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Role of conformational dynamics in kinetics of an enzymatic cycle in a nonequilibrium steady state

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Enzyme is a dynamic entity with diverse time scales, ranging from picoseconds to seconds or even longer. Here we develop a rate theory for enzyme catalysis that includes conformational dynamics as cycling on a two-dimensional (2D) reaction free energy surface involving an intrinsic reaction coordinate (X) and an enzyme conformational coordinate (Q). The validity of Michaelis–Menten (MM) equation, i.e., substrate concentration dependence of enzymatic velocity, is examined under a nonequilibrium steady state. Under certain conditions, the classic MM equation holds but with generalized microscopic interpretations of kinetic parameters. However, under other conditions, our rate theory predicts either positive (sigmoidal-like) or negative (biphasic-like) kinetic cooperativity due to the modified effective 2D reaction pathway on X-Q surface, which can explain non-MM dependence previously observed on many monomeric enzymes that involve slow or hysteretic conformational transitions. Furthermore, we find that a slow conformational relaxation during product release could retain the enzyme in a favorable configuration, such that enzymatic turnover is dynamically accelerated at high substrate concentrations. The effect of such conformation retainment in a nonequilibrium steady state is evaluated. © *2009 American Institute of Physics*. [DOI: 10.1063/1.3207274]

I. INTRODUCTION

Recent advances have allowed single-molecule enzymatic turnovers to be monitored in real time under the nonequilibrium steady-state condition,[1–3] in which a constant flux from reactant to products is maintained under constant concentrations of substrates. Unlike the conventional ensemble-averaged measurements, this condition closely resembles in the situation of living cell where the steady state is maintained by constant supply of free energy.[4–7] Theoretical discussions on enzyme kinetics often start with the classic Michaelis–Menton (MM) equation that gives the catalysis rate v in terms of substrate concentration $[S]$ as follows:

$$v = k_{\text{cat}}[S]/([S] + K_M), \tag{1}$$

where k_{cat} is the maximum turnover rate and K_M is the Michaelis constant. It is now known that protein conformational dynamics often controls and slaves the catalysis.[8] However, this important effect of conformational motions has not been explicitly considered in the classic MM equation. Moreover, both positive (sigmoidal-like) and negative (biphasic-like) cooperativities of the dependence of v on $[S]$ have been observed for many monomeric enzymes that involve slow or hysteretic conformational transitions[9–15] and termed as kinetic coopertivity.[16] Such a non-MM behavior from monomeric enzymes cannot be explained by MM equation or the existing cooperativity theories [Monod-Wyman-Changeux (MWC) or Koshland-Nemethy-Filmer (KNF) models] that invoke multiple subunits or binding sites.[8,16,17]

Here we develop an alternative approach that includes the effects of conformational fluctuations on enzyme catalysis. We consider a nonequilibrium conformational cycling on a two-dimensional (2D) reaction free energy surface spanned by an intrinsic reaction coordinate (IRC) (X) and a conformational coordinate (Q) that describes enzyme's motion. The resulting analytic theory shows that the experimentally observed $[S]$ cooperativity could naturally arise from a competition between the slow conformational dynamics and the thermodynamic substrate influx toward the enzyme, which modifies the nonequilibrium reaction pathway on 2D X-Q surface in a nontrivial way. MM relation, positive kinetic cooperativity, and negative kinetic cooperativity on substrate concentration are found to belong to different limits of the same unified theory, which can explain non-MM dependence previously observed on many monomeric enzymes.[9–16] Interestingly, we find that the enzyme could be retained in a favorable conformation due to the slow conformational relaxation during product release, such that the overall enzymatic cycling is effectively accelerated in a nonequilibrium steady state.

