Figure 1, for example, if the unperturbed energy profile *and* the effect of the field would be symmetric, the time scale for absorbing a small quantity of energy from the external perturbation would be identical with the time scale for dissipating that energy, and the reaction could not be driven by a fluctuating field. However, any asymmetry separates the time scales such that energy is absorbed somewhat faster than it is dissipated. This allows for the effect of the fluctuating field to accumulate over a long time and thus to have a net effect on the overall reaction.

Qualitative Description. The effect of the oscillating perturbation on the intermediate and transition states can be schematically shown in terms of a kinetic barrier diagram[8] as seen in Figure 1, where the filled black arrows indicate the effect of an increase in potential ($+\psi$) and the open arrows indicate the effect of a decrease in potential ($-\psi$). If μ_S and μ_P are equal we might expect from thermodynamics that no net change will occur in [S] or [P] since their molar free energies do not depend on the perturbation. Nevertheless, the oscillating perturbation can drive the reaction, as can be understood in the following.

In the positive phase of the perturbation, denoted by the filled black arrows in Figure 1, the free energy of ES decreases relative to that of the other states, with the effect that binding of both S and P becomes favorable. Since the transition state energy between E + S and ES decreases, while that between E + P and ES does not, S initially binds more rapidly than P. If the positive field were left on for much longer than the relaxation time, the total amount of P that binds would equal the total amount of S that binds. However, the field switches before then, leaving the chemical potential of P slightly higher than the chemical potential of S. During the negative phase of the perturbation, denoted by unfilled arrows in Figure 1, the free energy of ES is increased relative to the unbound form. Thus, dissociation of both S and P is favored during this phase of perturbation. Since the activation barrier between ES and E + S is unchanged but that between ES and E + P is reduced, P dissociates faster than S. Once again,

(5) Astumian, R. D.; Chock, P. B.; Tsong, T. Y.; Westerhoff, H. V. *Phys. Rev. A* **1989**, *39*, 6416–6435.
(6) Weaver, J. C.; Astumian, R. D. *Science* **1990**, *247*, 459–462.
(7) Markin, V. S.; Tsong, T. Y.; Astumian, R. D.; Robertson, B. *J. Chem. Phys.* **1990**, *93*, 5062–5066.
(8) Burbaum, J. J.; Raines, R. T.; Alberry, W. J.; Knowles, J. R. *Biochemistry* **1989**, *28*, 9293–9305.

if the negative perturbation would remain on for a long time, the total amount of S dissociated eventually would be the same as the total amount of P dissociated. But the perturbation switches back positive before this can happen. Thus, the net effect of a cycle of the field is to increase slightly the chemical potential of P at the expense of a slight decrease in the chemical potential of S (see Figure 2a).

The magnitude of the effect clearly depends on the frequency of cycling the field (see Figure 3). At very low frequencies the field does not cause the reaction to go away from equilibrium since the amounts of S and P bound and dissociated in the positive and negative phases will be the same. At high frequencies, kinetic effects can take over as described above. The concentration change per cycle is of course no greater than the total enzyme concentration E_T and is usually much less. However, the effect accumulates over many cycles of the field (see Figure 2b) until the concentration difference between P and S just counterbalances the differences in intrinsic rates of association and dissociation in the positive and negative phases of the field, respectively. When this happen, the number of substrate molecules converted to product averaged over a cycle of the field is identically zero. The concentrations that enforce this condition can be quite different from the equilibrium concentrations.

The applied perturbation provides the energy source. The interaction with the intermediate and transition states provides the mechanism by which some of this energy is absorbed by the enzyme. Finally, the asymmetry of interaction between the different transition states governs the direction that the ac perturbation drives the reaction. If the perturbation affected the transition state between ES and E + P instead of the transition state between E + S and ES, the arguments would be exactly the same except that the directions would be opposite. The required asymmetry can come either from intrinsic asymmetry in the binding of substrate vs product of, as in Figure 1, from asymmetric interaction of the intermediate and transition states with the applied perturbation, or from both. We expect asymmetry to be the rule rather than the exception, particularly for energy transducing membrane proteins. Jencks[9] has discussed the phenomenological importance of binding asymmetry for several ion pumps.

"Resonant" Activation of Flux for a Two-Intermediate Enzyme. Consider the slightly more complex reaction, with two intermediates,

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E^*P \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} E + P \qquad (11)$$

Here, there are two relaxation times characterizing the kinetics. For the case where the second, conformational transition, step is fast, they can be given simple physical interpretation. The fast process, characterized by the inverse relaxation time

$$\tau_f^{-1} = k_2 + k_{-2} \qquad (12)$$

relates to the redistribution between the two bound enzyme species, and the slow process, with inverse relaxation time

$$\tau_s^{-1} = k_1[S] + k_{-3}[P] = \frac{k_{-1} + k_3K_2}{1 + K_2} \qquad (13)$$

relates to redistribution between bound and unbound forms. In Figure 4, we see a plot of the flux for this system as a function of frequency. At frequencies less than τ_s^{-1} or greater than τ_f^{-1} the sign of the flux is the same as the sign of the affinity. In the intermediate frequency regime, however, a "resonant" activation is seen. It is important to note that this is not a resonant phenomenon in the classical sense. The frequency bandwidth is quite broad, governed by the difference between the fast and slow relaxation times of the system.

