Effect of the internal rotations of the reactants in diffusion-controlled chemical reactions: An application to the enzyme kinetic problems

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A theoretical model describing the effect of orientational constraints in diffusion-controlled enzymatic reactions is developed. It involves a rototranslational diffusion problem with mixed boundary conditions, which is solved in a diffusion-jump approximation. In this approximation the reactant molecules are partitioned in two classes. One class (I) includes the molecules which have their internal rotational angles, with respect to the enzyme binding site, within a given angular region C. All the other molecules belong to the second class (II). The relative population of the two classes changes according to interconversion rates which, in the present theory, is related to the true rotational diffusion coefficients of the reactants. The molecules of class I are adsorbed inside of a small circular region of the enzyme, while the ones belonging to class II are reflected everywhere at the surface. The solution of this problem leads to a set of integral equations from which the flux of adsorbed reactant molecules can be calculated. The influence of different physical parameters (rotational and translational diffusion coefficients, size of the enzyme binding region, range of the orientational region C) on the total flux has been investigated by numerical calculations, and some interesting limiting cases have been examined.

I. INTRODUCTION

Several methods have been developed to study translational diffusion in various biological problems. In particular, Wiegel has developed analytical mathematical models to study reactant-receptor interactions at the membrane surface for different forms and sizes of the binding site of the receptor.[1,2] De Lisi developed mathematical models for estimating reaction rates on and off the membrane in terms of cell size, receptor density, and translational diffusion coefficients.[3] In a recent review, McCammon *et al.* showed how molecular-dynamics simulation techniques can be used to face enzymatic diffusion-controlled reactions.[4] Solc and Stockmayer treated the more complex problem of rototranslational diffusion motions in chemical reactions.[5] Similar formalisms have been extended to biological problems by some authors, e.g., by Schmitz and Schurr,[6] who developed an interesting simplified procedure to study ligand-receptor interactions at the membrane surface and by Szabo *et al.*,[7] who faced similar problems. By a different approach based on the theory of statistical thermodynamics, Hill studied orientational effects in diffusion-controlled ligand-protein reactions.[8]

In a recent paper[9] (hereinafter referred to as I) we developed a simple analytical model to study rototranslational effects in diffusion-controlled enzyme reactions, or, more generally, in reactant-receptor interactions. The solution of this problem was obtained by solving the rototranslational diffusion equation with proper boundary conditions. In I we assumed that the reactant molecules are adsorbed (or chemically transformed) at the enzyme surface, which has been simulated by a sphere whose reactive region has been smeared out over all the surface. The reactant molecules can freely diffuse in the surrounding medium by a rototranslational Brownian motion and react with the enzyme surface only if their internal rotational angles lie within a given range, otherwise they are reflected.

This model well describes the fact that the chemically active moiety of the reactants is located in a specific region of the molecule. In this paper we try to remove the drastic assumption of a uniform distribution of reactive sites over the enzyme (or cell) surface. This approximation could be a fairly good picture for the receptors distribution over the cellular membrane (the number of specific receptors per cell is about 10^5, (Ref. 3) even if the large distance between the receptors [10^2-10^3 Å (Ref. 3)] makes questionable the uniform approximation). When one considers isolated enzymes this picture is totally wrong, because as a rule there is only one active site generally located in a narrow region of the enzyme proteic structure. Consequently, the present model, imposing severe constraints both on the reactants orientation and

on the size of the chemically active region of the enzyme, could be a useful tool for describing diffusion-controlled reactions in biological systems.

In Sec. II we develop the mathematical model that lead to an analytical expression for the flux across the enzyme binding site. In Sec. III we show how previous analytical models reobtained as limiting cases of our treatment. Finally, Sec. IV is concerned with numerical results that show the dependence of the flux on the various parameters of the model.

II. THE MODEL

Since the reactive site of the enzyme is a small fraction of the total surface, we describe this region as a small circle with radius a lying over a flat infinite surface (see Fig. 1). In the steady-state conditions, the reactants are continuously released at a very large distance from the enzyme surface ($z \to \infty$) and diffuse across the medium. When they touch the reactive site they are adsorbed, provided the orientation of the reactant's internal frame, with respect to the enzyme's one, lies within a given range ($-\phi_0 \le \phi \le \phi_0$). The molecules having orientations outside this range are reflected on the whole surface.

Under the usual assumption of independent rotational and translational motions, the diffusion equation in cylindrical coordinates (see Fig. 1) can be written as[10]

$$D_T \left[\frac{\partial^2}{\partial r^2} + \frac{1}{r}\frac{\partial}{\partial r} + \frac{\partial^2}{\partial z^2} \right] P(r,z,\phi) + D_R \frac{\partial^2}{\partial \phi^2} P(r,z,\phi) = 0 , \tag{1}$$

where D_T and D_R are the translational and rotational diffusion coefficients, respectively, and $P(r,z,\phi)$ is the reactant concentration. Because of the axial symmetry of the system, the ψ dependence has been dropped in Eq. (1).

