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Role of Diffusion in the Kinetics of Reversible Enzyme-catalyzed Reactions†

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The accurate expression for the steady-state velocity of an irreversible enzyme-catalyzed reaction obtained by Shin and co-workers (*J. Chem. Phys.* **2001**, *115*, 1455) is generalized to allow for the rebinding of the product. The amplitude of the power-law ($t^{-1/2}$) relaxation of the free- and bound-enzyme concentrations to steady-state values is expressed in terms of the steady-state velocity and the intrinsic (chemical) rate constants. This result is conjectured to be exact, even though our expression for the steady-state velocity in terms of microscopic parameters is only approximate.

Key Words : Diffusion-influenced reaction, Enzyme-catalyzed reaction, Michaelis-Menten kinetics

Introduction

In enzyme-catalyzed reaction kinetics, the diffusive motion of the substrate leads to deviations from the hyperbolic Michaelis-Menten concentration dependence of the steady-state velocity.[1] The first sophisticated many-body theory of this effect was developed by Shin and co-workers[2] within the framework of their "renormalized kinetic theory." Recently computer simulations[3] have shown that their result for the steady-state velocity is remarkably accurate. The purpose of this note is to generalize their expression to the reversible case, where the substrate and product concentrations are both maintained at fixed, but nonequilibrium values. Specifically, we consider the influence of diffusion on the following kinetic scheme

$$\mathrm{E} + \mathrm{S} \underset{\kappa_{-1}}{\overset{\kappa_1}{\rightleftharpoons}} \mathrm{C} \underset{\kappa_2}{\overset{\kappa_{-2}}{\rightleftharpoons}} \mathrm{E} + \mathrm{P} \qquad (1)$$

where E, S, and P denote the enzyme, substrate, and product, respectively; C is a complex of the enzyme with either the substrate or the product; and κ_1, κ_{-1}, κ_2, and κ_{-2} denote the intrinsic (chemical) rate constants for substrate and product binding and unbinding. While this generalization can be implemented within the framework of Shin's kinetic theory, here, following Park and Agmon,[3] we will use the self-consistent relaxation time approximation.[4]

This generalization is also of interest in a different biophysical context, namely, the diffusional transport of ions through a transmembrane channel with an obligatory internal binding site.[5] The reason is that the kinetic schemes that describe the two processes are formally the same. Briefly, the enzyme corresponds to the internal binding site, while the substrate and product correspond to ions on the two sides of the internal site which have a higher and lower electrochemical potential, respectively. The expressions for the steady-state ion flux and enzymatic velocity have the identical mathematical structure.

Theory

We consider the following microscopic model. All molecules are spherical and their dynamics is diffusive. When an enzyme and a substrate (or product) come in contact (i.e., their separation is the sum of their radii, a), they may react to form a complex with intrinsic rate constant κ_1 (or κ_2). The complex dissociates with rate constant $\kappa_{-1} + \kappa_{-2}$, to form either an enzyme-substrate contact pair with probability $\kappa_{-1}/(\kappa_{-1} + \kappa_{-2})$ or an enzyme-product contact pair with probability $\kappa_{-2}/(\kappa_{-1} + \kappa_{-2})$. The ratios κ_{-1}/κ_1 and κ_{-2}/κ_2 are the equilibrium dissociation constants of the substrate and product, respectively, and will be denoted as K_{d1} and K_{d2}. The substrate and product are assumed to be in excess over the enzyme, so that their concentrations are effectively fixed, at [S] and [P], respectively, which in general are nonequilibrium values. The fixed values of [S] and [P] allow the bimolecular steps in the kinetic scheme of Eq. (1) to be treated as pseudo-first order. We further assume that the enzyme and the complex (both are referred to as macromolecules) have the same diffusion constant, and that the substrate and product (both are referred to as ligands) also have the same diffusion constant. Thus there is only a single relative diffusion constant, denoted as D, between a macromolecule and a ligand.

