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When Does the Michaelis−Menten Equation Hold for Fluctuating Enzymes?

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Enzymes are dynamic entities: both their conformation and catalytic activity fluctuate over time. When such fluctuations are relatively fast, it is not surprising that the classical Michaelis−Menten (MM) relationship between the steady-state enzymatic velocity and the substrate concentration still holds. However, recent single-molecule experiments have shown that this is the case even for an enzyme whose catalytic activity fluctuates on the $10^{-4}-10$ s range. The purpose of this paper is to examine various scenarios in which slowly fluctuating enzymes would still obey the MM relationship. Specifically, we consider (1) the quasi-static condition (e.g., the conformational fluctuation of the enzyme−substrate complex is much slower than binding, catalysis, and the conformational fluctuations of the free enzyme), (2) the quasi-equilibrium condition (when the substrate dissociation is much faster than catalysis, *irrespective* of the time scales or amplitudes of conformational fluctuations), and (3) the conformational-equilibrium condition (when the dissociation and catalytic rates depend on the conformational coordinate in the same way). For each of these scenarios, the physical meaning of the *apparent* Michaelis constant and catalytic rate constant is provided. Finally, as an example, the theoretical analysis of a recent single-molecule enzyme assay is considered in light of the perspectives presented in this paper.

1. Introduction

Ever since its introduction in 1913,[1] the Michaelis−Menten (MM) equation has provided a highly satisfactory description of the steady-state kinetic behavior of many enzymes.[2−4] In the simplest formulation, a substrate, S, binds reversibly to an enzyme, E, forming an enzyme−substrate complex, ES, that undergoes unimolecular decomposition to form a product, P, and the original enzyme (E):

$$\mathrm{E} + \mathrm{S} \underset{k_{-1}}{\overset{k_1^0}{\rightleftharpoons}} \mathrm{ES} \xrightarrow{k_2} \mathrm{E} + \mathrm{P}$$

When steady state (i.e., the concentration of ES no longer depends on time) is established, the rate of product formation (v) is characteristically hyperbolic in the substrate concentration, that is, $v = k_2[\mathrm{S}]/([\mathrm{S}] + K_\mathrm{M})$, where the Michaelis constant is $K_\mathrm{M} \equiv (k_{-1} + k_2)/k_1^0$.[2−4]

The discovery of slow fluctuations in the catalytic activity of an enzyme using single-molecule techniques[5] was unexpected in part because it seemed incompatible with the nearly universal applicability of the MM equation. If all conformational fluctuations of the enzyme and enzyme−substrate complex were much faster than catalysis, there would be no problem, but it is now well-established that proteins fluctuate on essentially all time scales.[6−15] Indeed, turnover fluctuations on the $10^{-4}-10$ s time scale have been observed for several systems.[6−15] For β-galactosidase, English et al.[15] demonstrated that MM behavior of the average number of turnovers per unit time still holds even in the presence of slow protein fluctuations.

In general, the steady-state kinetics of a fluctuating enzyme is non-MM,[16,17] providing a potential mechanism for positive and negative kinetic cooperativity of monomeric enzymes.[18] The goal of this paper is to explore various scenarios in which the steady-state kinetics of a fluctuating enzyme can be described by the MM relation. Recently, in separate publications,[16,17] we have in part considered this question, but our focus was primarily on the situation when conformational dynamics was much slower than catalysis. In this paper, we will not only clarify the relationship between our studies but also extend them to consider more general scenarios in which the MM equation holds in the presence of conformational fluctuations occurring on all time scales.