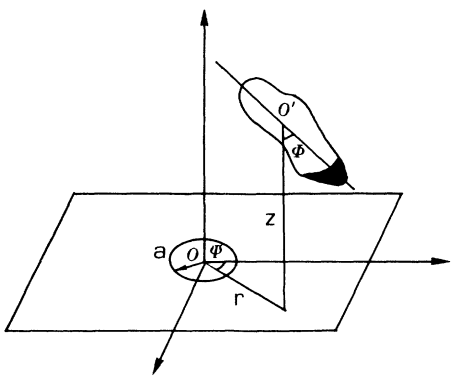

FIG. 1. Schematic drawing of a reactant-enzyme complex. The cylindrical coordinates of the reactant center of mass O' are r, z, and ψ, and the black region represents the chemically active moiety of the reactant. The reactant rotation axis passes through O' and is perpendicular to the plane OrO', however, the rotator may lie on every plane passing through the straight line rO' (plane rotator approximation). The enzyme surface is represented by an infinite plane having a small circular binding site of radius a whose center is located at $r=z=0$.

Rotational and translational motions are coupled through the boundary conditions, which, in the present model, are

$$P(r,z,\phi)|_{z\to\infty} = P(r,z,\phi)|_{r\to\infty} = P_0 , \tag{2a}$$

$$P(r,z,\phi)|_{z=0} = 0 \quad \begin{cases} 0 \le r \le a \\ \phi \le \phi_0 , \end{cases} \tag{2b}$$

$$\frac{\partial}{\partial z} P(r,z,\phi)|_{z=0} = 0 \quad \begin{cases} a \le r \le \infty \\ 0 \le \phi \le \pi , \end{cases} \tag{2c}$$

$$\frac{\partial}{\partial z} P(r,z,\phi)|_{z=0} = 0 \quad \begin{cases} 0 \le r \le a \\ \phi \ge \phi_0 , \end{cases} \tag{2d}$$

The first condition (2a) imposes a uniform distribution of reactant molecules very far from the enzyme binding site. Equation (2b) is the adsorption condition limited to the reactant molecules having the "correct" orientation within the binding site. Equations (2c) and (2d) impose reflection for reactants having "incorrect" orientation within the binding site (2d) or for molecules touching the enzyme surface outside the reactive area (2c). The general solution of Eq. (1) is

$$P(r,z,\phi) = P_0 + \sum_{k=0}^{\infty} \int_0^{\infty} A_k(\lambda) J_0(\lambda r) \times \exp[-(\lambda^2 + s^2 k^2)^{1/2} z] \times \cos(k\phi) d\lambda , \tag{3}$$

where $J_0(\lambda r)$ is a Bessel function of zeroth order and $s^2 = D_R / D_T$.

The coefficients $A_k(\lambda)$ are to be determined by the boundary conditions (2). Unfortunately, the expansion procedure followed in paper I cannot be easily extended to the present model because of the more complex boundary conditions. The number of algebraic linear equations for the expansion coefficients is too large to be useful in practice, even for numerical applications. Here we propose a simpler approximate procedure, and in Appendix B we apply this method to the case of a spherical enzyme with a uniformly reactive surface for which the exact numerical solution has been obtained in I. Both methods give almost the same results, making us confident on the reliability of our approximate procedure. Since we are interested in the total flux across the enzyme binding site and since it differs from zero only for reactant molecules having the correct internal orientation, we have partitioned the reactants population in two classes: the first one, characterized by the concentration $P^+(r,z)$, contains all the molecules that have correct orientational angles ($|\phi| < \phi_0$); the second one, with concentration $P^-(r,z)$ contains all the remaining molecules having incorrect orientational angles ($|\phi| > \phi_0$). Obviously, the total concentration is $P^+(r,z) + P^-(r,z)$. The interconversion between these two classes is regulated by a rate constant, which in Appendix A will be related to the rotational diffusion coefficient. This procedure transforms the diffusion Eq. (1) into two coupled jump-diffusion equations:

$$D_T\left[\frac{\partial^2}{\partial r^2}+\frac{1}{r}\frac{\partial}{\partial r}+\frac{\partial^2}{\partial z^2}\right]P^+(r,z)+k_{-\to+}P^-(r,z) -k_{+\to-}P^+(r,z)=0\ , \quad (4a)$$