Starting from the many-body equations that describe the dynamics of our model and integrating over all the positions of the molecules, one can show that the bulk concentrations, [E] and [C], of the enzyme and the complex satisfy the rate equations

†This paper is to commemorate Professor Kook Joe Shin's honourable retirement.

$$\frac{d[\mathrm{E}]}{dt}=-\frac{d[\mathrm{C}]}{dt}=-\kappa_1\rho_{\mathrm{ES}}(a,t)-\kappa_2\rho_{\mathrm{EP}}(a,t)+(\kappa_{-1}+\kappa_{-2})[\mathrm{C}] \quad (2a)$$

where t denotes time; $\rho_{\mathrm{ES}}(r,t)$ is the probability density for an enzyme-substrate pair (E···S), in which an enzyme molecule is in one volume element and a substrate molecule is in another and the two volume elements are separated by r; and $\rho_{\mathrm{EP}}(r,t)$ is defined in a analogous way for an enzyme-product pair (E···P). At $r = \infty$, one has $\rho_{\mathrm{ES}}(r,t) = [\mathrm{E}][\mathrm{S}]$ and $\rho_{\mathrm{EP}}(r,t) = [\mathrm{E}][\mathrm{P}]$. If these equalities were assumed to hold at $r = a$, Eq. (2a) would reduce to the ordinary rate equation of chemical kinetics,

$$\frac{d[\mathrm{E}]}{dt}=-\frac{d[\mathrm{C}]}{dt}=-\kappa_1[\mathrm{E}][\mathrm{S}]-\kappa_2[\mathrm{E}][\mathrm{P}]+(K_{\mathrm{d1}}\kappa_1+K_{\mathrm{d2}}\kappa_2)[\mathrm{C}] \quad (2b)$$

From this, one could easily find the steady-state concentrations $[\mathrm{E}]_{\mathrm{ss}}$ and $[\mathrm{C}]_{\mathrm{ss}}$. The resulting steady-state velocity, v_{ss}, of the enzyme-catalyzed reaction would be[6]

$$v_{ss}=\kappa_1[\mathrm{E}]_{\mathrm{ss}}[\mathrm{S}]-\kappa_{-1}[\mathrm{C}]_{\mathrm{ss}} \quad (3a)$$

$$=\frac{\kappa_1\kappa_2(K_{\mathrm{d2}}[\mathrm{S}]-K_{\mathrm{d1}}[\mathrm{P}])[\mathrm{E}]_{\mathrm{T}}}{\kappa_1([\mathrm{S}]+K_{\mathrm{d1}})+\kappa_2([\mathrm{P}]+K_{\mathrm{d2}})} \quad (3b)$$

where $[\mathrm{E}]_{\mathrm{T}} \equiv [\mathrm{E}] + [\mathrm{C}]$ is the total enzyme concentration. By writing κ_1K_{d1} as κ_{-1} and κ_2K_{d2} as κ_{-2}, we recover the familiar Michaelis-Menton expression for the irreversible enzyme-catalyzed reaction when $\kappa_2[\mathrm{P}]$ is set to zero.

In general the pair probability densities $\rho_{\mathrm{ES}}(r,t)$ and $\rho_{\mathrm{EP}}(r,t)$ deviate from [E][S] and [E][P]; the ratios $\rho_{\mathrm{ES}}(r,t)/[\mathrm{E}][\mathrm{S}]$ and $\rho_{\mathrm{EP}}(r,t)/[\mathrm{E}][\mathrm{P}]$ are known as pair distribution functions. Because of flux conservation, the pair probability densities satisfy

$$4\pi Da^2\frac{\partial\rho_{\mathrm{ES}}(r,t)}{\partial r}\bigg|_{r=a}=\kappa_1\rho_{\mathrm{ES}}(a,t)-\kappa_{-1}[\mathrm{C}] \quad (4a)$$

$$4\pi Da^2\frac{\partial\rho_{\mathrm{EP}}(r,t)}{\partial r}\bigg|_{r=a}=\kappa_2\rho_{\mathrm{EP}}(a,t)-\kappa_{-2}[\mathrm{C}] \quad (4b)$$

Equations (2a) and (4) are formally exact for the model but they are not closed. To make progress, the pair probability densities must be approximated.

The self-consistent relaxation time approximation[4] is based on a set of linear reaction-diffusion equations, not for the pair probability densities themselves, but for their deviations from the bulk values (e.g., $\delta\rho_{\mathrm{ES}}(r,t) \equiv \rho_{\mathrm{ES}}(r,t) - [\mathrm{E}][\mathrm{S}]$). The reaction terms couple the deviations in probability density for the enzyme-substrate pair, E···S, and the complex-substrate pair (C···S), because E···S is transformed into C···S when the enzyme binds a ligand and vice versa when the complex dissociates. This coupling is described in a mean-field way by using effective rate constants that are determined self-consistently. Specifically the deviations in probability density for E···S and C···S, $\delta\rho_{\mathrm{ES}}(r,t)$ and $\delta\rho_{\mathrm{CS}}(r,t)$, are assumed to satisfy