The outline of this paper is as follows. In section 2, we present a statistical mechanical theory of the kinetics of a fluctuating enzyme. Our strategy is to gradually develop the formalism by starting with the description of the conformational dynamics of the free enzyme/enzyme−substrate complex and then successively incorporate the influence of substrate binding and finally catalysis. In section 3, we consider the quasi-static condition and show that MM behavior results when the conformational dynamics in either E or ES states is much slower than the other and slower than the binding and catalytic rates. In sections 4 and 5, we present two scenarios which lead to MM behavior irrespective of the time scale of conformational fluctuations of either E or ES. Finally, in section 6, we discuss

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the implications of our results on the interpretation of the recent single-enzyme experiments of English et al.[15]

2. Theory of the Kinetics of Fluctuating Enzymes

To construct the required theoretical framework, we begin by considering the conformational dynamics of the free enzyme (E) and the enzyme−substrate complex (ES) in the absence of substrate binding/dissociation and catalysis. The various conformations are specified by a multidimensional coordinate, **r**, which in principle can be continuous or discrete. Let $p_0(I,\mathbf{r},t)$ be the probability that this coordinate is **r** at time t for the system in state I, I = E or ES. The dynamics is described by an evolution equation of the form

$$\frac{\partial p_0(I,\mathbf{r},t)}{\partial t} = L_I p_0(I,\mathbf{r},t) \tag{1}$$

If the dynamics is described as diffusion on a free energy surface, $U_I(\mathbf{r})$, then the evolution operator is the Smoluchowski operator, $L_I = \nabla\cdot\mathbf{D}_I e^{-\beta U_I(\mathbf{r})}\cdot\nabla\, e^{\beta U_I(\mathbf{r})}$, where $\mathbf{D}_I$ is the diffusion tensor in state I (I = E or ES) and $\beta = (k_B T)^{-1}$. If the dynamics is described as transitions among a set of discrete conformations, then L_I is the corresponding rate matrix. In fact, the precise form of L_I is irrelevant for our purposes and thus our formalism is quite general. All we require is that eq 1 correctly describes the relaxation to the equilibrium Boltzmann distribution, $\exp(-\beta U_I(\mathbf{r}))$. Since the equilibrium distribution is stationary (i.e., its time derivative is zero), we have

$$L_I e^{-\beta U_I(\mathbf{r})} = 0, \quad I = \text{E, ES} \tag{2}$$

Next, let us consider the influence of substrate association and dissociation in the absence of catalysis (e.g., the substrate is a nonreactive transition-state analog). We assume that, for a given conformation, the binding kinetics can be described by the usual rate equations of chemical kinetics (i.e., the substrate concentration is low enough that the translational diffusion does not significantly influence the time course of the kinetics[19−21]) and that the binding of substrate is pseudo-first-order with the substrate concentration ([S]) being time independent. Then, the dynamics of the system is described by

$$\frac{\partial p(\text{E},\mathbf{r},t)}{\partial t} = [L_\text{E} - k_1^{\,0}(\mathbf{r})[\text{S}]]p(\text{E},\mathbf{r},t) + k_{-1}(\mathbf{r})\, p(\text{ES},\mathbf{r},t) \tag{3.a}$$

$$\frac{\partial p(\text{ES},\mathbf{r},t)}{\partial t} = [L_\text{ES} - k_{-1}(\mathbf{r})]p(\text{ES},\mathbf{r},t) + k_1^{\,0}(\mathbf{r})[\text{S}]\, p(\text{E},\mathbf{r},t) \tag{3.b}$$

where we have introduced the conformation-dependent association ($k_1^{\,0}(\mathbf{r})$) and dissociation ($k_{-1}(\mathbf{r})$) rate coefficients. When the free enzyme is in equilibrium with the enzyme−substrate complex, the time-independent equilibrium distributions ($p_{eq}(I,\mathbf{r})$) satisfy

$$[L_\text{ES} - k_{-1}(\mathbf{r})]p_{eq}(\text{ES},\mathbf{r}) + k_1^{\,0}(\mathbf{r})[\text{S}]\, p_{eq}(\text{E},\mathbf{r}) = 0 \tag{4.a}$$

$$[L_\text{ES} - k_1^{\,0}(\mathbf{r})[\text{S}]]p_{eq}(\text{E},\mathbf{r}) + k_{-1}(\mathbf{r})\, p_{eq}(\text{ES},\mathbf{r}) = 0 \tag{4.b}$$

