Theory and Simulation of the Influence of Diffusion in Enzyme-Catalyzed Reactions

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The Michaelis−Menten equation for the kinetics of a simple enzyme-catalyzed reaction is based on the assumption that the two steps of the reaction, (i) reversible formation of the enzyme−substrate complex (ES) by diffusional encounter and (ii) irreversible conversion of the substrate in ES to product, are both described by ordinary rate equations. It is well-known that the rate coefficient, $k(t)$, for enzyme−substrate binding is time dependent due to the influence of diffusion. Will the influence of diffusion lead to non-Michaelis−Menten kinetics? To address this question, three theoretical approaches to account for the influence of diffusion on the kinetics of enzyme-catalyzed reactions are discussed and tested on a model system. It is found that the restriction on the site for enzyme−substrate binding makes the time dependence of $k(t)$ sufficiently weak so that deviation from the Michaelis−Menten equation is unlikely to be observed. Within the range of parameters that is of practical interest, the three theories all predict that the effective rate constant for substrate association is given by $k(\infty)$ and the effective rate constant for substrate dissociation is given by $k_d k(\infty)/k(0)$, where k_d is the rate constant for ES to form a geminate pair. Previous work has shown that $k(\infty)/k(0)$ depends only weakly on interaction potential, hence favorable electrostatic interactions between enzyme and substrate, while enhancing the association rate constant $k(\infty)$ *significantly*, will suppress the effective dissociation rate constant only *marginally*. By analogy, the release of product is also expected to be marginally affected by electrostatic interactions. Enzymes are thus found to enjoy all the benefits of electrostatic interactions but suffer very little from their side effects.

I. Introduction

The standard model for the kinetics of enzyme-catalyzed reactions is given by the following scheme:[1−3]

$$\mathrm{E} + \mathrm{S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{ES} \xrightarrow{k_{cat}} \mathrm{E} + \mathrm{P} \tag{1.1}$$

The first step in this scheme describes the reversible formation of the enzyme−substrate complex ES by diffusional encounter, while the second step describes the irreversible formation of the product P. When the decrease in the substrate concentration [S] is insignificant and ES is in steady state, one obtains the Michaelis−Menten equation for the rate of production formation:

$$v \equiv \frac{\mathrm{d[P]}}{\mathrm{d}t} = \frac{k_{cat}[\mathrm{E}]_{total}[\mathrm{S}]}{K_M + [\mathrm{S}]} \tag{1.2}$$

where $[\mathrm{E}]_{total}$ is the total enzyme concentration and K_M is the Michaelis constant given by

$$K_M = \frac{k_{-1} + k_{cat}}{k_1} \tag{1.3}$$

The derivation of eq 1.2 is based on the assumption that the two steps of Scheme 1.1 obey ordinary rate equations. The forward process of the first step in Scheme 1.1 is a diffusion-influenced bimolecular reaction. It is well-known that for such a reaction one has to use a time-dependent rate coefficient $k(t)$ rather than a rate constant k_1 in the rate equation.[4,5] The focus of this paper is the influence of diffusion on the kinetics of enzyme-catalyzed reactions.

For the reversible diffusion-influenced reaction described by the first step of Scheme 1.1, Szabo[6] has discussed three approaches to account for the influence of diffusion on its kinetics. In addition, he has shown how unimolecular decay pathways can be incorporated in these approaches. One may view the second step of Scheme 1.1 as unimolecular decay of the species ES. However, unlike in the case of monomer−excimer kinetics treated by Szabo, one recovers the species E upon the decay of ES. This complication can be readily taken care of in two of the approaches discussed by Szabo. In both of these two approaches, the kinetics is determined by a rate equation involving the time-dependent rate coefficient $k(t)$ for the irreversible bimolecular reaction. This is obtained either by simply modifying the rate equation for the above process or by using a superposition approximation. The third approach is based on an inexact convolution relation. We will develop this approach further to treat the kinetics of enzyme-catalyzed reactions.

The accuracy of these approaches is most easily tested by comparing with computer simulations. For this purpose we have chosen a model system that consists of a spherical enzyme and a collection of pointlike substrates (see Figure 1). We will show that the superposition approximation and convolution relation approaches provide lower and upper bounds, respectively, for the simulated rate of product formation. The modified rate equation approach is virtually indistinguishable from the superposition approximation approach. At steady state, these two approaches lead to eq 1.2 for the rate of product formation, with the forward and reverse rate constants for the first step of Scheme 1.1 given by

$$k_1 = k(\infty) \tag{1.4a}$$

$$k_{-1} = k_d k(\infty)/k(0) \tag{1.4b}$$

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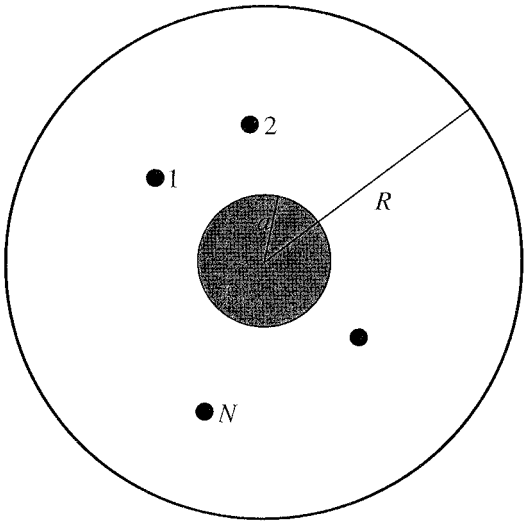

Figure 1. Enzyme−substrate model system. The enzyme is represented by the shaded circle at the center, and the substrates are represented by solid dots dispersed between the enzyme and the large circle representing the container.

where k_d is the rate constant for ES to dissociate into a geminate pair (rather than separated E and S). These are the classical expressions for the steady-state on and off rate constants of a reversible diffusion-influenced reaction.[7] Within the range of parameters that is of practical interest, the difference between the superposition approximation and convolution relation approaches is negligible. Consequently deviation from Michaelis−Menten kinetics due to the influence of diffusion is unlikely to be observed.

Attractive forces between an enzyme and its substrate will accelerate their diffusional encounter. It is well-known that a number of important enzymes exploit favorable electrostatic interactions with their substrates to enhance the rates of product formation.[8] The initial value of $k(t)$, $k(0)$, depends on the interaction potential through the average Boltzmann factor in a region around the active site where substrate binding is effective. Recently we have shown that the steady-state value of $k(t)$, $k(\infty)$, nearly follows the same dependence on the interaction potential.[9] This dependence has been demonstrated on a realistic model for the enzyme acetylcholinesterase.[8b] The nearly identical dependence of $k(0)$ and $k(\infty)$ on the interaction potential means that the effective rate constant k_{-1} for substrate dissociation given by eq 1.4b will have a very *weak* dependence on the interaction potential.

This conclusion is significant in view of recent concern that favorable electrostatic interactions between enzyme and substrate, while accelerating substrate binding, may impede product release. This concern in part led Gilson et al.[10] to propose a "back door" mechanism for product release. While product release has not been explicitly modeled in Scheme 1.1, it can be understood by analogy to substrate dissociation. On the basis of eq 1.4b, we expect that electrostatic interactions will have only a marginal effect on product release.

The outline for the rest of the paper is as follows. In section II we present results of the three theoretical approaches. Section III contains detailed descriptions of the enzyme−substrate model system and our simulation procedure. A comparison of simulation results and the theories is then presented in section IV. Finally, in section V, the practical impact of the findings of the present study is discussed.