$$\frac{\partial\delta\rho_{\mathrm{ES}}}{\partial t}=D\nabla^2\delta\rho_{\mathrm{ES}}-(k_1[\mathrm{S}]+k_2[\mathrm{P}])\delta\rho_{\mathrm{ES}}+(k_{-1}+k_{-2})\delta\rho_{\mathrm{CS}} \quad (5a)$$

$$\frac{\partial\delta\rho_{\mathrm{CS}}}{\partial t}=D\nabla^2\delta\rho_{\mathrm{CS}}+(k_1[\mathrm{S}]+k_2[\mathrm{P}])\delta\rho_{\mathrm{ES}}-(k_{-1}+k_{-2})\delta\rho_{\mathrm{CS}} \quad (5b)$$

where k_1 and k_{-1} (k_2 and k_{-2}) are the effective rate constants for substrate (product) binding and unbinding. They are stipulated to satisfy detailed balance:

$$\frac{k_{-i}}{k_i}=\frac{\kappa_{-i}}{\kappa_i}=K_{\mathrm{d}i};\ i=1,2 \quad (6)$$

but are otherwise undetermined for the moment. The boundary condition at $r = a$ satisfied by $\delta\rho_{\mathrm{ES}}(r,t)$ is obtained from Eq. (4a) after replacing $\rho_{\mathrm{ES}}(r,t)$ by $\delta\rho_{\mathrm{ES}}(r,t) + [\mathrm{E}][\mathrm{S}]$. Since C cannot bind another S or P, $\delta\rho_{\mathrm{CS}}(r,t)$ satisfies the reflecting boundary condition at $r = a$. By definition, at $r = \infty$, $\delta\rho_{\mathrm{ES}}(r,t) = \delta\rho_{\mathrm{CS}}(r,t) = 0$.

The analogous deviations $\delta\rho_{\mathrm{EP}}(r,t)$ and $\delta\rho_{\mathrm{CP}}(r,t)$ satisfy the same equations, Eqs. (5), governing $\delta\rho_{\mathrm{ES}}(r,t)$ and $\delta\rho_{\mathrm{CS}}(r,t)$ and the same boundary conditions, except for $\delta\rho_{\mathrm{EP}}(r,t)$ the boundary condition at $r = a$ involves κ_2 and κ_{-2} instead of κ_1 and κ_{-1} [cf. Eqs. (4a) and (4b)]. Equations (5) constitute the simplest approximation for the pair probability densities that leads to the exact asymptotics for the A + B $\rightleftarrows$ C and A + B $\rightleftarrows$ C + D reactions.[7,8] They [and the analogous equations for $\delta\rho_{\mathrm{EP}}(r,t)$ and $\delta\rho_{\mathrm{CP}}(r,t)$] can be solved subject to the above boundary conditions in a straightforward way.[4] The results for $\delta\rho_{\mathrm{ES}}(a,t)$ and $\delta\rho_{\mathrm{EP}}(a,t)$ can then be used to close Eq. (2a). Fortunately all the required algebra has been done by Park and Agmon[3] and we can go directly to the final results by reinterpreting their parameters.

The final results for the concentrations are remarkably simple in Laplace space. We denote the Laplace transform of $f(t)$ by $\hat{f}(s)=\int_0^\infty dt e^{-st}f(t)$. All one has to do is to take the ordinary chemical rate equation, Eq. (2b) in Laplace space, and replace the intrinsic rate constants by s-dependent kernels that are expressed in terms of the Smoluchowski diffusion-controlled rate coefficient $\hat{k}_{\mathrm{D}}(s)$ for an irreversible reaction. Specifically, by replacing k_i by $\hat{\mathcal{K}}_i(s)$, we have

$$-[\mathrm{E}]_0+s[\hat{\mathrm{E}}]=[\mathrm{C}]_0-s[\hat{\mathrm{C}}]=-\hat{\mathcal{K}}_1(s)[\hat{\mathrm{E}}][\mathrm{S}]-\hat{\mathcal{K}}_2(s)[\hat{\mathrm{E}}][\mathrm{P}]+(K_{\mathrm{d1}}\hat{\mathcal{K}}_1(s)+K_{\mathrm{d2}}\hat{\mathcal{K}}_2(s))[\hat{\mathrm{C}}] \quad (7)$$