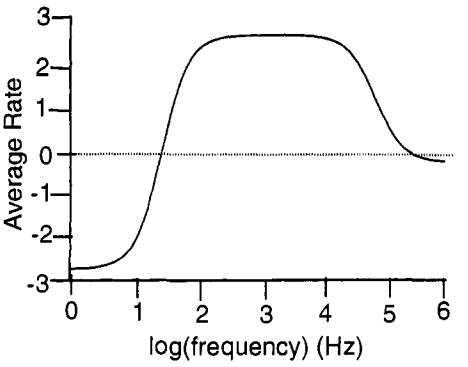

Figure 4. "Resonant" activation of a catalytic flux. The parameters used as $k_1^0 = 10$, $k_{-1}^0 = 200$, $k_2^0 = 20000$, $k_{-2}^0 = 500000$, $k_3^0 = 500$, and $k_{-3}^0 = 1$; $z_{+1} = -2$, $z_{+2} = 1$, $z_{-2} = -1$, and $z_{-1} = z_{+3} = z_{-3} = 0$ elementary charges; [S] = 1 and [P] = 1.3; and $E_T = 0.1$ and $eF\psi_1/RT = 0.5$. The equilibrium constant $K = 1$, and the affinity is $0.26RT$.

Notice that free energy is transduced from the oscillating field when the energy absorbing step (ES ⇌ E*P) is very fast compared to the oscillation, so that the energy input is reversible. This is achieved by having τ_f^{-1} large. For energy accumulation over many cycles of the field to be achieved, it is necessary that the overall system be out of equilibrium with the applied field. This is achieved by having τ_s^{-1} small. With $\tau_s^{-1} \ll \omega \ll \tau_f^{-1}$, effective energy transduction is achieved as seen in Figure 4. Effective energy coupling is achieved most readily with an enzyme that has several conformational states where the molecular properties such as dipole moment are very different, but which have similar free energies and are separated by very small rate constants. This has as a limiting case the reaction in eq 2, where the change in the parameter z occurs continuously along the reaction coordinate, which is what would be achieved in the limit $\tau_f^{-1} \to \infty$. It is seen that proteins do indeed have many conformational states separated by very small energy barriers[10] (i.e., large rate constants). These states are kinetically undetectable at room temperature by normal kinetic methods but contribute to non-exponential relaxation at lower temperatures.

Discussion and Conclusions

There are two major points of our paper. The first is that the overall affinity (or, equivalently, the free energy difference between reactants and products) does not necessarily predict the direction of any non-elementary reaction sequence (even "elementary-complex" reactions defined by Keizer[2]) if the thermodynamic parameters of the system depend on time. In general, if the free energies of any intermediates in a chemical reaction depend on some thermodynamic parameter, oscillation of that parameter may change the direction of the average net reaction.

The second point is that an enzyme can be designed, either through protein engineering or by evolution, to couple a fluctuating signal as an energy source to drive a reaction away from equilibrium. For example, a protein that has a conformational transition involving charge movement can couple electric energy to drive even a totally non-electrogenic chemical reaction. The interaction is governed solely by intrinsic properties of the enzyme. It follows that Coulombic interactions between two enzymes or between two subunits of the same enzyme may provide a mechanism by which two non-electrogenic reactions may be coupled.

While our results are general, they will find the greatest applicability for membrane enzymes and transporters. Reactions catalyzed by proteins embedded in membranes are likely to have

(9) Jencks, W. P. *Methods Enzymol.* **1989**, *171*, 145–164.

(10) Fraunfelder, H.; Wolynes, P. G. *Science* **1985**, *229*, 337–341.

a significant electric field susceptibility for several reasons:[11] (1) Because a membrane has a large electrical resistance and the cytosol is a good ionic conductor, the field due to an applied potential will be much larger within the membrane than anywhere else. (2) Proteins are large and have significant dipole moments. Many membrane proteins in particular are known to involve very large charge movements in some conformational changes. (3) Proteins in membranes cannot rotate to escape the effect of the field since flipping involves a very large activation energy.

A number of experimental examples of the effect of an external electric field on membrane enzymes are known. The rate of transport of Na^+ and K^+ catalyzed by Na^+,K^+-ATPase in erythrocytes[12] and the rate of synthesis of ATP in plant protoplasts[13] depend on the frequency and amplitude of an applied oscillating electric field. Our theoretical results have successfully explained the frequency dependence of the ion transport[14] and the frequency and amplitude dependence of the ATP synthesis.[15]

Internally produced oscillations are important in many biological systems.[16,17] In vivo, large (>10 mV amplitude) oscillations[18-20] and fluctuations[21,22] of the membrane potential are common. Many membrane proteins and enzymes,[23] including voltage-gated channels,[24] are very sensitive to changes in the membrane potential.