II. Theoretical Results

We will study the influence of diffusion on the kinetics of enzyme-catalyzed reactions for times in which the decrease in [S] is insignificant. In general this is the time range that is of interest in experimental studies of enzyme kinetics. In this time range the forward process of the first step in Scheme 1.1 is pseudo-first-order.

A. Limiting Situations. When $k_{cat} \rightarrow 0$, Scheme 1.1 becomes one for a reversible diffusion-influenced reaction. We now briefly summarize the three approaches discussed by Szabo[6] to account for the influence of diffusion on the kinetics in this situation. In the simplest approach, the kinetics of the reversible reaction is determined by the rate equation

$$\frac{d[ES]}{dt} = k(t)[E][S] - \frac{k(t)}{k(0)} k_d[ES] \tag{2.1}$$

and the condition

$$[E] + [ES] = [E]_{total} \tag{2.2}$$

The rate coefficient $k_d k(t)/k(0)$ for the reverse process is fixed by ensuring that, at steady state, eq 2.1 gives the correct equilibrium concentrations. These are determined by the equilibrium dissociation constant

$$K_S = k_d/k(0) \tag{2.3}$$

A much more sophisticated approach is based on using a superposition approximation to truncate the hierarchy of equations satisfied by the reduced distribution functions of the reactant molecules. The final result can be written as

$$\frac{d[ES]}{dt} = k_f(t)[E][S] - k_d[ES] \tag{2.4}$$

where

$$k_f(t) = k(t) - K_S \int_0^t \phi(t - t') \frac{dk(t')}{dt'} dt' \tag{2.5}$$

with

$$\phi(t) = [ES(t)]/[E(t)][S] \tag{2.6}$$

By further making the approximation $\phi(t - t') \approx \phi(t)$ in eq 2.5, eq 2.4 reduces to eq 2.1.

The third approach is based on the assumption that each time the complex ES dissociates to form a geminate pair of E and S at contact, it is surrounded by an equilibrium distribution of S. The concentration of ES at time t is then given by a convolution relation:

$$[ES(t)] = [1 - S(t|eq)][E]_{total} - k_d \int_0^t [ES(t')] S(t - t'|gem)\, dt' \tag{2.7}$$

where $S(t|eq)$ is the survival probability of an E surrounded by an equilibrium distribution of S, given by

$$S(t|eq) = \exp\{-[S] \int_0^t k(t')\, dt'\} \tag{2.8}$$

and $S(t|gem)$ is the survival probability of an E in the presence

of both its geminate partner and an equilibrium distribution of S, given by

$$S(t|\text{gem}) = \frac{k(t)}{k(0)} S(t|\text{eq}) \tag{2.9}$$

Straightforward extensions of these three approaches will be made below to study the kinetics of enzyme-catalyzed reactions. Here we note that, in the limit $k_{cat} \rightarrow 0$, the exact kinetics of the first step in Scheme 1.1 is irrelevant to the rate of product formation. In this limit, destruction of ES by product formation is so infrequent that its concentration always has time to reach the equilibrium value $[ES]_{eq} = [E]_{total}[S]/(K_S + [S])$. The rate of product formation, $v = d[P]/dt$, is thus

$$v = k_{cat}[ES]_{eq} = \frac{k_{cat}[E]_{total}[S]}{K_S + [S]} \tag{2.10}$$

When $k_{cat} \rightarrow \infty$, Scheme 1.1 effectively becomes one for an irreversible bimolecular reaction. In this limit, the concentration of ES will approach zero and the concentration of E will approach $[E]_{total}$. The rate of product formation will approach the rate of the forward process of the first step, given by

$$v = k(t)[E]_{total}[S] \tag{2.11}$$

When the substrate concentration $[S] \rightarrow \infty$, the enzyme will always be found in a complex with a substrate and thus $[ES] = [E]_{total}$. Then the rate of product formation is

$$v = k_{cat}[E]_{total} \equiv v_{max} \tag{2.12}$$

The exact results given in the last three equations will be used to check the theories below.

(B) Modified Rate Equation Approach. According to Szabo,[6a] the unimolecular decay pathway described by the second step of Scheme 1.1 can be taken into consideration by viewing eq 2.1 as an ordinary rate equation for the first step of the Scheme. Then

$$\frac{d[ES]}{dt} = k(t)[E][S] - \frac{k(t)}{k(0)} k_d[ES] - k_{cat}[ES] \tag{2.13}$$

This should again be solved in conjunction with eq 2.2. The rate of product formation is

$$v = \frac{d[P]}{dt} = k_{cat}[ES] \tag{2.14}$$

By checking against eqs 2.10−2.12, it is easy to confirm that eq 2.13 correctly predicts the rate of production when $k_{cat} \rightarrow 0$ or ∞ or when $[S] \rightarrow \infty$. As noted by Szabo, eq 2.1 is not exact for the kinetics of a reversible diffusion-influenced reaction in the limit $[S] \rightarrow 0$. Similarly, eq 2.13 is not exact for the kinetics of an enzyme-catalyzed reaction in this limit (see below). When $t \rightarrow \infty$, eq 2.13 predicts that the steady-state concentration of ES is

$$[ES(\infty)] = \frac{[E]_{total}[S]}{K_S + k_{cat}/k(\infty) + [S]} \tag{2.15}$$

The steady-state rate of product formation is then given by eq 1.2.

(C) Superposition Approximation Approach. The generalization of eq 2.4 to account for the second step of Scheme 1.1 is again straightforward. The result is

$$\frac{d[ES]}{dt} = k_f(t)[E][S] - k_d[ES] - k_{cat}[ES] \tag{2.16}$$

with $k_f(t)$ specified by eqs 2.5 and 2.6. Of course eq 2.13 can be obtained from eq 2.16 by making the approximation $\phi(t - t') \approx \phi(t)$. The superposition approximation approach correctly predicts the rate of production when $k_{cat} \rightarrow 0$ or ∞ or when $[S] \rightarrow \infty$. It also correctly predicts the rate of production when $[S] \rightarrow 0$ (see below). The predicted steady-state rate of product formation is again given by eq 1.2.

(D) Convolution Relation Approach. As in the case of a reversible diffusion-influenced reaction,[6c] this approach is best illustrated by working with an isolated pair of E and S. There are two distinct species of unbound E. In one of them, denoted by E_1, E is accompanied by S at some separation. The other, denoted by E_0, is just E by itself as S has already been converted to product. There is only one bound species of E, which will be denoted by ES. The aim is to find relations among the probabilities for finding the three species. We will denote these probabilities by the symbols of the species enclosed by angular brackets. If the pair of E and S is prepared in a volume V, then the concentration of a species, say ES, is given by $[ES] = \langle ES\rangle/V$.