$$D_T\left[\frac{\partial^2}{\partial r^2}+\frac{1}{r}\frac{\partial}{\partial r}+\frac{\partial^2}{\partial z^2}\right]P^-(r,z)+k_{+\to-}P^+(r,z) -k_{-\to+}P^-(r,z)=0\ , \quad (4b)$$

where $k_{i\to j}$ the transition rates from the generic ith to the jth state. The ratio $k_{+\to-}/k_{-\to+}$ can be determined by knowing the equilibrium distribution at the infinity, where we have $P^+(r,z)|_\infty=(2\phi_0/2\pi)P_0\equiv P^+_\infty$ and $P^-(r,z)|_\infty=(2\pi-2\phi_0)/2\pi\equiv P^-_\infty$, then

$$\frac{k_{+\to-}}{k_{-\to+}}=\frac{2\pi-2\phi_0}{2\phi_0}\ . \quad (5)$$

The set of equations (4) describes a typical composite Markov process with uncorrelated variables. The implicit assumptions are: (i) during the jump from i to j the r,z position of the reactant does not change; (ii) the jump probabilities $k_{i\to j}$ are independent of the reactant's position; (iii) the D_T coefficient is independent of the ith and jth states.[11]

Introducing the new variables $C^\pm(r,z)=P^\pm_0-P^\pm(r,z)$ in the jump-diffusion model, the boundary conditions (2) become

$$C^\pm(r,z)|_{z=\infty}=C^\pm(r,z)|_{r=\infty}=0\ , \quad (6a)$$

$$C^+(r,z)|_{z=0}=P_0\frac{2\phi_0}{2\pi},\quad r\in I_1 \quad (6b)$$

$$\frac{\partial}{\partial z}C^+(r,z)|_{z=0}=0,\quad r\in I_2 \quad (6c)$$

$$\frac{\partial}{\partial z}C^-(r,z)|_{z=0}=0,\quad 0\le r\le\infty \quad (6d)$$

where I_1 and I_2 are a more compact notation for the intervals $0\le r\le a$ and $a\le r\le\infty$. The general solution of Eqs. (4) is

$$C^\pm(r,z)=\int_0^\infty A^\pm(\lambda)J_0(\lambda r)\exp(-\gamma z)d\lambda\ , \quad (7)$$

where the $A^\pm(\lambda)$ coefficients are to be determined by boundary conditions. Inserting Eq. (7) into Eqs. (4), we obtain

$$(-\lambda^2+\gamma^2)A^+(\lambda)+\mu A^-(\lambda)-\nu A^+(\lambda)=0\ , \quad (8a)$$

$$(-\lambda^2+\gamma^2)A^-(\lambda)+\nu A^+(\lambda)-\mu A^-(\lambda)=0\ , \quad (8b)$$

where the shortened notation $\mu\equiv k_{-\to+}/D_T$ and $\nu\equiv k_{+\to-}/D_T$ has been used. The eigenvalues and eigenvectors of the 2×2 system of equations (8) are

$$\gamma_1=\pm\lambda\ , \quad (9a)$$

$$\gamma_2=\pm[(\mu+\nu)+\lambda^2]^{1/2}\ , \quad (9b)$$

and

$$A_1^+(\lambda)=\frac{\mu}{\nu}A_1^-(\lambda)\ , \quad (10a)$$

$$A_2^+(\lambda)=-A_2^-(\lambda)\ , \quad (10b)$$

respectively. Only the positive roots of Eqs. (9) will be retained in order to satisfy the boundary condition (6a). Making use of Eqs. (7), (9), and (10), the solution of the coupled equations (4) becomes

$$C^+(r,z)=\int_0^\infty\left[\frac{\mu}{\nu}A_1^-(\lambda)e^{-\lambda z}-A_2^-(\lambda)e^{-s(\lambda)z}\right]\times J_0(\lambda r)d\lambda\ , \quad (11a)$$

$$C^-(r,z)=\int_0^\infty[A_1^-(\lambda)e^{-\lambda z}+A_2^-(\lambda)e^{-s(\lambda)z}]J_0(\lambda r)d\lambda\ , \quad (11b)$$

where

$$s(\lambda)\equiv\gamma_2(\lambda)=[(\mu+\nu)+\lambda^2]^{1/2}\ .$$

Combining Eqs. (11) with the boundary conditions (6), one obtains

$$C^+(r,z)|_{z=0}=P_0\frac{2\phi_0}{2\pi}=\int_0^\infty\left[\frac{\mu}{\nu}A_1^-(\lambda)-A_2^-(\lambda)\right]J_0(\lambda r)d\lambda\ , \quad r\in I_1 \quad (12a)$$

$$\frac{\partial}{\partial z}C^+(r,z)|_{z=0}=0=\int_0^\infty\left[-\frac{\mu}{\nu}\lambda A_1^-(\lambda)+s(\lambda)A_2^-(\lambda)\right]J_0(\lambda r)d\lambda\ , \quad r\in I_2 \quad (12b)$$

$$\frac{\partial}{\partial z}C^-(r,z)|_{z=0}=0=\int_0^\infty[-\lambda A_1^-(\lambda)-s(\lambda)A_2^-(\lambda)]J_0(\lambda r)d\lambda\ , \quad 0\le r\le\infty\ . \quad (12c)$$