where $[\mathrm{E}]_0$ and $[\mathrm{C}]_0$ are the initial concentrations of E and C. The kernels are given by

$$\frac{1}{\hat{\mathcal{K}}_i(s)}=\frac{1}{\kappa_i}+\frac{\mu}{s\hat{k}_D(s)}+\frac{1-\mu}{(s+\lambda)\hat{k}_{\mathrm{D}}(s+\lambda)} \quad (8a)$$

where

$$\lambda=k_1[\mathrm{S}]+k_2[\mathrm{P}]+k_{-1}+k_{-2}=k_1([\mathrm{S}]+K_{\mathrm{d1}})+k_2([\mathrm{P}]+K_{\mathrm{d2}}) \quad (8b)$$

$$\mu=(k_{-1}+k_{-2})/\lambda=(k_1K_{\mathrm{d1}}+k_2K_{\mathrm{d2}})/\lambda \quad (8c)$$

It is interesting to note that $\hat{\mathcal{K}}_i(s)$ has the same structure (and in fact proportional to) the Laplace transform of the rate coefficient for an irreversible stochastically-gated reaction[9] in which the macromolecule, or ligand, switches between an open or reactive state (analogous to E···S and E···P) and a closed or nonreactive state (analogous to C···S and C···P), with interconversion rates $k_1[\mathrm{S}] + k_2[\mathrm{P}]$ and $k_{-1} + k_{-2}$.

The effective rate constants k_1 and k_2 remain to be determined [k_{-1} and k_{-2} can be then obtained *via* Eq. (6)]. The simplest possibility is to equate k_i and the intrinsic rate constants κ_i. This would give the analogue of the linearized extended superposition approximation for the A + B ⇄ C reaction.[8] A better approximation is to determine them self-consistently[4] by solving the implicit equation

$$k_i=\hat{\mathcal{K}}_i(0); \qquad i=1, 2 \tag{9}$$

One attractive consequence of this self-consistency condition is that the steady-state value of [E] or [C] is the same as that given by ordinary chemical kinetics when κ_i are replaced by k_i. Put differently, k_i can be interpreted as the effective binding rate constants at steady state. However, as explained below, k_i depend on the diffusion constant and the ligand concentrations. Note that, once k_i are determined, the kernels $\hat{\mathcal{K}}_i(s)$ at all s can be calculated using Eq. (8a).

For the present spherical model, $s\hat{k}_D(s)=4\pi Da[1+(sa^2/D)^{1/2}]$. Combining Eqs. (8a) and (9) leads to

$$\frac{1}{k_i}=\frac{1}{\kappa_i}+\frac{\mu}{4\pi Da}+\frac{1-\mu}{4\pi Da[1+(\lambda a^2/D)^{1/2}]} \tag{10}$$

Since μ and λ depend on the substrate and product concentrations [see Eqs. (8b) and (8c)], the effective binding rate constants will also depend on these concentrations. At low ligand concentrations, $k_i \to 4\pi Da\kappa_i/(4\pi Da+\kappa_i)$, which are the familiar steady-state rate constants (denoted hereafter as k_{ssi}) for irreversible diffusion-influenced binding to the enzyme by the substrate and product (i.e., the long-time limits of the irreversible time-dependent rate coefficients). At very high ligand concentrations, binding almost always occurs from the contact distance, and therefore $k_i \to \kappa_i$.

Note that the second and third terms on the right-hand side of Eq. (10) are independent of i. Subtracting out these two terms, we find

$$\frac{1}{k_1}-\frac{1}{k_2}=\frac{1}{\kappa_1}-\frac{1}{\kappa_2} \tag{11}$$

This result will find use below.

We believe that if the reversible Michaelis-Menten problem is tackled by the kinetic theory formalism of Shin and co-workers, the final result would formally be the same as that given by Eqs. (7) and (8), except that k_i in Eqs. (8b) and (8c) would be replaced by $\hat{\mathcal{K}}_i(s)$ (i.e., the effective rate constants would be s-dependent). Then Eq. (8a) would be solved self-consistently for each value of s. Clearly, for steady-state properties this would lead to the same results as our procedure. However, for time-dependent problems (e.g., the amplitude of the power-law relaxation to steady state) there would be differences.