II. CONVENTIONAL 1D PICTURE

The thermodynamics and dynamics of enzyme catalysis are intimately related. From a thermodynamic perspective, most biochemical reactions *in vivo* occur under nonequilibrium conditions,[4–7] in which substrate is continuously converted to product driven by a chemical driving force. In ad-

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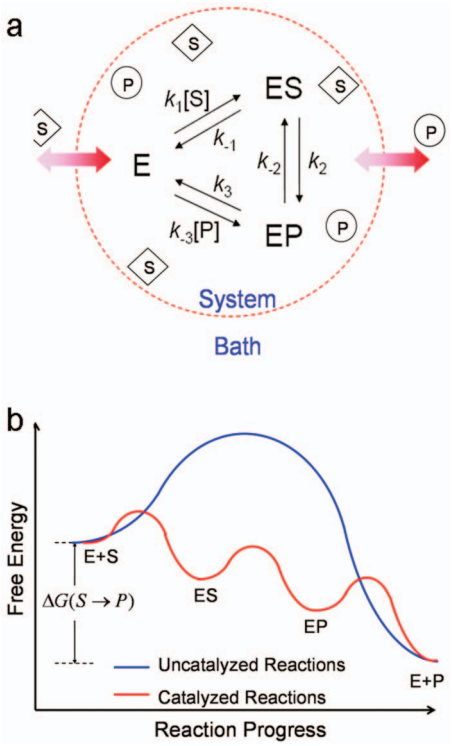

FIG. 1. (a) Enzymatic turnovers under nonequilibrium steady state. The dashed circle denotes an open thermodynamic system, in which substrates are continuously converted to products but their concentration $[S]$ and $[P]$ are held constant due to the exchange with the bath. (b) The traditional 1D free energy diagram of catalysis. In this picture, the activation barriers come from both the enzyme conformational coordinate (for the first and third barriers) and intrinsic reaction coordinate (for the second barrier). A 2D reaction free energy surface is needed to study their coupling.

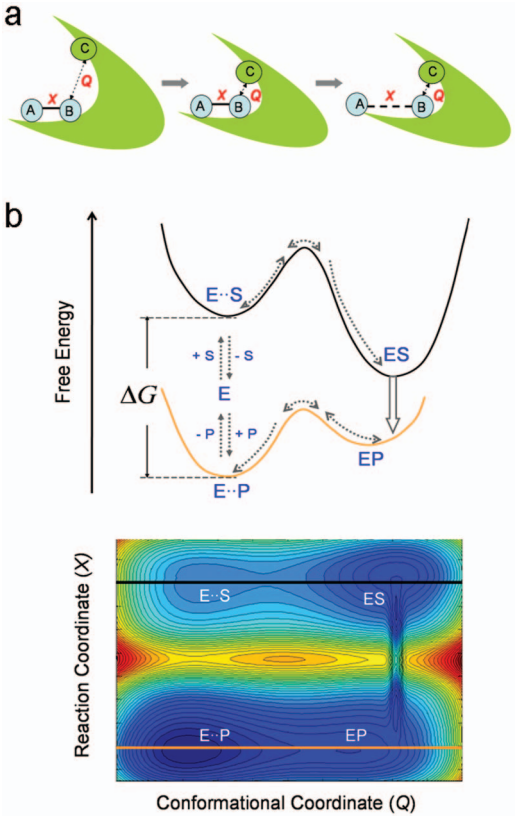

FIG. 2. (a) A cartoon for illustration of X-Q pair in enzyme catalysis. X coordinate is the conventional 1D coordinate describing the distance between atoms A and B, while the slow Q coordinate is the physical distance between atom B and a nearby catalytic residue C that serves to catalyze this bond breaking reaction. Only when the Q coordinate reaches certain configuration, the activation barrier along X is significantly lowered and the reaction could progress. (b) The new 2D reaction free energy surfaces of catalysis. The catalytic zone is a narrow region of Q for which the activation barrier for X coordinate is much lowered compared to other Q configurations. Color bar in the contour plot is designed to reflect the relative free energy levels.

dition, due to the exchange with the bath, substrate and product concentrations and their chemical potential remain time invariant in the system, representing a steady state.[6] Meanwhile, enzyme is a dynamic entity with diverse time scales, ranging from picoseconds to seconds or even longer.[2,3,18–20] During the continuous catalytic cycling, the slow parts of conformational motions could, in some cases, lag behind the fast *substrate influx*. As a result, working enzymes may exist in a nonequilibrium conformation, which has indeed been observed recently.[21,22] These experiments show that enzyme's conformation, while it is performing catalysis under high $[S]$, is distinctly different from that in the nonworking state (at zero $[S]$).