Since the coefficients of $J_0(\lambda r)$ appearing in the integrals (12) are independent of r, recalling that condition (12c) must be valid for all r values, it follows that Eq. (12c) is satisfied only if $-\lambda A_1^-(\lambda)-s(\lambda)A_2^-(\lambda)=0$.

Inserting this result into Eqs. (12a) and (12b), we find

$$\int_0^\infty A_1^-(\lambda)J_0(\lambda r)\left[\frac{\mu}{\nu}+\frac{\lambda}{s(\lambda)}\right]d\lambda=P_0\frac{2\phi_0}{2\pi},\quad r\in I_1 \quad (13a)$$

$$\int_0^\infty A_1^-(\lambda)J_0(\lambda r)\lambda\,d\lambda=0,\quad r\in I_2\ . \quad (13b)$$

Introducing the new adimensional variables

$$\rho=r/a,\quad \tilde\lambda=\lambda a,\quad B(\tilde\lambda)=\frac{A_1^-(\tilde\lambda)\tilde\lambda}{P_0(2\phi_0/2\pi)(\nu/\mu)} \quad (14a)$$

we can rewrite Eqs. (13) in a more compact form

$$\int_0^\infty B(\tilde{\lambda})J_0(\tilde{\lambda}\rho)[1+q(\tilde{\lambda})]\tilde{\lambda}^{-1}d\tilde{\lambda}=1, \quad 0\le\rho\le 1 \tag{14b}$$

$$\int_0^\infty B(\tilde{\lambda})J_0(\tilde{\lambda}\rho)d\tilde{\lambda}=0 , \quad 1\le\rho\le\infty \tag{14c}$$

where $q(\tilde{\lambda})$ is an arbitrary function of $\tilde{\lambda}$, which in the present case is given by

$$q(\tilde{\lambda})=\frac{\nu}{\mu}\frac{\tilde{\lambda}}{[(\mu+\nu)+\tilde{\lambda}^2]^{1/2}} . \tag{14d}$$

The dual integral equations (12a) and (12b) have been investigated by several authors assuming different expressions for the function $q(\tilde{\lambda})$.[12,13] We refer to those original papers for the theory, and here we quote only the final equation for the coefficients $B(\tilde{\lambda})$:

$$B(\tilde{\lambda})=\tilde{\lambda}^{1/2}\sum_{m=0}^{\infty} p_m J_{2m+1/2}(\tilde{\lambda}) , \tag{15}$$

where p_m are obtained by solving the linear system of equations

$$p_n+\sum_{k=0}^{\infty} L_{kn}p_k=(4n+1)\left[\frac{2}{\pi}\right]^{1/2}\delta_{n0} , \tag{16}$$

the L_{kn} matrix elements being defined as

$$L_{kn}=(4n+1)\int_0^\infty q(\tilde{\lambda})\tilde{\lambda}^{-1}J_{2k+1/2}(\tilde{\lambda})J_{2n+1/2}(\tilde{\lambda})d\tilde{\lambda} . \tag{17}$$

Solving the system of equations (16), inserting Eq. (15) into Eq. (11), and taking into account that the coefficients $B(\tilde{\lambda})$ and $A_1^-(\tilde{\lambda})$ are related through Eq. (14a), we obtain analytical expressions for $C^+(r,z)$ and $C^-(r,z)$.

Since only the reactant molecules having the correct orientational angles are adsorbed, we can easily calculate the total flux of reactants across the enzyme binding site

$$J=-D_T\int_s\frac{\partial}{\partial z}C^+(r,z)|_{z=0}ds$$

$$=-2\pi D_T\int_0^a\frac{\partial}{\partial z}C^+(r,z)|_{z=0}r\,dr . \tag{18}$$

Calculating the derivative of $C^+(r,z)$ and performing the integration over dr, eventually we find

$$J=2\pi D_T P_0 a\left[\frac{2}{\pi}\right]^{1/2}\sum_{m=0}^{\infty} p_m\frac{\Gamma(m+1)}{\Gamma(-m+1)} , \tag{19}$$

where $\Gamma(x)$ is the gamma function. Equation (19) describes the dependence of the reactants flux at the enzyme active region as a function of the parameters of the model (D_T, $k_{i\to j}$, a, ϕ_0, and P_0). Numerical results will be shown in Sec. IV.