In the above formalism, the **r** dependence of $k_1^{\,0}(\mathbf{r})$ and $k_{-1}(\mathbf{r})$ cannot be arbitrary. They are constrained by the requirement that at equilibrium $p_{eq}(I,\mathbf{r})$ must be proportional to the Boltzmann distribution, $\exp(-\beta U_I(\mathbf{r}))$. Substituting $p_{eq}(I,\mathbf{r}) = \alpha_I \exp(-\beta U_I(\mathbf{r}))/\int\exp(-\beta U_I(\mathbf{r}))\, d\mathbf{r}$ into eqs 4.a and 4.b and using eq 2, we see that such a trial function is actually a solution of these equations if and only if

$$\alpha_\text{E} k_1^{\,0}(\mathbf{r})[\text{S}]e^{-\beta U_\text{E}(\mathbf{r})}/\int e^{-\beta U_\text{E}(\mathbf{r})}\, d\mathbf{r} = \alpha_\text{ES} k_{-1}(\mathbf{r})e^{-\beta U_\text{ES}(\mathbf{r})}/\int e^{-\beta U_\text{ES}(\mathbf{r})}\, d\mathbf{r} \tag{5}$$

This is essentially the condition of detailed balance for reversible substrate binding. In general, the principle of detailed balance places limitations on the nature of transitions between different classical or quantum states, even at the single-molecule level.[22,23]

Since α_I is independent of **r**, integrating over all **r**, we find that $\alpha_\text{E}[\text{S}]\langle k_1^{\,0}\rangle_\text{E} = \alpha_\text{ES}\langle k_{-1}\rangle_\text{ES}$ where we have defined the conformational-equilibrium average in state I as

$$\langle(...)\rangle_I = \frac{\int(...)e^{-\beta U_I(\mathbf{r})}\, d\mathbf{r}}{\int e^{-\beta U_I(\mathbf{r})}\, d\mathbf{r}} \tag{6}$$

Combining this with eq 5, we have

$$\frac{k_1^{\,0}(\mathbf{r})e^{-\beta U_\text{E}(\mathbf{r})}}{\langle k_1^{\,0}\rangle_\text{E}\int e^{-\beta U_\text{E}(\mathbf{r})}\, d\mathbf{r}} = \frac{k_{-1}(\mathbf{r})e^{-\beta U_\text{ES}(\mathbf{r})}}{\langle k_{-1}\rangle_\text{ES}\int e^{-\beta U_\text{ES}(\mathbf{r})}\, d\mathbf{r}} \tag{7}$$

This is the form of the detailed balance condition given in ref 17 (see their eq 6.35). Using the above relations and requiring that the equilibrium distributions are normalized as $\int(p_{eq}(\text{E},\mathbf{r}) + p_{eq}(\text{ES},\mathbf{r}))\, d\mathbf{r} = 1$, we find

$$p_{eq}(\text{E},\mathbf{r}) = \frac{\langle k_{-1}\rangle_\text{ES}}{\langle k_1^{\,0}\rangle_\text{E}[\text{S}] + \langle k_{-1}\rangle_\text{ES}} \frac{e^{-\beta U_\text{E}(\mathbf{r})}}{\int e^{-\beta U_\text{E}(\mathbf{r})}\, d\mathbf{r}} \tag{8.a}$$

and

$$p_{eq}(\text{ES},\mathbf{r}) = \frac{\langle k_1^{\,0}\rangle_\text{E}[\text{S}]}{\langle k_1^{\,0}\rangle_\text{E}[\text{S}] + \langle k_{-1}\rangle_\text{ES}} \frac{e^{-\beta U_\text{ES}}(\mathbf{r})}{\int e^{-\beta U_\text{ES}(\mathbf{r})}\, d\mathbf{r}} \tag{8.b}$$

These expressions have a physically transparent structure: the equilibrium population for a given coordinate, **r**, is the product of Boltzmann distribution and the fractional occupancy obtained from simple kinetics using rate coefficients averaged over conformational fluctuations.