However, the conventional one-dimensional (1D) reaction free energy profile [Fig. 1(b)] does not contain an enzyme conformational coordinate. As a result, the cycling in the corresponding kinetic scheme [Fig. 1(a)] is always made to follow the *same pathway*, no matter how fast or slow the substrate influx and the conformational motion are. Therefore, the conventional kinetic scheme [Fig. 1(a)] is generally inadequate in addressing the coupling between the substrate influx and enzyme conformational dynamics.

The 1D scheme is expected to be valid in the following two limiting cases. (i) When the conformational motion is fast enough to always follow the reaction path. (ii) The conformational motion is sufficiently slow so as not to move during the reaction, which is essentially the lock-and-key mechanism. In either of the two cases, conformational fluctuation need not be considered, and the catalysis rate is determined solely by the substrate influx and the intrinsic catalytic rate, as envisaged in the classic MM relation, Eq. (1). However, this is clearly not the general situation.

III. 2D REACTION FREE ENERGY SURFACE

To overcome the deficiency of the conventional 1D scheme (Fig. 1), we shall theoretically analyze a 2D reaction free energy surface where one more coordinate is introduced to describe the enzyme conformational motion. We shall show that even this simple generalization brings in a host of new features. As elaborated in Ref. 23, the IRC X may denote the length of the bond to be broken or formed in the reaction, and Q denotes the collective enzyme conformational coordinate, describing *the conformational dynamics* that facilitates the catalytic reaction along X. A specific example for X-Q pair in a bond breaking reaction is sketched in Fig. 2(a). When the Q dynamics is slower than that of X, the rate of catalysis is partly "slaved" to enzyme fluctuations.

We identify the following four distinct states on X-Q surface: (i) the unbound contact pair $E\cdots S$ (where E and S

are in close proximity); (ii) the bound enzyme-substrate complex ES; (iii) the bound enzyme-product complex EP, formed after the reaction but before the product release; (iv) the final unbound contact pair $E\cdots P$. All the four states have been illustrated in Fig. 2(b). Transition from $E\cdots P$ back to $E\cdots S$ completes the enzymatic cycle which occurs through the diffusion of P away from E and the appearance of a new substrate S close to E.

The free energy difference between $E\cdots S$ and $E\cdots P$ nearly equals to that in chemical potential between S and P: $U_{E\cdots S}(Q)-U_{E\cdots P}(Q)=\Delta G(S\rightarrow P)$. The equilibrium positions $Q_{I,e}$ often satisfy $Q_{E\cdots S,e}\approx Q_{E\cdots P,e}\neq Q_{ES,e}\neq Q_{EP,e}$. The transition from $E\cdots S$ to ES and vice versa, that is, binding or dissociation of S to or from E, is described by a diffusive barrier crossing process in Q coordinate. The binding configuration Q_{binding} serves as the dividing surface between $E\cdots S$ and ES as in Kramers theory.[24] So is true for EP and $E\cdots P$ states.

The coupling between Q-X is crucial in performing the transition state stabilization for catalysis.[23] The stabilization of the activation barrier along X in the ES complex needs to be significant to considerably accelerate the rate compared to uncatalyzed reactions. This is often possible only for a limited range of Q configurations. Quantitatively, the catalytic reaction along the IRC (X coordinate) is described by a reaction zone in the (X-Q) plane whose width in Q space is rather narrow. We can thus make a further simplification: $k_{\text{IRC}}(Q)\rightarrow k_{\text{IRC}}\delta(Q-Q_{\text{catalysis}})$ which defines a delta-function sink[25] with finite integrated decay rate constant k_{IRC} located at $Q_{\text{catalysis}}$. The magnitude of this k_{IRC} is determined through the remnant activation barrier and barrier crossing dynamics, which can be obtained through molecular dynamics and/or quantum chemical calculations as in many studies. However, such calculations only provide values of k_{IRC} at the favorable geometry already reached by ES complex (the position marked as ES in Figs. 2 and 3), but neglect the dynamic process needed to reach there.