III. LIMITING CASES

Simple analytical expressions for the total flux can be obtained for some interesting limiting cases.

Case (i): $2\phi_0=2\pi$. When all the reactant molecules are adsorbed over the active site, independently of their internal orientational angles ($2\phi_0\to 2\pi$), we have [see Eq. (5)]

$$\nu/\mu=k_{+\to-}/k_{-\to+}=\lim_{2\phi_0\to 2\pi}[(2\pi-2\phi_0)/2\phi_0]=0 .$$

Then, $q(\tilde{\lambda})=0$ and $L_{kn}=0$ [see Eqs. (14d) and (17)]. Consequently, Eq. (16) reduces to $p_n=(2/\pi)^{1/2}\delta_{n0}$ and the flux calculated by Eq. (19) becomes

$$J=4D_T P_0 a . \tag{20}$$

Case (ii): $k_{+\to-}=k_{-\to+}=0$. In this case we have $\mu=\nu=0$ (but $\mu/\nu\neq 0$ [see Eq. (5)]) then $q(\tilde{\lambda})\to\tilde{\lambda}^{-1}$. The resulting integral appearing in Eq. (17) now can be calculated analytically[14] and the matrix elements L_{kn} reduce to $L_{kn}=(\nu/\mu)\delta_{kn}\delta_{n0}$. Inserting this result into the equations system (16), we find

$$p_n=(2\phi_0/2\pi)(2/\pi)^{1/2}\delta_{n0}$$

and the flux (19) becomes as follows:

$$J=4D_T P_0 a\frac{2\phi_0}{2\pi} . \tag{21}$$

Case (iii): $k_{+\to-}=k_{-\to+}=\infty$. In this limiting case we have $q(\tilde{\lambda})\to 0$, and we re-obtain the same result found for case (i).

Case (iv): $a\to\infty$. Also in this case $p_n=(2/\pi)^{1/2}\delta_{n0}$ [see case (i)] and the flux (19) tends to the infinity, as expected.

Equation (20) is identical to that obtained by different authors who considered the purely translational diffusion of a reactant.[1,7,15] In our jump-diffusion model this means that all the molecules belong to the $C^+(r,z)$ class. Case (ii) takes into account the presence of a very high barrier between the $C^+(r,z)$ and $C^-(r,z)$ states (i.e., the interconversion rate is zero). Since the statistical weight of $C^+(r,z)$ is $2\phi_0/2\pi$, we expect the flux to be proportional to the $2\phi_0/2\pi$ ratio, as found in Eq. (21). Finally, when the interconversion rate tends to the infinity [case (iii)], all the molecules near the enzyme reactive site suddenly reach the proper orientation [the $C^+(r,z)$ state] and then are adsorbed. This is equivalent to an adsorption over the entire range of internal orientational angles ($2\phi_0=2\pi$) and explains the identical analytical results obtained for cases (i) and (iii).

IV. RESULTS AND DISCUSSION

Making use of Eq. (19), we calculated the reactants flux across an enzyme binding site for different values of the physical parameters contained in our model. To have a more meaningful description of the system, we related the interconversion constants $k_{+\to-}$ and $k_{-\to+}$ to the rotational diffusion coefficients of the reactants by a simple approximate analytical procedure described in Appendix A. In Figs. 2(a) and 2(b) we report the flux J versus the width of the reactive internal angles ϕ_0; the curves were calculated for different values of the rotational diffusion coefficients D_R. The size of the enzyme active region was set equal to 5 Å [Fig. 2(a)] and 10 Å [Fig. 2(b)], and the translational diffusion coefficient D_T was always maintained constant and set equal to 10^{-6} $cm^2\,sec^{-1}$, a typical value for large molecules. The flux was normalized to that calculated at $2\phi_0=2\pi$, i.e., to the

maximum flux obtained when all the impinging molecules are adsorbed independently of their orientation. The numerical results show an almost linear relationship between J and ϕ_0 for small values of D_R ($\sim 10^8$ rad^2 sec^{-1}), whereas for higher values of D_R, a very nonlinear behavior is found. For larger binding sites the nonlinear region spans over a wider range than for the small sites [Fig. 2(b)]. When D_R tends to the infinity, the flux suddenly reaches the step-function shape.

The general trend of the present results is not very far from that calculated in paper I, obtained assuming a uniformly reactive spherical surface. However, when the enzyme active site is limited to a small fraction of the surface, the curves of J versus ϕ_0 are flatter and the nonlinear behavior is reached only for quite high values of D_R.