We are finally in a position to incorporate the influence of catalysis described by a conformation-dependent rate coefficient for irreversible product formation, $k_2(\mathbf{r})$. To obtain the evolution equations for the probability distributions, all that one has to do is to replace $k_{-1}(\mathbf{r})$ in eq 4 by $k_{-1}(\mathbf{r}) + k_2(\mathbf{r})$, because now the substrate in the ES complex can either dissociate or be converted into a product:

$$\frac{\partial p(\text{E},\mathbf{r},t)}{\partial t} = [L_\text{E} - k_1^{\,0}(\mathbf{r})[\text{S}]]p(\text{E},\mathbf{r},t) + [k_{-1}(\mathbf{r}) + k_2(\mathbf{r})]p(\text{ES},\mathbf{r},t) \tag{9.a}$$

$$\frac{\partial p(\text{ES},\mathbf{r},t)}{\partial t} = [L_\text{ES} - k_{-1}(\mathbf{r}) - k_2(\mathbf{r})]p(\text{ES},\mathbf{r},t) + k_1^{\,0}(\mathbf{r})[\text{S}]\, p(\text{E},\mathbf{r},t) \tag{9.b}$$

The steady-state probabilities, $p_{ss}(\text{E},\mathbf{r})$ and $p_{ss}(\text{ES},\mathbf{r})$, satisfy

$$[L_\text{E} - k_1^{\,0}(\mathbf{r})[\text{S}]]p_{ss}(\text{E},\mathbf{r}) + [k_{-1}(\mathbf{r}) + k_2(\mathbf{r})]p_{ss}(\text{ES},\mathbf{r}) = 0 \tag{10.a}$$

$$[L_{ES} - k_{-1}(\mathbf{r}) - k_2(\mathbf{r})]p_{ss}(ES,\mathbf{r}) + k_1^0(\mathbf{r})[S]\, p_{ss}(E,\mathbf{r}) = 0 \quad (10.b)$$

The steady-state distributions are normalized as $\int(p_{ss}(E,\mathbf{r}) + p_{ss}(ES,\mathbf{r}))\, d\mathbf{r} = 1$. The above equations must in general be solved numerically even for the steady-state distributions.

The steady-state enzymatic velocity, which is equal to the average number of turnovers per unit time or to the reciprocal of the mean time between successive catalytic events, is given by

$$v = \int k_2(\mathbf{r})\, p_{ss}(ES,\mathbf{r})\, d\mathbf{r} \quad (11)$$

In this paper, we focus primarily on the dependence of v on substrate concentration. The formalism to calculate other quantities (e.g., the distribution of the time between consecutive turnovers) has been presented elsewhere.[16,17]

In general, the dependence of v on substrate concentration for a fluctuating enzyme cannot be described by the MM equation. In the next three sections, we discuss when it can be.

3. Quasi-static Condition

Let us assume that the conformational dynamics in one of the states (E or ES) is much slower than all other processes including the conformational dynamics in the other state (ES or E). We now consider two cases: (1) E is essentially static and (2) ES is essentially static.

For case 1, we can set $L_E = 0$ in eq 10.a. The sum of eqs 10.a and 10.b says that $L_E p_{ss}(E,\mathbf{r}) + L_{ES}p_{ss}(ES,\mathbf{r}) = 0$. It then follows that $L_{ES}p_{ss}(ES,\mathbf{r}) = 0$ and hence $p_{ss}(ES,\mathbf{r})$ must be proportional to $\exp(-\beta U_{ES}(\mathbf{r}))$ as a result of eq 2. Using the ansatz $p_{ss}(ES,\mathbf{r}) = \alpha \exp(-\beta U_{ES}(\mathbf{r}))$ in eq 10 together with $L_E = 0$, we find that

$$v = \frac{\chi_2[S]}{[S] + C_M} \quad (12)$$

where the apparent catalytic rate (χ_2) and apparent Michaelis constant (C_M) are given by

$$\chi_2 = \langle k_2\rangle_{ES}, \quad C_M = \langle K_M\rangle_{ES} \quad (13)$$

where $K_M(\mathbf{r}) = (k_{-1}(\mathbf{r}) + k_2(\mathbf{r}))/k_1^0(\mathbf{r})$ is the Michaelis constant for fixed conformational coordinate.