We now present results for (1) the steady-state velocity and (2) the time course of the relaxation to steady state. The steady-state velocity of the enzymatic reaction is obtained by substituting k_i for κ_i in Eq. (3b), predicted by ordinary chemical kinetics. The result is

$$v_{ss}=\frac{k_1k_2(K_{d2}[S]-K_{d1}[P])[E]_T}{k_1([S]+K_{d1})+k_2([P]+K_{d2})} \tag{12}$$

As expected, the velocity is zero at equilibrium (i.e., when $K_{d2}[S] = K_{d1}[P]$). When $[P] = 0$, this expression with the definitions of k_i in Eq. (10) reduces to the result obtained by Shin and co-workers.[2] The analogue of Eq. (12) was used recently[5] in the context of ion transport through a transmembrane channel with an obligatory internal binding site. The present work provides a theoretical foundation for the use of Eq. (12) in that study. There, k_i were replaced by k_{ssi} (the steady-state rate constants for irreversible ion binding to the internal site from either side). This replacement is justified given the low ion concentrations relevant for the transmembrane channel.

To find how [E] and [C] approach their steady-state values, we solve Eq. (7) near $s = 0$. Upon inverse Laplace transforming we find that as $t \to \infty$

$$\frac{[C]-[C]_{ss}}{[E]_T-[C]_{ss}} \approx \frac{k_1k_2(k_1-k_2)(K_{d2}[S]-K_{d1}[P])}{(k_1([S]+K_{d1})+k_2([P]+K_{d2}))^2 4\pi D(\pi Dt)^{1/2}} \tag{13}$$

The $t^{-1/2}$ dependence is consistent what Shin and co-workers[2] found in the absence of product rebinding. When $k_2 \to 0$ and $K_{d2}k_2 = k_{-2}$ is identified with k_p, this reduces to Eq. (3.11) of Park and Agmon.[3] Note that, in addition to the equilibrium condition, the amplitude of the $t^{-1/2}$ term vanishes also when $k_1 = k_2$. [E] and [C] then relax with the usual $t^{-3/2}$ asymptotic behavior.

The amplitude in Eq. (13) depends on the effective rate constants k_i, which are only approximate. Hence the numerical value of the amplitude is also approximate. However, using Eqs. (6), (11), and (12), we may rewrite Eq. (13) as

$$\frac{[C]-[C]_{ss}}{[E]_T-[C]_{ss}} \approx \frac{(\kappa_1-\kappa_2)(v_{ss}/[E]_T)^2}{\kappa_1\kappa_{-2}[S]-\kappa_2\kappa_{-1}[P]} \frac{1}{4\pi D(\pi Dt)^{1/2}} \tag{14}$$

Remarkably the effective rate constants k_i have been subsumed into the steady-state velocity and all that remains are the intrinsic rate constants and the bulk concentrations of S and P. It seems too much of a coincidence for this to happen and it is tempting to conjecture that the functional form of Eq. (14) is exact, even though it was derived in the framework of an approximate theory. It will be interesting to see whether this conjecture is supported by computer simulations and if it seems to be, how it can be proved.

Concluding Remarks

We considered how the diffusive motion of ligands influences the kinetics of a reversible enzyme-catalyzed reaction. The steady-state velocity has the same functional form as that obtained from ordinary chemical kinetics but with effective rate constants that depend both on the macromolecule-ligand relative diffusion constant and on the substrate and product concentrations. The free- and bound-enzyme concentrations relax to their steady-state values as

$t^{-1/2}$ as found by Shin and co-workers in the absence of product rebinding. We were able to express the amplitude of this relaxation in terms of the steady-state velocity and conjectured that the resulting functional form is exact.

This work can be extended in a number of ways. It is straightforward to allow the substrate and product (and similarly the free- and bound-enzyme) to have different diffusion constants. More importantly, our simplifying assumption of uniformly reactive, spherically symmetric molecules can be readily relaxed. Since our reversible formalism requires only the Laplace transform of the irreversible diffusion-controlled rate coefficients, one can handle any problem for which a good approximation for the latter has been obtained. These include the presence of arbitrary centrosymmetric interaction potentials,[10] planar reactive regions of arbitrary shape embedded in an inert surface,[11] a buried site connected to the surface by a cylindrical tunnel,[12] and reactive patches on a plane, cylinder, and sphere in the presence of nonspecific binding.[13] In fact, in the context of ion transport through a transmembrane channel with an obligatory internal binding site, some of these features have been dealt with recently in the low ion concentration limit.[5]

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