Let us first consider the situation that $\langle ES\rangle = 1$ and $\langle E_1\rangle = \langle E_0\rangle = 0$ at $t = 0$. As noted by Agmon and Szabo,[6c] the key to obtain $\langle E_1\rangle$ at time t is the observation that it is the accumulation of the fraction of geminate pair, generated by substrate dissociation at earlier times, that stays unbound. The probability that a geminate pair generated at time t' is still unbound at time $t > t'$ is

$$S(t - t'|\text{gem}) = \frac{k(t - t')}{k(0)} \tag{2.17}$$

Since between time t' and $t' + dt'$ the amount of geminate pair generated by substrate dissociation is $k_d\langle ES(t')\rangle dt'$, the probability for finding E_1 at time t is

$$\langle E_1(t)\rangle = k_d \int_0^t \langle ES(t')\rangle S(t - t'|\text{gem})\, dt' \tag{2.18}$$

The population of ES decays either by substrate dissociation to form geminate pair or by substrate conversion to product. The total amount of ES lost at time t by these mechanisms is

$$(k_d + k_{cat}) \int_0^t \langle ES(t')\rangle\, dt'$$

Of the amount of geminate pair generated between t' and $t' + dt'$, the fraction that is rebound at time t is obviously $1 - S(t - t'|\text{gem})$. Hence

$$\langle ES(t)\rangle = 1 + k_d \int_0^t \langle ES(t')\rangle[1 - S(t - t'|\text{gem})]\, dt' - (k_d + k_{cat}) \int_0^t \langle ES(t')\rangle\, dt'$$

$$= 1 - k_d \int_0^t \langle ES(t')\rangle S(t - t'|\text{gem})\, dt' - k_{cat} \int_0^t \langle ES(t')\rangle\, dt' \tag{2.19}$$

The amount of E_0, generated by substrate conversion to product

in the bound pair, is

$$\langle E_0(t)\rangle = k_{cat}\int_0^t \langle ES(t')\rangle\, dt' \tag{2.20}$$

It can be easily confirmed that $\langle E_1(t)\rangle + \langle E_0(t)\rangle + \langle ES(t)\rangle = 1$.

We are actually interested in the situation that initially E and S are unbound with their separation distributed according to the equilibrium probability. Then one has to allow for the possibility that the unbound pair can exist with S never binding to E. The probability for this is

$$S(t|eq) = 1 - \frac{\int_0^t k(t')\, dt'}{V} \tag{2.21}$$

The generalizations of eq 2.18 and 2.19 are

$$\langle E_1(t)\rangle = S(t|eq) + k_d\int_0^t \langle ES(t')\rangle S(t-t'|gem)\, dt' \tag{2.22}$$

$$\langle ES(t)\rangle = 1 - S(t|eq) - k_d\int_0^t \langle ES(t')\rangle S(t-t'|gem)\, dt' - k_{cat}\int_0^t \langle ES(t')\rangle\, dt' \tag{2.23}$$

No change is required for eq 2.20.

The rate of product formation is the same as the rate of the appearance of E_0. It depends on $\langle ES\rangle$ according to eq 2.20. By taking the time derivative of this equation and then dividing by V, one obtains eq 2.14. The value of $\langle ES\rangle$ is determined by eq 2.23. By taking the time derivative and then dividing by V, this equation becomes

$$d[ES]/dt = k(t)[E]_{total}[S] - K_S\int_0^t [ES(t-t')](dk(t')/dt')dt' - k_d[ES] - k_{cat}[ES] \tag{2.24}$$

where $[E]_{total} = 1/V$ and $[S] = 1/V$ are the initial enzyme and substrate concentrations in the present situation. This gives the exact result for the concentration of ES in the limit $[S] \to 0$. It can be checked that eq 2.16 correctly reduces to this limiting equation.

When an E is surrounded by an equilibrium distribution of S at time $t = 0$, no simple convolution relations hold at $t > 0$ between the probability for finding the species with an unbound E surrounded by a certain number of S and that for finding the species with a bound E surrounded by the same or a different number of S. Agmon and Szabo[6c] introduced the approximation that each geminate pair newly generated by substrate dissociation is surrounded by an equilibrium distribution of S. We extend this by making the additional approximation that each E newly freed by substrate conversion to product is surrounded by an equilibrium distribution of S. Under these approximations, the problem of calculating $\langle ES(t)\rangle$, the probability for finding all the species with a bound E, is completely analogous to that in the case of an isolated pair. The details of the derivation are given in the Appendix. The final result is

$$[ES(t)] = (1 - S(t|eq))[E]_{total} - k_d\int_0^t [ES(t')] \times S(t-t'|gem)\, dt' - k_{cat}\int_0^t [ES(t')]S(t-t'|eq)\, dt' \tag{2.25}$$

This generalizes eq 2.7 by the addition of the last term involving k_{cat}. The rate of product formation, d[P]/dt, is obtained from $[ES(t)]$ through eq 2.14.

In the limit $[S] \to 0$, $S(t|eq) \approx 1 - [S]\int_0^t k(t')\, dt'$, and $S(t|gem) \approx k(t)/k(0)$. In the opposite limit $[S] \to \infty$, $S(t|eq) = S(t|gem) = 0$. These results can be used to confirm that eq 2.25 correctly predicts d[P]/dt in the two limits of [S]. It is also easy to see that the correct result for d[P]/dt is obtained when $k_{cat} \to 0$, as the counterpart of eq 2.25 in this limit, eq 2.7, gives the correct equilibrium value for [ES]. However, when $k_{cat} \to \infty$, eq 2.25 does not predict the correct result for d[P]/dt given by eq 2.11. In this limit, the approximation that an E newly freed by substrate conversion to product is always surrounded by an equilibrium distribution of S becomes very poor.

The steady-state concentration of ES predicted by eq 2.25 is

$$[ES(\infty)] = \frac{[E]_{total}[S]}{K_S + \alpha k_{cat}/k(\infty) + [S]} \tag{2.26}$$

where

$$\alpha = k(\infty)[S]\int_0^\infty S(t|eq)dt = \int_0^\infty e^{-[S]\int_0^t k(t')dt'}\, dt \Big/ \int_0^\infty e^{-[S]\int_0^t k(\infty)dt'}\, dt \tag{2.27}$$

If $\alpha = 1$, eq 2.26 is identical with eq 2.15, which is the prediction of the two earlier approaches. In general α depends the substrate concentration and thus the steady-state rate of product formation in the present approach deviates from the Michaelis−Menten equation. The value of α is always less than or equal to 1. This can be seen by rewriting eq 2.27 as $\alpha = \int_0^\infty e^{-[S]\int_0^t [k(t')-k(\infty)]dt'} e^{-[S]\int_0^t k(\infty)dt'}\, dt / \int_0^\infty e^{-[S]\int_0^t k(\infty)dt'}\, dt$. Since $k(t) \geq k(\infty)$, the first factor of the integrand in the numerator cannot be greater than 1. This factor and hence α are equal to 1 only when $[S] \to 0$ or when $k(t)$ is a constant over the whole time range. When $k(t)$ is a constant, all the three approaches become identical.

III. Enzyme−Substrate Model and Simulation Procedure

The model system that we have simulated to test the different theories is shown in Figure 1. In this system, N substrates, with diffusion constant D, are freely diffusing within a spherical container with radius R. Fixed at the center of the container is a spherical enzyme (with radius a). After diffusing to the surface of the enzyme, a substrate may bind to the enzyme if it is free. The binding is modeled by a partially reflecting or radiation boundary condition[11] with an intrinsic association rate constant k_a:

$$4\pi Da^2 \frac{\partial[p(r,t)/r^2]}{\partial r}\Big|_{r=a} = k_a[p(a,t)/a^2] \tag{3.1}$$

where $p(r, t)$ is the probability density for finding the substrate at a distance r from the center of the enzyme at time t. If the enzyme is already bound with a substrate, its surface becomes reflecting to any other substrate and then

$$\frac{\partial[p(r,t)/r^2]}{\partial r}\Big|_{r=a} = 0 \tag{3.2}$$

A bound substrate may either dissociate, with a rate constant k_d, to any point on the surface of the enzyme or form product with a rate constant k_{cat}. This model is chosen because extensive analytical results for it are available[5,6] and its simulations are relatively easy to carry out. A similar model has recently been used to study the effect of conformational gating on irreversible ligand binding.[12]

We take advantage of the spherical symmetry of the model and deal only with the radial distance r of each substrate. Diffusion in the three-dimensional space is equivalent to

diffusion along r under an effective potential $U(r) = -2\ln(r)/k_BT$.[13] The movement of each substrate is generated by[14]

$$r = r_0 + 2D\Delta t/r_0 + (2D\Delta t)^{1/2}z \tag{3.3}$$

where Δt is the time step and z is a normally distributed random number. Movement near a boundary (at $r = a$ or R) has to be handled with care. The outer boundary $r = R$ is taken care of by assuming that there the effective potential $U(r)$ is constant. Then any move generated by eq 3.3 that brings the substrate across the boundary is simply reflected back.