Next, consider case 2 where the conformational dynamics in the ES state is essentially static. Now, one can set $L_{ES} = 0$ in eq 10.b and consequently $L_E p_{ss}(E,\mathbf{r}) = 0$. Using the ansatz $p_{ss}(E,\mathbf{r}) = \alpha \exp(-\beta U_E(\mathbf{r}))$ in eq 10 together with $L_{ES} = 0$, we find that v has the same MM form in eq 12 but now the apparent coefficients are given by

$$\chi_2 = \left\langle \frac{k_2}{K_M} \right\rangle_E \Big/ \left\langle \frac{1}{K_M} \right\rangle_E, \quad \frac{1}{C_M} = \left\langle \frac{1}{K_M} \right\rangle_E \quad (14)$$

When $U_E(\mathbf{r}) = U(\mathbf{r})$, this result is the same as that in eq 6.58 of Gopich and Szabo[17] that was derived under more restrictive conditions. When $U(\mathbf{r}) = 0$, it is the continuum limit of eq 30 of Kou et al.[16] that used a different notation. To make the relationship explicit, let us define a weigh function, $w(\mathbf{r})$,

$$w(\mathbf{r}) \equiv \frac{k_2(\mathbf{r})e^{-\beta U_E(\mathbf{r})}}{K_M(\mathbf{r})} \Big/ \int \frac{k_2(\mathbf{r})e^{-\beta U_E(\mathbf{r})}}{K_M(\mathbf{r})}\, d\mathbf{r} \quad (15)$$

Then, the apparent rate constants in eq 14 can be written in the form

$$\frac{1}{\chi_2} = \int \frac{w(\mathbf{r})}{k_2(\mathbf{r})}\, d\mathbf{r}, \quad C_M = \chi_2 \int \frac{w(\mathbf{r})\, K_M(\mathbf{r})}{k_2(\mathbf{r})}\, d\mathbf{r} \quad (16)$$

that was given by Kou et al.[16] It is based on this representation that they concluded that χ_2 is related to the "harmonic mean" of the conformationally dependent catalytic rate ($k_2(\mathbf{r})$). However, because the weigh function ($w(\mathbf{r})$) depends on $k_2(\mathbf{r})$, this may be misleading and we now prefer the representation of eq 14.

It should be pointed out that the above quasi-static condition does not simply assume that the conformational dynamics in *both* the E and ES states is much slower than binding and catalysis. For example, if the conformational dynamics in both states is very slow but occur on the same time scale, one does not get MM behavior.[17] In fact, our quasi-static condition assumes that there is time scale separation in the conformational fluctuations of the E and ES states.

4. Quasi-equilibrium Condition

The quasi-equilibrium condition (i.e., product formation is much slower than substrate dissociation, $k_2(\mathbf{r}) \ll k_{-1}(\mathbf{r})$) has a history as long as that of enzymology. It was invoked in 1902 by Henri who gave the first rate equation for reactions involving enzymes.[24] Eleven years later, still using this condition, Michaelis and Menten confirmed Henri's experimental work and presented what is now called the MM equation.[1] It was not until 1925 that Briggs and Haldane proposed the steady-state approximation[25] that did not require the restriction of equilibrium imposed by Henri, Michaelis, and Menten.[1,24]

We will show that, under the quasi-equilibrium condition, fluctuating enzymes will always obey MM kinetics *irrespective* of time scales of conformational fluctuations in the free enzyme and enzyme−substrate complex states. If the condition $k_2(\mathbf{r}) \ll k_{-1}(\mathbf{r})$ is satisfied, the equations that determine the steady-state distributions ($p_{ss}(I,\mathbf{r})$) (i.e., eq 10) and the equilibrium distributions in the absence of catalysis (i.e., eq 4) are identical and hence $p_{ss}(I,\mathbf{r}) = p_{eq}(I,\mathbf{r})$, $I = E, ES$. This is the very reason that $k_2(\mathbf{r}) \ll k_{-1}(\mathbf{r})$ is termed as the quasi-equilibrium condition.