A remarkable conclusion can be expressed as follows. Consider a simple one-transition elementary reaction, $S \rightleftharpoons P$, at equilibrium. If the chemical potentials of S and P are independent of electric potential, for example, the system will remain in equilibrium even in an ac electric field. No energy is absorbed from the field. When an enzyme that catalyzes the reaction is added, however, the overall system will no longer be in equilibrium if the conformational transitions of the enzyme involve the movement of charge. If the frequency of the applied field is not too slow, the chemical reaction $S \rightleftharpoons P$ may be driven away from equilibrium.

The rate of departure from equilibrium and the final displacement from equilibrium can be significant. The point at which the rate is zero is independent of enzyme concentration so long as the rate of the uncatalyzed reaction is negligible. For the symmetric case studied here, where $k^0_{-1} = k^0_2 = k_{cat}$ and $k^0_1 = k^0_{-2}$, and $-z_{+1} = z_{+2} = 1$ and $z_{-2} = z_{-2} = 0$, we find that with [S] = [P] = k^0_{-1}/k^0_1 (i.e. half saturation) the initial rate at which the reaction proceeds from equilibrium is $(d[P]/dt)_{int} = k_{cat}E_T(\psi_1/RT)^2/8$. This relation says that for $\psi_1 = 25$ mV the rate of departure from equilibrium is a bit greater than 12% of the maximum velocity ($v_{max} = k_{cat}E_T$) for that enzyme. The final steady state of the catalyzed reaction in the ac field is [P]/[S] = $1.5K$.

These results are important for understanding how proteins facilitate free energy transduction in biological systems.[25,26] In enzyme catalysis, a simple chemical reaction such as hydrolysis of ATP is coupled to the conformational transitions of a protein. This coupling provides thermodynamic leverage that allows perturbations that influence the protein conformational transitions to drive the chemical reaction away from equilibrium. Such coupling can be achieved in vivo through local interactions between two or more enzymes or subunits.[27] Effective coupling can be achieved most readily with large flexible enzymes that have very rapid conformational transitions. The size provides for a large interaction energy since it is easier to imagine a significant displacement charge or ΔV for a large molecule than for a small one. The rapidity of the conformational changes allows for the energy input to be almost reversible. This may be one reason why enzymes have evolved to be so large and to have such a rich hierarchy of rapidly equilibrating conformational states.[10]

Acknowledgment. We thank Drs. John Kasianovich, Joe Hubbard, and Jeff Sachs of NIST, Dr. John Ross of Stanford University, Dr. Jim Weaver of Harvard/MIT Division of Health Sciences Technology, and Drs. David Oxtoby, Keith Moffat, Ted Steck, John Westly, Marvin Makinen, Carey Bagdesarian, and especially Howard Taget of the University of Chicago for very helpful discussions during the course of this work. This work was supported in part by NIH Grant RO1ES06010.

(11) Astumian, R. D.; Robertson, B. *J. Chem. Phys.* **1989**, *91*, 4891–4901.
(12) Liu, D. S.; Astumian, R. D.; Tsong, T. Y. *J. Biol. Chem.* **1990**, *265*, 7260–7267.
(13) Graziana, A.; Ranjeva, R.; Tessié, J. *Biochemistry* **1990**, *29*, 8313–8318.
(14) Robertson, B.; Astumian, R. D. *J. Chem. Phys.* **1991**, *94*, 7414–7419.
(15) Robertson, B.; Astumian, R. D. *Biochemistry* **1992**, *31*, 138–141.
(16) Richter, P.; Ross, J. *Science* **1981**, *211*, 715–719.
(17) Lazar, J. G.; Ross, J. *Science* **1990**, *247*, 189–193.
(18) Dean, P. M.; Mathews, E. K. *J. Physiol.* **1970**, *210*, 255–265.
(19) Rapp, P. E.; Berridge, M. J. *J. Exp. Biol.* **1981**, *93*, 119–130.
(20) Rapp, P. *Prog. Neurobiol.* **1987**, *29*, 267–305.
(21) DeFelice, L. J. *Introduction to Membrane Noise*; Plenum: New York, 1981.
(22) Astumian, R. D.; Chock, P. B.; Tsong, T. Y.; Chen, Y.-D.; Westerhoff, H. V. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 434–439.
(23) DeWeer, P.; Gadsby, D. C.; Rakowski, R. F. *Annu. Rev. Physiol.* **1988**, *50*, 225–242.
(24) Catterall, W. A. *Annu. Rev. Biochem.* **1986**, *55*, 953–962.
(25) Tsong, T. Y.; Astumian, R. D. *Bioelectrochem. Bioenergy.* **1986**, *15*, 457–476.
(26) Westerhoff, H. V.; Tsong, T. Y.; Chock, P. B.; Chen, Y.-D.; Astumian, R. D. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *83*, 4734–4738.
(27) Kamp, F.; Astumian, R. D.; Westerhoff, H. V. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 3792–3797.