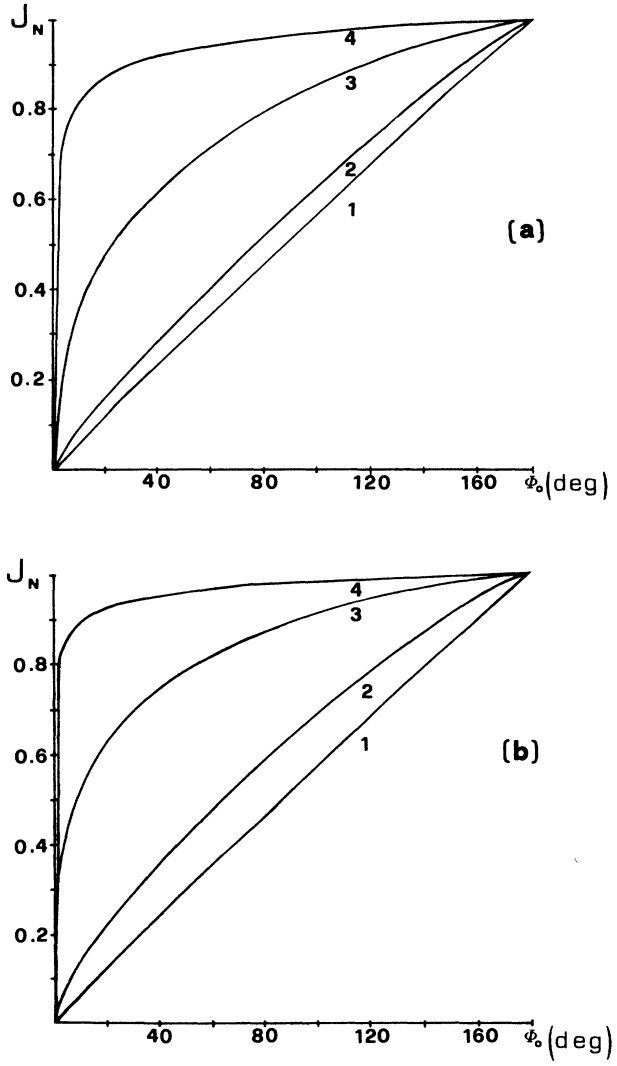

FIG. 2. Flux (normalized to its maximum value) vs the width of the reactant capture angle ϕ_0 (degrees). The size of the enzyme site is 5 Å [Fig. 2(a)] and 10 Å [Fig. 2(b)]. The translational diffusion coefficient D_T is 10^{-6} cm^2 sec^{-1}. The curves are plotted for different values of the reactant-rotational-diffusion coefficient D_R. Curve 1, $D_R = 10^6$; curve 2, $D_R = 10^8$; curve 3, $D_R = 10^{10}$; curve 4, $D_R = 10^{12}$ (units are rad^2 sec^{-1}).

In Fig. 3 the dependence of the flux on the width of the enzyme site a is reported. The curves have been calculated setting $\phi_0 = 20°$ and $D_T = 10^6$ cm^2 sec^{-1}. All the calculated values of J lie between the two straight lines obtained by using Eqs. (20) and (21), which correspond to the limiting cases $D_R \to \infty$ [case (iii)] and $D_R = 0$ [case (ii)], respectively.

It is interesting to compare our exact solution of Eq. (4), subject to the boundary conditions (6), with that obtained using an approximate procedure developed by Szabo *et al.* and successfully applied to various problems involving diffusion-controlled chemical reactions.[7,16] Following this method, one assumes that

$$\left.\frac{\partial C^+}{\partial z}\right|_{z=0} = \begin{cases} Q & r \in I_1 \\ 0 & r \in I_2 \end{cases}, \tag{22}$$

where the constant Q can be determined by imposing the adsorption condition

$$\int_0^a C^+|_{z=0} r\, dr = \frac{2\pi}{2\phi_0} P_0 \,. \tag{23}$$

Numerical values obtained by using the "constant flux" approximation are reported in Fig. 4. The agreement between the two methods is fairly good suggesting that the approximate method could be a useful tool in dealing with more complex problems, which cannot be easily handled by the exact procedure here developed.