Like in protein folding and biomolecular binding,[26,27] a "funnel-like" energy landscape may guide the ES conformation toward its most stable configuration. This is so because the driving force is the favorable interaction energy contacts between the substrate and enzyme groups while the opposing force is the entropy loss due to the substrate-enzyme confinement. The situation is also similar to dynamic allosteric effect[7] because catalysis involves the transfer of free energy released by the "induced fit" along Q to generate the "strain energy" on the bond along X.[8,19]

IV. DYNAMICAL RATE THEORY

We next present a rate theory based on our 2D free energy surface. This theory is partly in the spirit of MM approach but incorporates dynamical effects mentioned above. We start by discretizing the entire catalytic processes into five distinct stages on the reaction free energy surfaces. They are denoted by a, b, c, d, and e, as indicated in Fig. 3. Similarly, transition rate constants k_{ab}, k_{ba}, k_{bc}, k_{cd}, k_{de}, and k_{ea} describe conformation fluctuations necessary for substrate binding, conformation relaxation before substrate binding (with activation barrier $\Delta G^{\neq}_{\text{binding}}$), conformational relaxation from Q_{binding} to $Q_{\text{catalysis}}$, k_{IRC} at $Q_{\text{catalysis}}$, conformational relaxation after catalysis, and substrate diffusion to form the encounter pair, respectively. k_{bc}, k_{cd}, k_{de} are usually much faster than their respective reversible counterparts due to the large free energy drops indicated in Fig. 3. Moreover, k_{ea} is also expected to be dominating over k_{ae} because of the relatively low product concentration $[P]$.

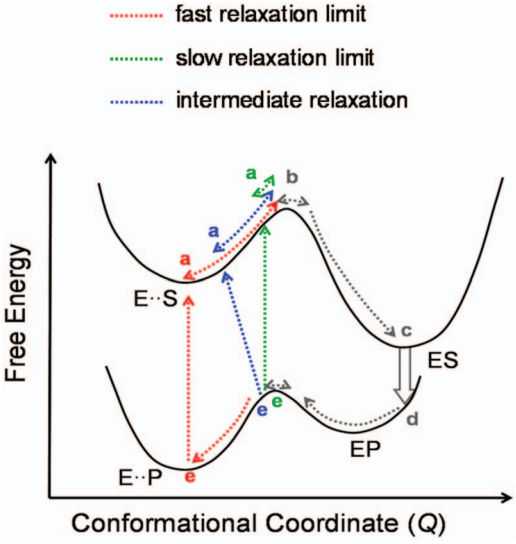

FIG. 3. Nonequilibrium enzyme probability flow on reaction free energy surfaces. When the enzyme relaxation dynamics is much faster than the time scale of substrate encounter latter, enzyme encounters a new substrate after it relaxes down to the equilibrium position of $E\cdots P$ surface (red arrow). In the opposite situation, enzyme encounters a new substrate immediately after it starts its relaxation on $E\cdots P$ surface (green arrow). When the enzyme relaxation dynamics on $E\cdots P$ surface is comparable to the time scale of substrate encounter, the enzyme encounters a new substrate molecule *on its way relaxing back* to its equilibrium position of $E\cdots P$ surface. This is denoted by the tilted arrow pointing from stage e to stage a (blue arrow). Unlike the case of fast relaxation limit, enzyme conformation can be retained in a favorable nonequilibrium configuration for fast cycling in both the slow and intermediate relaxation limits.

The dynamics of the entire turnover is now determined by the following master equation:

$$\frac{d}{dt}\begin{pmatrix}\rho_a(t)\\ \rho_b(t)\\ \rho_c(t)\\ \rho_d(t)\\ \rho_e(t)\end{pmatrix}=\begin{pmatrix}-k_{ab} & k_{ba} & 0 & 0 & k_{ea}\\ k_{ab} & -k_{ba}-k_{bc} & 0 & 0 & 0\\ 0 & k_{bc} & -k_{cd} & 0 & 0\\ 0 & 0 & k_{cd} & -k_{de} & 0\\ 0 & 0 & 0 & k_{de} & -k_{ea}\end{pmatrix}\times\begin{pmatrix}\rho_a(t)\\ \rho_b(t)\\ \rho_c(t)\\ \rho_d(t)\\ \rho_e(t)\end{pmatrix}. \quad (2)$$

The solution for the steady-state catalysis rate is found to be

$$\frac{1}{v} = \frac{1}{k_{\mathrm{IRC}}} + \frac{1}{k_{\mathrm{enz}}} + \frac{1}{k_{ea}}, \tag{3}$$

where k_{cd} is replaced by k_{IRC}, and the cumulative enzyme fluctuation rate k_{enz} is

$$\frac{1}{k_{\mathrm{enz}}} \equiv \frac{1}{k_{ab}} + \frac{k_{ba}}{k_{ab}k_{bc}} + \frac{1}{k_{bc}} + \frac{1}{k_{de}}, \tag{4}$$

which is the effective total time spent during conformational fluctuation.