The inner boundary $r = a$ is given a much more elaborate treatment. This treatment is invoked whenever the initial distance r_0 is between a and a larger distance a_1. The latter is chosen so that the chance of moving from it to $r \le a$ in a single step according to eq 3.3 is practically zero. If the enzyme is free initially, there is a certain probability that the substrate may bind to it after time Δt. This is given by

$$p_{bnd} = \frac{\kappa}{\kappa+1}\frac{a}{r_0}e^{-x^2}[\varphi(x) - \varphi(x + (1+\kappa)\sqrt{D\Delta t}/a)] \tag{3.4}$$

where $\kappa = k_a/4\pi Da$, $x = (r_0 - a)/2(D\Delta t)^{1/2}$, and $\varphi(x) = \exp(x^2)\mathrm{erfc}(x)$. Whether binding occurs in a particular step is decided by comparing p_{bnd} with a uniformly distributed random number. If the random number is larger, the substrate stays unbound. Then the probability density for finding it at r is

$$p(r) = \frac{r}{r_0^2 x_3}(e^{-x_1^2} + e^{-x_2^2}) - \frac{r}{ar_0}(1+\kappa)e^{-x_2^2}\varphi(x_2 + (1+\kappa)\sqrt{D\Delta t}/a) \tag{3.5}$$

normalized by $1 - p_{bnd}$. In eq 3.5, we have introduced the notations $x_1 = (r - r_0)/2(D\Delta t)^{1/2}$, $x_2 = (r + r_0 - 2a)/2(D\Delta t)^{1/2}$, and $x_3 = 2(\pi D\Delta t)^{1/2}/r_0$.

The distribution $p(r)$ is generated by the rejection method using different comparison functions in $a < r < a_1$ and $r > a_1$. Which region to put the substrate is determined by comparing $p_{r\ge a_1}/(1 - p_{bnd})$ with a uniformly distributed random number, where

$$p_{r\ge a_1} = {}^1/_2 e^{-y_1^2}[\varphi(y_1) + x_3/\pi] + {}^1/_2 e^{-y_2^2}[(1 - 2\kappa a/(1+\kappa)r_0)\varphi(y_2) - x_3/\pi + 2(a_1/r_0 - a/(1+\kappa)r_0)\varphi(y_2 + (1+\kappa)\sqrt{D\Delta t}/a)] \tag{3.6}$$

is the integration of $p(r)$ over the region $r > a_1$. In eq 3.6 we have introduced the notations $y_1 = (a_1 - r_0)/2(D\Delta t)^{1/2}$ and $y_2 = (a_1 + r_0 - 2a)/2(D\Delta t)^{1/2}$. If the random number is larger, then the substrate is put in $a < r < a_1$. Here the comparison function is

$$q_1(r) = \frac{a}{r_0^2 x_3}(e^{-x_1^2} + e^{-x_2^2}) \tag{3.7}$$

This is the distribution for a free one-dimensional Brownian particle in the presence of a reflecting boundary. The comparison function in $r > a_1$ is

$$q_2(r) = \frac{2r}{r_0^2 x_3}e^{-x_1^2} = \frac{2x_1}{r_0\sqrt{\pi}}e^{-x_1^2} + \frac{2}{r_0 x_3}e^{-x_1^2} \tag{3.8}$$

The first term of eq 3.8 represents an exponential distribution for x_1^2 and the second term represents a normal distribution for x_1. Both can be generated using standard algorithms.[15] This concludes our prescription for simulating the movement of a substrate when the enzyme is free before the time step. The situation that the enzyme is bound by another substrate before the time step can be handled analogously with κ set to zero.

When the enzyme is bound with a substrate, it may become free again by two mechanisms: substrate dissociation to form geminate pair or substrate conversion to product. Which of these events actually occurs is determined as follows. After each time step Δt, $\exp[-(k_d + k_{cat})\Delta t]$ is compared with a uniformly distributed random number. If the random number is larger, then the enzyme becomes free. The mechanism that has freed the enzyme is then determined by comparing $k_d/(k_d + k_{cat})$ with another uniformly distributed random number. If this random number is smaller, then the bound substrate is dissociated and its radial distance is assigned to a. Otherwise, the substrate is destroyed and the formation of a product is recorded.

The overall design of the simulation is as follows. At $t = 0$, N substrates are assigned radial distances between a and R from the distribution $p(r) = 3r^2/(R^3 - a^3)$, which corresponds to a uniform distribution in the three-dimensional space. The substrates are then moved one by one. Each substrate has four possible moves. If initially it is unbound, it may move either to another distance between a and R or inside the enzyme (provided the enzyme is available for binding). If initially the substrate is bound to the enzyme, it may dissociate to $r = a$ or convert to product. After each time step, the status (free or bound) of the enzyme and the number (0 or 1) of product formed are recorded. The substrates are followed until either they have been converted to product or the desired number of time steps has been completed. This procedure is repeated many times.

IV. Test of Theories

For the model system simulated in the previous section, the time-dependent rate coefficient for the irreversible formation of ES by diffusional encounter is[5]

$$k(t) = \frac{k_a}{\kappa+1}[1 + \kappa\varphi((1+\kappa)\sqrt{\tau})] \tag{4.1}$$

where $\kappa = k_a/4\pi Da$ and $\tau = Dt/a^2$. The survival probability $S(t|\mathrm{eq})$ is given by $\exp[-\rho i(\tau)]$, where $\rho = 4\pi a^3[S]$ and

$$i(\tau) = \frac{\kappa\tau}{\kappa+1} + \frac{\kappa^2}{(\kappa+1)^3}[2(1+\kappa)\sqrt{\tau/\pi} - 1 + \varphi((1+\kappa)\sqrt{\tau})] \tag{4.2}$$

The governing equations for $[ES(t)]$ in the three theoretical approaches, eqs 2.13, 2.16, and 2.25, can be numerically solved by using the central difference rule to evaluate the time derivative and using the trapezoidal rule to evaluate the time integral. Integration of $[ES(t)]$ over time then yields the product concentration $[P(t)]$. The natural units of time and concentration in the model system are $t_0 = a^2/D$ and $c_0 = 1/4\pi a^3$, respectively. For a typical enzyme (e.g., acetylcholinesterase) catalyzing the reaction of a small substrate (e.g., acetylcholine), the appropriate values of a and D are roughly 40 Å and 50 Å^2/ns, respectively. The above units thus correspond to 30 ns and 2 mM.