The results obtained in this work confirm the hypothesis that double constraints, both on the size of the

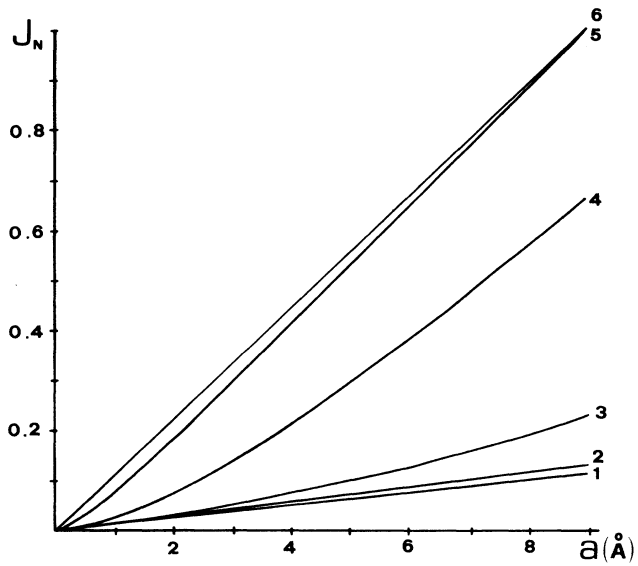

FIG. 3. Flux (scaled to an arbitrary constant) vs the radius a (Å) of the enzyme binding site. D_T is 10^{-6} cm^2 sec^{-1} and $\phi_0 = 20°$. The curves have been calculated for different values of D_R. Curve 1, $D_R = 0$; curve 2, $D_R = 10^6$; curve 3, $D_R = 10^8$; curve 4, $D_R = 10^{10}$; curve 5, $D_R = 10^{12}$; curve 6, $D_R \to \infty$ (units are rad^2 sec^{-1}).

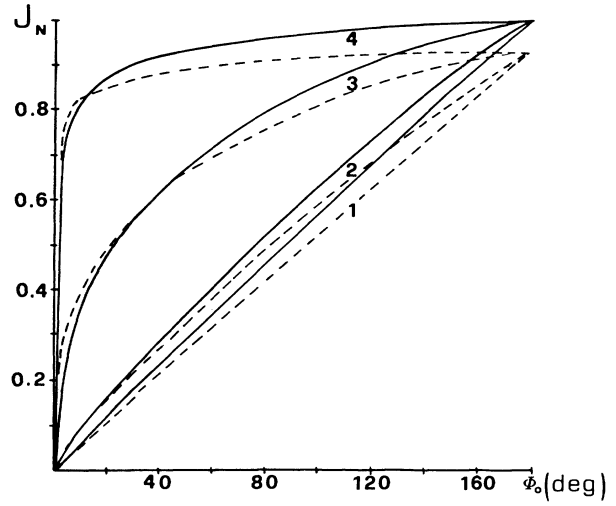

FIG. 4. Flux (normalized to its maximum value) vs the width of the reactant capture angle ϕ_0 (degrees) calculated by the exact model (solid lines) and the "constant flux" model (dashed lines). The translational diffusion coefficient D_T is 10^{-6} cm^2 sec^{-1} and the radius of the enzyme binding site is 5 Å. The curves have been calculated for different values of the reactant-rotational diffusion coefficient D_R. Curve 1; $D_R=10^6$; curve 2, $D_R=10^8$; curve 3, $D_R=10spu10$; curve 4, $D_R=10^{12}$ (units are rad^2 sec^{-1}).

enzyme active region and on the orientation of the reactants, strongly modulate the reactivity in diffusion-controlled biological interactions. For a more realistic description of these systems, all the three Euler angles defining the reactant orientation in the enzyme reactive pocket should be taken into account, as well as the role of the intermolecular forces, and the competition between the active sites should be considered. Work is in progress along this line.

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APPENDIX A

Diffusion and jump motions are different processes; then, generally, it is impossible to find a rigorous relationship between the interconversion constants and diffusion coefficients. In the case of two states [$C^+(t)$ and $C^-(t)$] we have attempted to find an approximate relationship between $k_{i\to j}$ and D_R by comparing the mean value of the time-evolution probability calculated in the two processes. In more detail, since the stationary solution ($t\to\infty$) for jump and diffusion equations must be identical and assuming that at $t=0$ the same distribution holds for both processes, then we may impose the condition that the two time-averaged probabilities are equal. This leads to an approximate relationship between rotational-diffusion coefficients and interconversion constants

$$\int_0^\infty \int_{-\phi_0}^{+\phi_0} P(\phi,t)d\phi\,dt=\int_0^\infty C^+(t)dt\ , \tag{A1}$$

where $C^+(t)$ and $\int_{-\phi_0}^{+\phi_0}P(\phi,t)d\phi$ are the concentration of the reactant molecules having the correct orientational angles to bind to the enzyme site and calculated according to the jump and diffusion model, respectively:

$$\frac{d}{dt}C^+(t)=k_{-\to+}C^-(t)-k_{+\to-}C^+(t)\ , \tag{A2a}$$

$$\frac{d}{dt}C^-(t)=k_{+\to-}C^+(t)-k_{-\to+}C^-(t)\ , \tag{A2b}$$

and

$$\frac{\partial}{\partial t}P(\phi,t)=D_R\frac{\partial^2}{\partial\phi^2}P(\phi,t)\ . \tag{A3}$$