The classic MM equation now naturally follows as a limiting form of the general equations (3). Among all the rate constants in Eq. (3), k_{IRC} is strictly independent of $[S]$. k_{enz} is essentially $[S]$ independent, in the cases of fast (red arrow in Fig. 3) and slow (green arrow in Fig. 3) enzyme relaxation dynamics on $E\cdots P$ surface, compared to the time scale of substrate encounter. In the limit of such time scale separation, $[S]$ enters into Eq. (3) only through rate constant k_{ea}. According to Smoluchowski theory,

$$k_{ea} = 4\pi R D_S [S], \tag{5}$$

where R is the reaction radius and D_S is the sum of diffusion constants of substrate and enzyme in solution phase. Therefore, Eqs. (3)–(5) together reproduce the MM relation (1) but with new definitions of k_{cat} and K_M,

$$k_{\mathrm{cat}} = \frac{1}{1/k_{\mathrm{IRC}} + 1/k_{\mathrm{enz}}}, \quad K_M = \frac{1}{1/k_{\mathrm{IRC}} + 1/k_{\mathrm{enz}}} \frac{1}{4\pi R D_S}. \tag{6}$$

Equation (6) can be regarded as a generalized MM expression but includes effects of fast or slow enzyme conformational motions.[28–30]

The more general scenario, where the enzyme relaxation rate on $E\cdots P$ surface is comparable to substrate encounter rate, gives rise to a nonequilibrium situation. As denoted by the tilted blue arrow pointing from stage e to stage a in Fig. 3, the enzyme encounters a new substrate molecule *on its relaxation pathway back* to the equilibrium position of $E\cdots P$ surface. As the complete relaxation back becomes unnecessary, enzymatic turnover is dynamically facilitated in this nonequilibrium steady state.

We now seek for a mean-field-like treatment for the above general time-scale-overlapping condition. First we approximate the expression of k_{enz} in Eq. (4). Due to the thermodynamic stability of ES complex, state c is further down away from state b in the free energy surfaces, compared to from state b to state a. Thus, we assume that the relaxation rate from b to c is slower than that from b to a: $k_{bc} \ll k_{ba}$. In addition, the activation rate k_{ab} is much slower than the relaxation rate k_{ba}: $k_{ab} \ll k_{ba}$. Based on these two facts, Eq. (4) simplifies to

$$\frac{1}{k_{\mathrm{enz}}} \approx \left(\frac{k_{ba}}{k_{ab}}\right)\frac{1}{k_{bc}} + \frac{1}{k_{de}}. \tag{7}$$

The ratio k_{ba}/k_{ab} is related to the effective, nonequilibrium binding activation barrier $\Delta G^{\neq}_{\mathrm{binding},ne}$,

$$k_{ba}/k_{ab} = \exp(\Delta G^{\neq}_{\mathrm{binding},ne}/k_B T). \tag{8}$$

In the limit of fast enzyme relaxation, $\Delta G_{\mathrm{binding},ne}$ equals to the equilibrium full activation free energy from the minimum of the $E\cdots S$ surface. However, under nonequilibrium conditions of a working enzyme, this barrier can be reduced considerably, as the enzyme is closer to the binding-ready conformation.