In Figure 2, we present the comparison of the result for $[P(t)]$ from the simulations and those from the superposition approximation and convolution-relation approaches at $[S]/c_0 = 0.023$, $\kappa = 20$, $k_d t_0 = 0.45$, and $k_{cat}t_0 = 0.01$, 0.1, and 1. At the smallest of the three k_{cat} values, both theories agree with the simulation very well. As k_{cat} is increased, the growth of

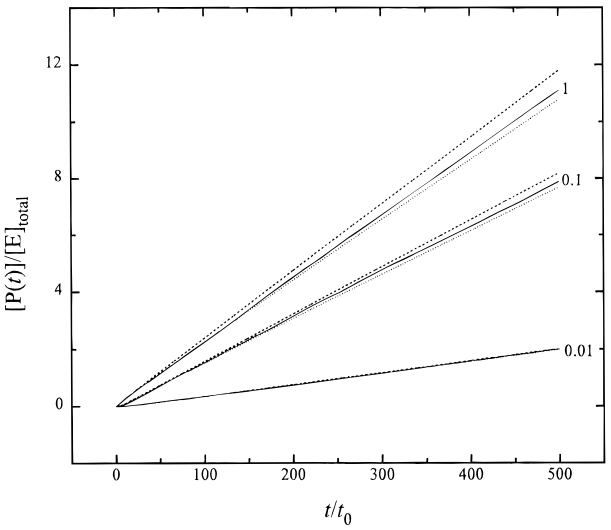

Figure 2. Growth of the product concentration at $[S]/c_0 = 0.023$, $\kappa = 20$, and $k_d t_0 = 0.45$. The values of $k_{cat}t_0$ are shown. Solid lines are obtained by simulating 1000 substrates in the region $a < r < R = 51a$ using a time step $\Delta t/t_0 = 0.1$, whereas dotted and dashed lines are predictions of the superposition approximation and convolution relation approaches, respectively.

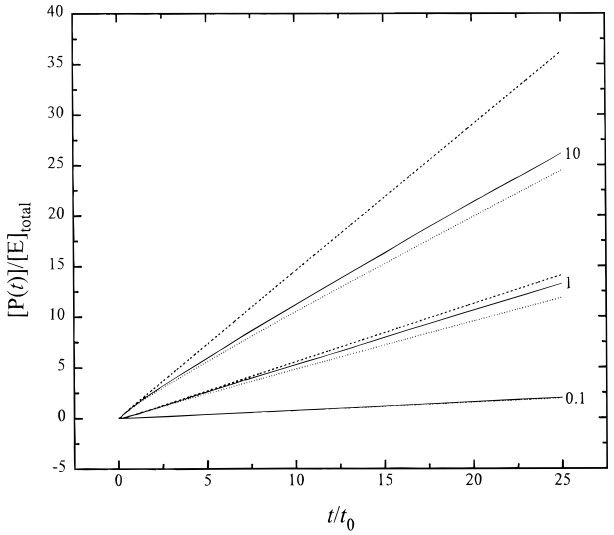

Figure 3. As in Figure 2, but the parameters are $[S]/c_0 = 1.13$, $\kappa = 5$, and $k_d t_0 = 1$. The values of $k_{cat}t_0$ are shown. The simulations involved 500 substrates moving in the region $a < r < R = 11a$ with a time step $\Delta t/t_0 = 0.001$.

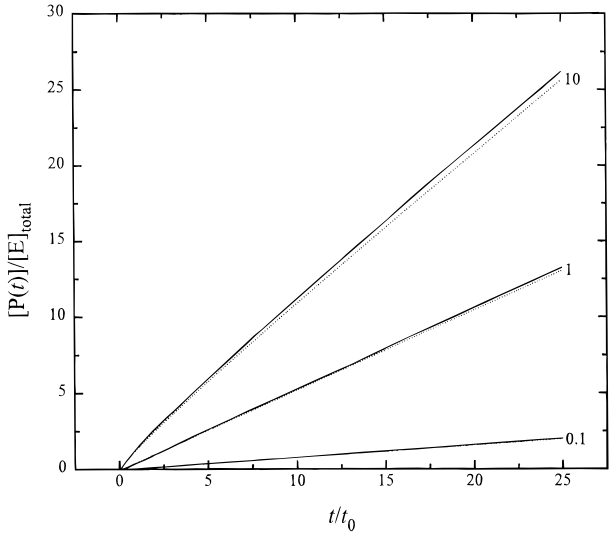

Figure 4. Comparison between the simulations (solid lines) and the predictions of eq 4.3 (dotted lines) for the growth of the product concentration. The parameters are the same as in Figure 3.

[P(t)] predicted by the superposition approximation approach becomes too slow, while that by the convolution relation approach becomes too fast. Hence the two theories provide lower and upper bounds, respectively, for d[P(t)]/dt. As k_{cat} is increased further and further, the accuracy of the superposition approximation approach improves again, consistent with the fact that this theory is exact in the limit $k_{cat} \rightarrow \infty$. On the other hand, the accuracy of the convolution relation approach continues to deteriorate, as the underlying assumption in this theory is very poor when $k_{cat} \rightarrow \infty$.

The prediction for [P(t)] by the modified rate equation approach is virtually indistinguishable from that by the superposition approximation approach. For example, $[P(500t_0)]/[E]_{total} = 1.986$, 7.684, and 10.78 at $k_{cat}t_0 = 0.01$, 0.1, and 1, respectively, in the former theory. These are 1.979, 7.680, and 10.78 in the latter theory. Close agreement between these two theories is observed over a very wide range of the parameters of the model. Thus for practical purpose the modified rate equation approach can replace the more complicated and more computationally demanding superposition approximation approach. The difference between these two theories is more pronounced in the case of reversible diffusion-influenced reactions.[16] The additional step in Scheme 1.1 thus obscures this difference.

The qualitative features shown in Figure 2 are also observed over a wide range of the parameters. The growth of the product concentration at a much higher substrate concentration, $[S]/c_0 = 1.13$, and $\kappa = 5$, $k_d t_0 = 1$, and $k_{cat}t_0 = 0.1$, 1, and 10 is shown in Figure 3. Again the superposition approximation approach provides the lower bound and the convolution relation approach provides the upper bound. One may expect that a proper combination of the two theories will provide a better prediction for [$P(t)$]. One possible combination is

$$[P(t)] = \frac{k_{cat}[P(t)]_{superp} + (K_S + [S])k(\infty)[P(t)]_{convol}}{k_{cat} + (K_S + [S])k(\infty)} \qquad (4.3)$$

where $[P(t)]_{superp}$ and $[P(t)]_{convol}$ refer to the predictions from the two individual theories. This is indeed in better agreement with the simulation than either of the two theories. The comparison between the predictions of eq 4.3 and the simulation results at $[S]/c_0 = 1.13$, $\kappa = 5$, $k_d t_0 = 1$, and $k_{cat}t_0 = 0.1$, 1, and 10 is presented in Figure 4.