Thus, to determine the velocity (v), all we have to do is to use eq 8.b in eq 11. This immediately leads to the MM relation given in eq 12 with

$$\chi_2 = \langle k_2\rangle_{ES}, \; C_M = \langle k_{-1}\rangle_{ES}/\langle k_1^0\rangle_E \quad (17)$$

Note that these are the same as obtained from simple enzyme kinetics using the conformationally averaged rate coefficients $\langle k_1^0\rangle_E$, $\langle k_{-1}\rangle_{ES}$, and $\langle k_2\rangle_{ES}$.

The quasi-equilibrium scenario would not be applicable to a "perfectly evolved enzyme"[26] where each binding event almost always leads to product (i.e., $k_2(\mathbf{r}) \gg k_{-1}(\mathbf{r})$). Thus, it would be interesting to perform single-molecule experiments on such an enzyme to see whether large amplitude fluctuations of the catalytic rate and MM behavior of the steady-state rate would still coexist.

5. Conformational-Equilibrium Condition

We have seen in the previous section that, if $k_2(\mathbf{r}) \ll k_{-1}(\mathbf{r})$, the steady-state populations of the E and ES states are

proportional to the Boltzmann distributions of these states. For the sake of completeness, we shall now show that this is also the case when the ratio of the catalytic and dissociation rates does not depend on the enzyme−substrate conformation (i.e., $k_2(\mathbf{r})/k_{-1}(\mathbf{r}) = q$ independent of $\mathbf{r}$). To see this, note that eq 10 can be reduced to eq 4 with $k_{-1}(\mathbf{r})$ being replaced by an effective $k_{-1}^{\text{eff}}(\mathbf{r}) \equiv k_{-1}(\mathbf{r}) + k_2(\mathbf{r}) = (1 + q)k_{-1}(\mathbf{r})$. Then, all the steps in the derivation of the previous section remain unaltered. In this way, one obtains the MM relation, but now

$$\chi_2 = \langle k_2 \rangle_{\text{ES}}, \quad C_{\text{M}} = (\langle k_{-1} \rangle_{\text{ES}} + \langle k_2 \rangle_{\text{ES}})/\langle k_1^{\,0} \rangle_{\text{E}} \qquad (18)$$

Physically, the independence of $k_2(\mathbf{r})/k_{-1}(\mathbf{r})$ on the conformational coordinate ($\mathbf{r}$) simply means that the free energies of the transition states for catalysis and for substrate dissociation have the same dependence on the conformational coordinate. Even though these processes involve breaking of different kinds of interactions, this scenario would be relevant if both transition states occurred early along the reaction path (i.e., the transition states were reactant-like).

6. An Example: Single-Molecule β-Galactosidase Assay

We now consider a recent single-enzyme experiment[15,27] in light of the results presented above. English et al.[15] have demonstrated that while the catalytic rate of a single β-galactosidase fluctuates over a broad range of time scales (10^{-3}−10 s), the average turnover velocity nevertheless exhibits MM behavior. In this work, the quasi-static condition was invoked to reconcile MM behavior and dynamic fluctuations.[14−16] However, as shown in section 3, this scenario requires that the conformational dynamics in the ES state to be not only much slower than binding and catalysis but also slower than the dynamics in the E state (or vice versa). This requirement appears to be somewhat stringent in light of the current view that protein conformational fluctuations occur on essentially all time scales.

Here, we will consider whether the quasi-equilibrium condition (see section 4) provides a more plausible explanation. This condition requires only that the catalytic rate ($k_2(\mathbf{r})$) be much smaller than the substrate dissociation rate ($k_{-1}(\mathbf{r})$) irrespective of the time scale of conformational fluctuations. The parameters used by English et al.[15] to fit their data ($k_{-1}(\mathbf{r}) = 18\,300$ s^{-1} and apparent $k_2(\mathbf{r}) = 730$ s^{-1}) do satisfy the quasi-equilibrium condition. However, since the fitting may be model dependent and since the dissociation rate has apparently not been measured directly, we adopt the following strategy. In the quasi-equilibrium scenario, the ratio of the apparent k_2 (i.e., χ_2) and the apparent K_{M} (i.e., C_{M}) is $\chi_2/C_{\text{M}} = \langle k_1^{\,0} \rangle_{\text{E}}(\langle k_2 \rangle_{\text{ES}}/\langle k_{-1} \rangle_{\text{ES}}) \ll \langle k_1^{\,0} \rangle_{\text{E}}$. If we assume that the association rate is nearly diffusion-controlled (i.e., $\langle k_1^{\,0} \rangle_{\text{E}} \geq 10^8$ M^{-1} s^{-1}), then we can check whether this inequality is consistent with experiment. English et al.[15] measured χ_2 and C_{M} to be around 730 s^{-1} and 380 μM, respectively. Thus, χ_2/C_{M} is about 2×10^6 s^{-1} M^{-1}, indicating that in this case the quasi-equilibrium scenario is a plausible explanation for the coexistence of conformational dynamics on all time scales and simple MM dependence of the mean enzymatic velocity on substrate concentration.