Next we find the $[S]$-dependence for $\Delta G^{\neq}_{\mathrm{binding},ne}$. After the enzyme crosses over to the stage e at time zero, that is, $Q_{E\cdots P,0} = Q_e$, as described in Fig. 3. The subsequent relaxation on $E\cdots P$ harmonic surface is given by solution of generalized Smoluchowski equation as

$$\rho_{E\cdots P}(Q,t|Q_e,0) = \sqrt{\frac{m\omega^2_{E\cdots P}}{2\pi k_B T[1-C^2_{E\cdots P}(t)]}} \times \exp\left\{-\frac{m\omega^2_{E\cdots P}[Q - Q_e C_{E\cdots P}(t)]^2}{2k_B T[1-C^2_{E\cdots P}(t)]}\right\}, \tag{9}$$

where $C_{E\cdots P}(t)$ is the normalized time correlation function of the dynamics on $E\cdots P$ surface, given by $\langle Q(0)Q(t)\rangle / \langle Q^2(t)\rangle$. For convenience, here we adopt a exponential function for this relaxation: $C_{E\cdots P}(t) = \exp(-k_{E\cdots P,\mathrm{relax}} \cdot t)$.

We then assume that the average time period τ for enzyme-substrate encounter to occur *after product release* is given by the reciprocal of the Smoluchowski rate: $\tau = 1/4\pi R D_S [S]$. During this τ, the conformational distribution evolves to a new distribution $\rho_{E\cdots P}(Q,\tau|Q_e,0)$ in Q. This evolved distribution at time τ will serve as the *initial ground state distribution of the activation process on* $E\cdots S$ surface immediately after substrate encounter. Thus, we can obtain $\Delta G^{\neq}_{\mathrm{binding},ne}$ by averaging over this distribution: $\Delta G^{\neq}_{\mathrm{binding},ne} \approx \int_{-\infty}^{Q_B} \rho_{E\cdots P}(Q,\tau|Q_e,0)(m\omega^2_{E\cdots S}/2)(Q_b^2 - Q^2)dQ$. In this way we obtain the result,

$$\Delta G^{\neq}_{\mathrm{binding},ne} \approx \frac{m\omega^2_{E\cdots S}}{2}\left[Q_b^2 - Q_e^2 \exp\left(-\frac{k_{E\cdots P,\mathrm{relax}}}{2\pi R D_S [S]}\right)\right]. \tag{10}$$

Finally, we arrive at the general $[S]$ dependence of the enzyme catalysis rate,

$$\frac{1}{v} \approx \frac{1}{4\pi R D_S [S]} + \frac{1}{k_{\mathrm{IRC}}} + \frac{1}{k_{de}} + \frac{1}{k_{bc}} \times \exp\left\{\frac{m\omega^2_{E\cdots S}}{2k_B T}\left[Q_b^2 - Q_e^2 \exp\left(-\frac{k_{E\cdots P,\mathrm{relax}}}{2\pi R D_S [S]}\right)\right]\right\}. \tag{11}$$

Hence, the $[S]$ dependence enters Eq. (11) through both the substrate encounter (the first term) and enzyme conformational activation (the last term), which would lead to nontrivial v-$[S]$ dependence in general, as demonstrated below. Note that Eq. (10) can also be derived by using first passage time calculation with an absorbing barrier at b and a reflecting barrier put on the left of a in Fig. 3.

Equation (11) contains information about enzyme conformational dynamics through k_{bc}, k_{de} $k_{E\cdots P,\mathrm{relax}}$, and the fre-