It is clear from Figures 2 and 3 that the difference between the superposition approximation and convolution relation approaches grows as time increases. When $t \rightarrow \infty$, the rate of product formation is k_{cat}[ES(∞)] in both theories, but with [ES(∞)] given by eq 2.15 in the former theory and by eq 2.26 in the latter one. For a given value of k_{cat}, the difference between these two expressions for [ES(∞)] are the largest when $k_d = 0$ and $\kappa \rightarrow \infty$. Under the last condition, substrate binding to enzyme is controlled by diffusion and

$$\alpha = 1 - \sqrt{[S]/c_0}\varphi(\sqrt{[S]/\pi c_0}) \qquad (4.4)$$

for the present model system. The value of α decreases from 1 to 0 as [S] is increased from 0 to ∞. When $[S] < 10^{-4}c_0 \sim 0.2$ μM, the value of α deviates from 1 by less than 1% and hence the difference between the superposition approximation and convolution relation approaches will be negligible for the model system under study. Since these theories have been shown to provide lower and upper bounds, respectively, for the

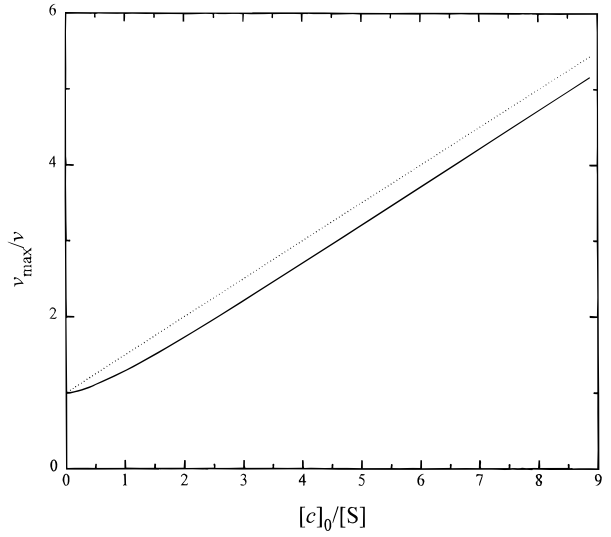

Figure 5. Comparison of the Michaelis−Menten equation (solid line) and eq 4.3 (dotted line) for the steady-state rate of product formation. The parameters are $\kappa = \infty$, $k_d = 0$, and $k_{cat}t_0 = 0.5$.

actual kinetics, the agreement between them means that both theories are accurate within the above range of substrate concentrations. An increase in k_d or a decrease in κ will enlarge the accuracy range for both theories.

The superposition approximation approach, with [ES(∞)] given by eq 2.15, leads to the Michaelis−Menten equation for the steady-state rate of product formation. In contrast, the convolution relation approach, with [ES(∞)] given by eq 2.15, predicts non-Michaelis−Menten steady-state kinetics. When the difference between these two theories is large, the actual kinetics, roughly given by eq 4.3, is likely to exhibit non-Michaelis−Menten behavior. Such behavior is illustrated in Figure 5, in which results for the steady-state rate of product formation from eq 4.3 and the Michaelis−Menten equation at $\kappa = \infty$, $k_d = 0$, and $k_{cat}t_0 = 0.5$ are compared. The infinite value for κ means $k_{cat}/K_M = 4\pi Da$. In conventional units, this is $\sim 10^{10}$ M^{-1} s^{-1}, which is an order of magnitude higher than the highest k_{cat}/K_M actually observed.[17] The value chosen for k_{cat} is $\sim 2 \times 10^7$ s^{-1}, which is roughly the highest k_{cat} actually observed.[17] When either κ or k_{cat} is reduced, the deviation of eq 4.3 at steady state from the Michaelis−Menten equation will diminish.

V. Discussion and Conclusion

We have studied three approaches to account for the influence of diffusion on the kinetics of enzyme-catalyzed reactions. It is found that the superposition approximation approach provides a lower bound for the rate of product formation whereas the convolution relation approach provides an upper bound. The modified rate equation approach is virtually indistinguishable from the superposition approximation approach. These two theories lead to the Michaelis−Menten equation for the steady-state kinetics, but the convolution relation approach does not. The deviation from the Michaelis−Menten equation is described by the factor α given in eq 2.27. This factor is determined by the substrate concentration and the rate coefficient $k(t)$ for substrate binding over the whole time range. In general the value of α is smaller than 1. Only when [S] → 0 or when $k(t)$ is a constant, α = 1 and then the Michaelis−Menten equation is recovered. On the basis of these results, we can now address several key issues that motivated the present study.

A. Non-Michaelis−Menten Kinetics due to Influence of Diffusion. Figure 5 indicates that the influence of diffusion by itself will lead to non-Michaelis−Menten steady-state kinetics. However, this result is obtained for a model in which the enzyme−substrate complex can be obtained by substrate binding to any part of the enzyme surface. In reality, only the part of the enzyme surface around the active site is useful for complex formation. The restriction on the site for substrate binding has two consequences on the rate coefficient $k(t)$ for substrate binding. The first is a well-known one, i.e., the amplitude of $k(t)$ is reduced. The second is that the time dependence of the rate coefficient is weakened. This has not been greatly appreciated but, as we now show, has a profound effect on the kinetics of the enzyme-catalyzed reaction. It makes the occurrence of non-Michaelis−Menten behavior unlikely.

To illustrate this, let us consider an enzyme that is again modeled by a sphere but with only a part of the surface effective for substrate binding. If this part is restricted to the region spanned by the polar angle from 0 to θ, an approximate analytic result can be obtained for the time-dependent rate coefficient $k(t)$.[18] This is given in terms of the Laplace transform, defined as $\hat{f}(s) = \int_0^\infty \exp(-st) f(t)\, dt$, by

$$\hat{k}(s) = \frac{1}{s} \frac{4\pi Da(1-\cos\theta)^2}{\sum_{l=0}^{\infty} \frac{[P_{l-1}(\cos\theta) - P_{l+1}(\cos\theta)]^2/(2l+1)}{l+1+xK_{l-1/2}(x)/K_{l+1/2}(x)}} \quad (5.1)$$

in the diffusion-controlled limit, where $x = (a^2 s/D)^{1/2}$, $P_l(x)$ are Legendre polynomials, and $K_{l+1/2}(x)$ are modified spherical Bessel functions of the third kind. The long and short time behavior of $k(t)$ can be found by letting $s \to 0$ and ∞, respectively. The results are

$$k(\infty) = \frac{4\pi Da(1-\cos\theta)^2}{\sum_{l=0}^{\infty} \frac{[P_{l-1}(\cos\theta) - P_{l+1}(\cos\theta)]^2}{(2l+1)(l+1)}} \equiv 4\pi Da f_\infty \quad (5.2)$$

$$k(t) = \frac{4\pi Da(1-\cos\theta)^2}{\sum_{l=0}^{\infty} \frac{[P_{l-1}(\cos\theta) - P_{l+1}(\cos\theta)]^2}{2l+1}} \sqrt{a^2/\pi Dt} \equiv 4\pi Da f_0 \sqrt{a^2/\pi Dt} \quad (5.3)$$

as $t \to 0$. When the whole enzyme surface is effective for substrate binding (i.e., $\theta = 180°$), $f_0 = f_\infty = 1$. However, when θ is small, f_0 is much smaller than f_∞. For example, for $\theta = 5°$, which corresponds to a disk-shaped binding site with a diameter of ~7 Å, $f_\infty = 2.8 \times 10^{-2}$, and $f_0 = 1.9 \times 10^{-3}$. The smaller value for f_0/f_∞ at $\theta = 5°$ means that the deviation of $k(t)$ at short times from $k(\infty)$ is reduced when compared to the situation at $\theta = 180°$. As a result, the deviation of α in eq 2.27 from 1 and thus the deviation of the steady-state kinetics in the convolution relation approach from the Michaelis−Menten equation are much reduced. Of course the Michaelis−Menten equation is predicted by the superposition approximation approach, and when there is agreement between it and the convolution-relation approach, one can be assured that both theories are accurate.