As additional support for this scenario, let us examine the substrate concentration dependence of the distribution of the time between consecutive catalytic events (i.e., the waiting time distribution, $f(t)$). It was recently predicted[17] that when $k_2(\mathbf{r}) \ll k_{-1}(\mathbf{r})$, $U_{\text{E}}(\mathbf{r}) = U_{\text{ES}}(\mathbf{r})$, and conformational dynamics is slower than catalysis, then the waiting time distribution can be described by a universal function of time for all substrate concentrations. Specifically, plots of $f(t)/\upsilon$ vs υt should superimpose for all [S].

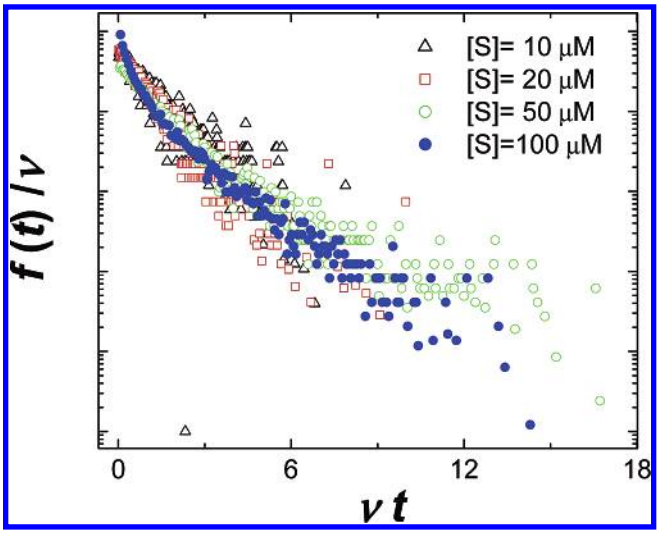

Figure 1. Scaling property of the normalized waiting time distributions ($f(t)$) for different [S]. The data of English et al.[15] are replotted as $f(t)/\upsilon$ vs υt for four different substrate concentrations: [S] = 10, 20, 50, and 100 μM, respectively. υ is the average number of turnovers (catalytic velocity) and is related to $f(t)$ via $\upsilon^{-1} = \int_0^\infty t\, f(t)\, \mathrm{d}t$.

As shown in Figure 1, experiment[15] tends to support this prediction, given the intrinsic errors. In light of this and the limited dynamic range at low substrate concentrations, it seems that it was premature to conclude that the time course of $f(t)$ changes from single-exponential to multiexponential with increasing substrate concentration.[15]

7. Concluding Remarks

In this paper, we have developed a theoretical formalism to describe the kinetics of a fluctuating enzyme that incorporates the condition of detailed balance for substrate binding and dissociation. Our focus is on the dependence of the steady-state rate of product formation on the substrate concentration, which can be influenced by the time scale and amplitude of enzyme fluctuations. Although a fluctuating enzyme does not in general exhibit MM steady-state kinetics, it does so in a large region of parameter space, albeit with apparent Michaelis and catalytic rate constants that have different microscopic interpretations. Thus, deviations from MM behavior may occur rarely and/or be small and difficult to detect experimentally. Many enzymes for which the MM relation holds on the ensemble level exhibit dynamic fluctuations in protein conformation and catalytic activity with a broad range of time scales on the single-molecule level. These notions have implications to biochemistry and cell biology, especially for systems containing a low copy number of enzyme molecules.

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