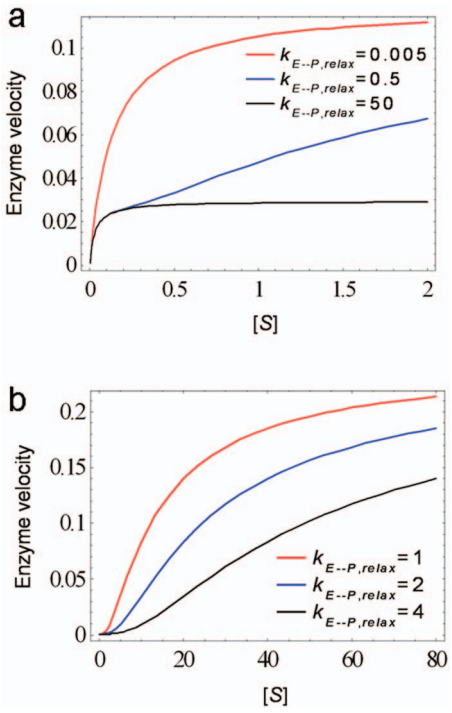

FIG. 4. Kinetic cooperativity and conformation retainment. (a) Negative kinetic cooperativity of v-$[S]$ curve, evaluated by Eq. (11). $4\pi RD_S=1$, $k_{bc}=1$, $1/k_{de}+1/k_{\mathrm{IRC}}=1$, $m\omega^2_{E\cdots S}Q_b^2/2=4k_BT$, $m\omega^2_{E\cdots S}Q_e^2/2=2k_BT$. When $k_{E\cdots P,\mathrm{relax}}$ is very fast (black curve) or very slow (red curve) compared to the substrate encounter rate, v-$[S]$ approaches simple MM relation. However, for blue curve, it exhibits an apparent biphasic (first fast and then slow) kinetics, which is generally regarded as negative kinetic cooperativity. (b) Positive kinetic cooperativity of v-$[S]$ curve, evaluated by Eq. (12). $k_{bc}=1000$, $m\omega^2_{E\cdots S}Q_b^2/2=12k_BT$, $m\omega^2_{E\cdots S}Q_e^2/2=6k_BT$. They all exhibit sigmoidal shapes, which is phenomenologically regarded as positive cooperativity. Note that, due to the mechanism of conformation retainment, a slower $k_{E\text{-}P,\mathrm{relax}}$ leads to a faster enzymatic velocity, as shown in both (a) and (b).

quency term $m\omega^2_{E\cdots S}$ which is closely related to binding activation energy. The three relaxation terms describe the average time taken to cross the conformational state points. While k_{bc} is a downhill relaxation as a simple description of induced fit, k_{de} is the rate of the dynamical event leading to product release. It is interesting to note (and we shall use this aspect below) that a large binding activation energy can make the last term in Eq. (11) really significant. Its $[S]$ dependence is nontrivial and is due to nonequilibrium effects.

V. CONFORMATION RETAINMENT AND KINETIC COOPERATIVITY

Observed kinetic cooperativity can easily (and in a physically appealing way) arise from this nonequilibrium rate theory. In Fig. 4(a), we plot three v-$[S]$ curves with different $k_{E\cdots P,\mathrm{relax}}$ of enzyme dynamics. When $k_{E\cdots P,\mathrm{relax}}$ is very fast (black curve) or very slow (red curve) compared to the substrate encounter rate, $\Delta G^{\neq}_{\mathrm{binding},ne}$ is essentially independent of $[S]$, and we recover the MM relation. This is precisely the time scale separation condition we mentioned earlier. However, in the intermediate time scale region where the relaxation on the product surface is comparable to the substrate encounter rate, non-MM behavior emerges. The blue curve in Fig. 4(a) exhibits an apparent biphasic (first fast and then slow) kinetics, which is generally regarded as a type of negative kinetic cooperativity.[8–17] It is the joint action of the substrate encounter, the first term in Eq. (11), and enzyme conformational activation, the last term in Eq. (11), that leads to the overall negative kinetic cooperativity.

We now discuss the conditions under which Eq. (11) gives rise to a positive kinetic cooperativity. Under a slow conformational gating condition that has a large binding activation barrier on the $E\cdots S$ surface, a fast enzyme relaxation and efficient catalytic reaction, Eq. (11) simplifies to

$$v \approx k_{bc}\exp\left\{-\frac{m\omega^2_{E\cdots S}}{2k_BT}\left[Q_b^2-Q_e^2\exp\left(-\frac{k_{E\cdots P,\mathrm{relax}}}{2\pi RD_S[S]}\right)\right]\right\}. \tag{12}$$

We have plotted the $[S]$ dependence of the rate predicted by Eq. (12) in Fig. 4(b). The typical "sigmoidal" shape is regarded as a typical of positive kinetic cooperativity.[8–17]

Interestingly, a slower conformational relaxation rate $k_{E-P,\mathrm{relax}}$ during product release process leads to a faster enzymatic velocity, as shown in both Figs. 4(a) and 4(b). This is because, by *avoiding* a complete relaxation back to its equilibrium for $E\cdots P$, enzyme could experience an effectively smaller activation energy barrier $\Delta G^{\neq}_{\mathrm{binding},ne}$ for $E\cdots S$ (green or blue arrows in Fig. 3) compared to the case of a complete conformational relaxation and reactivation (red arrow in Fig. 3). Hence, we term this mechanism as *conformation retainment*, which has a purely dynamic origin rooted in nonequilibrium steady state.