The dependence of α on the substrate concentration can be qualitatively understood by explicitly evaluating the integral in

eq 2.27 using the sum of eq 5.2 and 5.3 to approximate $k(t)$. The result is

$$a = 1 - \sqrt{f_0^2[\mathrm{S}]/f_\infty c_0}\,\varphi(\sqrt{f_0^2[\mathrm{S}]/\pi f_\infty c_0}) \qquad (5.4)$$

By comparing with eq 4.4, one can easily see that the range of substrate concentrations within which the value of α can be taken as 1 is now enlarged by a factor of f_∞/f_0^2. For $\theta = 5°$, $f_\infty/f_0^2 = 8 \times 10^3$ and the value of α estimated by eq 5.4 is between 0.99 and 1 if $[\mathrm{S}] < 0.8c_0 \sim 2$ mM.

Exact evaluation of α by the numerical Laplace inversion of eq 5.1 shows that the range of [S] in which α is between 0.99 and 1 is even wider than the above estimate. The upper bound of this concentration range is actually $\sim 10c_0$ or ~20 mM. Moreover, even if the substrate concentration is much higher than this upper bound, the difference between the results for the steady-state rate of product formation from the superposition approximation approach and the Michaelis−Menten equation is insignificant. For example, when $[\mathrm{S}] = 113c_0 \sim 240$ mM and $k_{\mathrm{cat}} = 0.5/t_0 \sim 2 \times 10^7\ \mathrm{s}^{-1}$, the steady-state rate of product formation is $0.864v_{\max}$ by using eq 2.15 and is $0.872v_{\max}$ by using eq 2.26. These differ by less than 1%. The difference is further reduced for lower values of k_{cat}. We thus conclude that, even though in principle the influence of diffusion can lead to non-Michaelis−Menten kinetics, such behavior is unlikely to be observed within the range of parameters that is of practical interest.

(B) Pre-Steady-State Kinetics. When $t = 10t_0 \sim 0.3$ μs, the value of $k(t)$ given by eq 5.1 for $\theta = 5°$ is within 0.5% of the steady-state value $k(\infty)$. This illustrates that, within the time range in which experimental studies of enzyme kinetics are usually carried out, which is above 0.1 ms, one can safely replace $k(t)$ by its steady-state value. Since we have concluded that both the superposition approximation and the convolution relation approaches are accurate for actual enzymes, we now replace $k(t)$ by $k(\infty)$ in the former theory and obtain kinetic equations appropriate for experimental conditions. These are

$$\frac{\mathrm{d}[\mathrm{ES}]}{\mathrm{d}t} = k(\infty)([\mathrm{E}]_{\mathrm{total}} - [\mathrm{ES}])[\mathrm{S}] - \frac{k(\infty)}{k(0)}k_{\mathrm{d}}[\mathrm{ES}] - k_{\mathrm{cat}}[\mathrm{ES}] \qquad (5.5a)$$

$$\frac{\mathrm{d}[\mathrm{P}]}{\mathrm{d}t} = k_{\mathrm{cat}}[\mathrm{ES}] \qquad (5.5b)$$

They are just ordinary rate equations for Scheme 1.1 with the effective rate constants for substrate association and dissociation given by

$$k_1 = k(\infty) \qquad (5.6a)$$

$$k_{-1} = k_{\mathrm{d}}k(\infty)/k(0) \qquad (5.6b)$$

Explicitly, k_1 is given by the steady-state value of the time-dependent rate coefficient for the irreversible formation of ES by diffusional encounter, and k_{-1} is determined by both the rate constant for ES to form a geminate pair and the initial and final values of $k(t)$.

(C) Effect of Electrostatic Interactions on Association and Dissociation Rate Constants. By requiring an active site for catalysis, the site at which substrate binding occurs is restricted and hence $k(\infty)$ is reduced. However, $k(\infty)$ can be elevated by favorable electrostatic interactions between enzyme and substrate. Recently we have shown that, to a good approximation, the effect of electrostatic interactions on $k(\infty)$ can be very simply accounted for.[8b,9] This, just like in the case for $k(0)$, is contained in the average Boltzmann factor within the binding site. If such a simple dependence on the interaction potential is true for $k(\infty)$, one can immediately realize from eq 5.6b that k_{-1} will be *independent* of the interaction potential (provided it does not affect k_{d}).

Experimental evidence for a weak dependence of k_{-1} on electrostatic interactions can be found in the literature. For example, Nolte et al.[19] studied the binding of *N*-methylacridinium to *Electrophorus electricus* acetylcholinesterase and found that there was strong electrostatic interactions between the enzyme and the ligand, as indicated by a factor of ~10 decrease in the association rate constant k_1 when the ionic strength was raised from 1.37 to 121 mM. However, the ionic screening of the electrostatic interactions was found to result in only a factor of ~2 increase in the dissociation rate constant k_{-1}. Very similar results have been obtained recently by Quinn et al.[20] for the hydrolysis of acetylcholine by *Torpedo californica* acetylcholinesterase. From 0−220 mM ionic strengths, k_1 is found to decrease by a factor of ~15, but k_{-1} is found to increase by a factor of only ~2.5.

Our conclusion for the effect of electrostatic interactions on the association and dissociation rate constants provides a simple explanation for recent results by Escobar et al.[21] on the kinetics of the blockade of Shaker K^+ channels by the peptide toxin Lq2. These authors found that a change in ionic strength from 25 to 200 mM decreases k_1 by a factor of over 40 but increases k_{-1} by a factor of merely 5. A charge mutation on the channel near the toxin binding site decreases k_1 by a factor of 5 but does not have any effect on k_{-1}. Escobar et al. have explained these results by suggesting that the toxin binding is activation controlled (i.e., $\kappa \to 0$) so that $k(\infty) \to k(0)$. However, the observed association rate constant at 25 mM ionic strength, $6 \times 10^8\ \mathrm{M}^{-1}\ \mathrm{s}^{-1}$, actually has the order of magnitude of a diffusion-controlled rate constant. More importantly, both the association and dissociation rate constants have been found previously by Miller[22] to scale with the solution viscosity. Hence the simplest explanation for the results of Escobar et al. is that the toxin binding is diffusion controlled to a large extent so that both k_1 and k_{-1} scale with the solution viscosity ($k_{\mathrm{d}}/k(0)$ is an equilibrium constant and thus does not depend on solution viscosity), but $k(\infty)$ and $k(0)$ have roughly the same dependence on the interaction potential so that k_1 has a strong dependence on the interaction potential whereas k_{-1} has only a weak one.[23]

(D) Effect of Electrostatic Interactions on Product Release. By analogy to substrate dissociation, we also expect that the rate constant for product release has only a weak dependence on electrostatic interactions. This conclusion is significant in view of recent concern that favorable electrostatic interactions between enzyme and product may impede product release. This concern in part led Gilson et al.[10] to propose a "back door" mechanism for product release. On the basis of the small effect of electrostatic interactions on the dissociation rate constant, we can state that such a concern is unnecessary. Enzymes are thus found to enjoy all the benefits of electrostatic interactions but suffer very little from their side effects.

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Appendix: Convolution-Relation Approach To Account for Diffusion in an Enzyme-Catalyzed Reaction

The basic assumption in this approach is that the distribution of substrates (S) surrounding a newly generated geminate pair

(by substrate dissociation) or a newly freed enzyme (E) (by substrate conversion to product) is the equilibrium one. This allows one to obtain convolution relations between the probability for finding the species with an unbound E surrounded by a certain number of S and that for finding the species with a bound E surrounded by the same or a different number of S.

Let us use E_n to denote the species with an unbound E surrounded by n substrates, and ES_n to denote the species with a bound E surrounded by $n-1$ substrates. The subscript thus signifies the number of substrates present in a species. We will denote the probability for finding a species by the symbol of the species enclosed in angular brackets. As only a single E will be considered, the reactant molecules will be restricted to a volume $V = 1/[E]_{total}$. The concentration of a species, say E_n, is related to the probability for finding it through $[E_n] = \langle E_n\rangle/V = \langle E_n\rangle[E]_{total}$.

Two mechanisms exist for generating E_n. One is by dissociating the bound S in ES_n, the other is by converting the bound S in ES_{n+1} to product. In analogy to eq 2.18, one has

$$\langle E_n(t)\rangle = k_d\int_0^t\langle ES_n(t')\rangle S_n(t-t'|\text{gem})\,dt' + k_{cat}\int_0^t\langle ES_{n+1}(t')\rangle S_n(t-t'|\text{eq})\,dt' \quad \text{(A1)}$$

where $S_n(t-t'|\text{gem})$ is the probability that a geminate pair generated at time t' surrounded by an equilibrium distribution of $n-1$ substrates is still unbound at time t, and $S_n(t-t'|\text{eq})$ is the corresponding quantity for an E newly freed through substrate conversion and surrounded by an equilibrium distribution of n substrates. These are

$$S_n(t|\text{gem}) = \frac{k(t)}{k(0)}[S(t|\text{eq})]^{n-1} \quad \text{(A2)}$$

$$S_n(t|\text{eq}) = [S(t|\text{eq})]^n \quad \text{(A3)}$$

where $S(t|\text{eq})$ is given by eq 2.21. A newly generated geminate pair or an E newly freed through substrate conversion may get bound again some time later. One thus has

$$\begin{aligned}\langle ES_n(t)\rangle &= k_d\int_0^t\langle ES_n(t')\rangle[1-S_n(t-t'|\text{gem})]\,dt' + k_{cat}\int_0^t\langle ES_{n+1}(t')\rangle[1-S_n(t-t'|\text{eq})]\,dt' - (k_d+k_{cat})\int_0^t\langle ES_n(t')\rangle\,dt' \\ &= -k_d\int_0^t\langle ES_n(t')\rangle S_n(t-t'|\text{gem})\,dt' + k_{cat}\int_0^t\langle ES_{n+1}(t')\rangle[1-S_n(t-t'|\text{eq})]\,dt' - k_{cat}\int_0^t\langle ES_n(t')\rangle\,dt' \quad \text{(A4)}\end{aligned}$$

Special attention has to be paid to $n = 0$ and N (the number of substrates present at $t = 0$). As there is no ES_0 species, when $n = 0$ eq A1 becomes

$$\langle E_0(t)\rangle = k_{cat}\int_0^t\langle ES_1(t')\rangle S_1(t-t'|\text{eq})\,dt' \quad \text{(A5)}$$

The equations for $n = N$ depend on the situation at $t = 0$. The initial situation that we are interested in is one in which an unbound E is surrounded by an equilibrium distribution of N substrates. Then for $n = N$ eq A1 changes to

$$\langle E_N(t)\rangle = S_N(t|\text{eq}) + k_d\int_0^t\langle ES_N(t')\rangle S_N(t-t'|\text{gem})\,dt' \quad \text{(A6)}$$

by analogy to eq 2.22. Similarly eq A4 changes to

$$\langle ES_N(t)\rangle = 1 - S_N(t|\text{eq}) - k_d\int_0^t\langle ES_N(t')\rangle S_N(t-t'|\text{gem})\,dt' - k_{cat}\int_0^t\langle ES_N(t')\rangle\,dt' \quad \text{(A7)}$$

by analogy to eq 2.23. Note that $S_N(t|\text{eq})$ and $S_N(t|\text{gem})$ become $S(t|\text{eq})$ and $S(t|\text{gem})$, respectively, in the thermodynamic limit N and $V \to \infty$ but $N/V = [S]$.

The total concentration of all the species with a bound E is given by

$$[ES]/[E]_{total} = \sum_{n=1}^{N}\langle ES_n\rangle \quad \text{(A8)}$$

The species ES_n or E_n contains n substrates and thus $N-n$ of the initial substrates have been converted to product. The total product concentration is thus given by

$$\begin{aligned}[P]/[E]_{total} &= \sum_{n=1}^{N}(N-n)(\langle ES_n\rangle + \langle E_n\rangle) + N\langle E_0\rangle \\ &= N - \sum_{n=1}^{N}n(\langle ES_n\rangle + \langle E_n\rangle) \quad \text{(A9)}\end{aligned}$$

In principle one can calculate [ES] by first finding $\langle ES_N\rangle$ from eq A7 and then using the result in eq A4 to find $\langle ES_{N-1}\rangle$, $\langle ES_{N-2}\rangle$, ..., and $\langle ES_1\rangle$. These can be used in eq A6, A1, and A5 to find $\langle E_N\rangle$, $\langle E_{N-1}\rangle$, ..., and $\langle E_0\rangle$ and calculate [P]. Such a procedure is hardly practical. We will now calculate [ES(t)] and [P(t)] for times sufficiently short so that only species with n close to N are substantially populated. In this time range, one can approximate $S_n(t|\text{eq})$ by $S_N(t|\text{eq}) = S(t|\text{eq})$ and $S_n(t|\text{gem})$ by $S_N(t|\text{gem}) = S(t|\text{gem})$. Summing eq A4 from $n = 1$ to $N-1$ and then then adding to eq A7, we find

$$[ES(t)] = [1-S(t|\text{eq})][E]_{total} - k_d\int_0^t[ES(t')]\times S(t-t'|\text{gem})\,dt' - k_{cat}\int_0^t[ES(t')]S(t-t'|\text{eq})\,dt' \quad \text{(A10)}$$

This is just eq 2.25. To calculate [P(t)], we first add eq A1 and A4 together,

$$\langle ES_n(t)\rangle + \langle E_n(t)\rangle = k_{cat}\int_0^t(\langle ES_{n+1}(t')\rangle - \langle ES_n(t')\rangle)\,dt' \quad \text{(A11)}$$

and eq A6 and A7 together

$$\langle ES_N(t)\rangle + \langle E_N(t)\rangle = 1 - k_{cat}\int_0^t\langle ES_N(t')\rangle\,dt' \quad \text{(A12)}$$

Multiplying eq A11 by n and eq A12 by N and then summing over n, we find

$$\sum_{n=1}^{N}n(\langle ES_n(t)\rangle + \langle E_n(t)\rangle) = N - k_{cat}\int_0^t[ES(t')]\,dt'/[E]_{total} \quad \text{(A13)}$$

Hence

$$[P(t)] = k_{cat}\int_0^t[ES(t')]\,dt' \quad \text{(A14)}$$

This is just the integral form of eq 2.14.

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(23) In fact the slight increase of k_{-1} at higher ionic strengths observed in the experimental studies[19−21] is expected. The decrease of $k(\infty)$ at higher ionic strengths is not exactly the same as but slightly less than that of $k(0)$, as indicated by our simulation work on a realistic model for acetylcholinesterase.[8b] Hence $k(\infty)/k(0)$ and thus k_{-1} are slightly larger at higher ionic strengths.