Equations (11) and (12) could account for the observed positive or negative kinetic cooperativity of many monomeric enzymes[9–16] for which the classic multisubunit cooperativity theories such as concerted model and sequential model[8,16,17] do not apply. Furthermore, earlier experimental evidence of the slow or hysteretic or mnemonic conformational fluctuations observed in these monomeric enzymes[9–16] further supports our dynamical theory.

VI. CONCLUSIONS AND DISCUSSIONS

Let us first summarize the main features of this work. We have based on a 2D free energy surface reported earlier[23] to develop a nonequilibrium rate theory of enzyme catalysis that includes the effects of slow enzyme conformational dynamics. There exist two distinct effects about how the conformational dynamics controls the overall enzymatic cycling. The dynamic facilitation, which occurs after the substrate binds bur before the catalysis, is a combination of Koshland's induced fit and Haldane's strain energy concepts.[31] On the other hand, the conformation retainment (Fig. 3) takes place in the product release process after the catalytic step but before the new substrate binding. The dynamic facilitation has been discussed in details in our previously paper.[23] Enzymatic cycling in a nonequilibrium steady state profoundly leads to the interesting phenomenon of conformation retainment, which is discussed in this paper.

The nonequilibrium effect enters through a competition between enzyme's substrate encounter rate and its rate of relaxation on the product release free energy surface. When the rate of encounter is large and/or enzyme relaxation is slow, a new substrate binding after the product release can

occur *before* the enzyme has time to completely relax back to its equilibrium conformation. This conformation retainment can contribute to faster enzymatic turnovers, as shown in Fig. 4. In such a case, the activation energy and hence the rate of binding become $[S]$ dependent. Therefore, the present theory suggests a dynamical mechanism that can modify the *effective reaction pathway on the 2D X-Q* surface. The theory also demonstrates why and how the MM relation breaks down due to this nonlinear competition. Equations (11) and (12) provide new and rather sophisticated expressions of the catalysis rate by such a nonequilibrium molecular machine, leading, on one hand, to classic MM under certain limiting conditions and to both positive and negative kinetic cooperativities of $[S]$ on the other. The theory described herein can account for the observed dynamic cooperativity of certain types of monomeric enzymes.[9–16]

The present 2D formalism is in a spirit similar to the ones employed in many other areas of chemical physics including barrierless isomerization reaction,[24] protein-ligand binding,[32] electron transfer reaction,[33] protein motor,[34,35] among others. It is also a generalization of previous kinetic modeling using discrete conformational states.[9–16,28,36] In all these models, functionally relevant motion is different from the actual or IRC, and in certain sense, all these treatments should be regarded as models of dynamical disorder, as discussed elegantly by Zwanzig.[37] The intrinsic merit of the approach adopted here is that, while the fast conformational dynamics (those occurring on nanoseconds or shorter time scales) can be included through a frequency dependent friction on X-coordinate,[38,39] the motion along Q serves as a control parameter much in the sense of a geometric bottleneck[40] that controls diffusion out of confinement. However, here the coordinate Q facilitates the enzymatic reaction and this facilitation is dynamic and nonequilibrium in nature. Without this dynamic facilitation, many enzymatic reactions would not occur.

We note that there is yet hardly any microscopic theory based on a reaction free energy function that can capture both the positive and the negative cooperativities of the substrate concentration dependence of the enzyme catalysis rate. The present work attempts to fill this lacuna. An essential aspect of the present explanation is that it is based explicitly on a nonequilibrium catalytic cycle. The explanation depends on the relative rates of relaxation on the free energy surface and also on the rates of product release and substrate capture which are also considered explicitly for the first time in a statistical mechanical theory. In this connection we note that free energy surface of a catalytic cycle for adenylate kinase catalysis of ATP and AMP to two ADP has been proposed by Whitford *et al.*[41] The present work would suggest a nonequilibrium modification by shortening the route of return to the free state of ADK.

The theory presented here can be generalized to more complex situations, such as a more detailed description of product release via a third dimension. The theoretical scheme is sufficiently general and can be modified to suit particular situations.

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