Solving the set of equations (A2) with the initial conditions $C^+(0)=1$, $C^-(0)=0$ and $C^+(\infty)=2\phi_0/2\pi$, $C^-(\infty)=(2\pi-2\phi_0)/2\pi$, one finds

$$C^+(t)=\frac{\phi_0}{\pi}+\frac{\pi-\phi_0}{\pi}\exp[-(k_{+\to-}+k_{-\to+})t]\ . \tag{A4}$$

According to the diffusion model, the previous initial conditions become

$$P(\phi,0)=\begin{cases}\dfrac{1}{2\phi_0}, & |\phi|\le\phi_0\\ 0, & |\phi|\ge\phi_0\ ,\end{cases}\qquad P(\phi,\infty)=\frac{1}{2\pi}\ .$$

Applying these conditions, the solution of Eq. (A3) is

$$P(\phi,t)=\frac{1}{2\pi}+\sum_{n\neq0}^{\infty}\frac{1}{2\pi\phi_0}\frac{\sin(n\phi_0)}{n}\exp(-in\phi)\times\exp(-n^2D_Rt)\ . \tag{A5}$$

Inserting Eqs. (A4) and (A5) into Eq. (A1), one obtains after simple algebra

$$\frac{1}{k_{+\to-}+k_{-\to+}}=\frac{\phi_0(\pi-\phi_0)}{3D_R}\ . \tag{A6}$$

Combining this latter result with Eq. (5), we find the desired relationship between $k_{-\to+}$ and D_R:

$$k_{-\to+}=\frac{3}{\pi}\frac{D_R}{\pi-\phi_0}\ . \tag{A7}$$

Applying the same procedure, but assuming that at $t=0$ are populated only those states having incorrect orientational angles [i.e., $C^-(t)$ and $\int_{|\phi|>\phi_0}P(\phi,t)d\phi$], one finds

$$k_{+\to-}=\frac{3}{\pi}\frac{D_R}{\phi_0}\ . \tag{A8}$$

The ratio between Eqs. (A8) and (A7) gives once again Eq. (5).

APPENDIX B

In order to check the validity of the present model, we performed a numerical comparison between the jump-

translational model and the rototranslational diffusion model developed in I. The comparison was tested on a simpler system which can be easily solved without any approximation in both models. In the considered system the enzyme is described as a reactive sphere with radius a. The reactant molecules diffuse across the medium and are adsorbed at the spherical surface provided their orientational angles lie within a given range, otherwise they are reflected. This problem has been solved exactly in I, here we develop an analytical procedure based on the jump-diffusion model whose equation in spherical coordinates is

$$D_T\left[\frac{\partial^2}{\partial r^2}+\frac{2}{r}\frac{\partial}{\partial r}\right]P^+(r)+k_{-\rightarrow+}P^-(r)-k_{+\rightarrow-}P^+(r)=0\ , \quad \text{(B1a)}$$

$$D_T\left[\frac{\partial^2}{\partial r^2}+\frac{2}{r}\frac{\partial}{\partial r}\right]P^-(r)+k_{+\rightarrow-}P^+(r)-k_{-\rightarrow+}P^-(r)=0\ , \quad \text{(B1b)}$$

where $P^+(r)$ are $P^-(r)$ are the concentrations of the reactant molecules calculated at point r having correct and incorrect orientational angles, respectively, and the other symbols have been previously defined [see Eq. (4)]. Making the change of variables $C^{\pm}(r)=P^{\pm}(\infty)-P^{\pm}(r)$, the boundary conditions (2) become

$$C^{\pm}(r)|_{r=\infty}=0\ , \quad \text{(B2a)}$$

$$C^{+}(r)|_{r=a}=\frac{2\phi_0}{2\pi}P_0\ , \quad \text{(B2b)}$$

$$\frac{\partial}{\partial r}C^{-}(r)|_{r=a}=0\ . \quad \text{(B2c)}$$

Solving Eqs. (B1) with the boundary conditions (B2), we obtain explicit equations for $C^+(r)$ and $C^-(r)$. The knowledge of $C^+(r)$ allows us to calculate the reactants flux across the enzyme surface, that is,

$$J=-4\pi a^2 D_T\frac{\partial}{\partial r}C^{+}(r)|_{r=a}\ , \quad \text{(B3)}$$

which, after simple algebra, becomes

$$J=4\pi a D_T P_0\frac{\phi_0}{\pi}\frac{1+\lambda a}{1+\lambda a\phi_0/\pi}\ , \quad \text{(B4)}$$

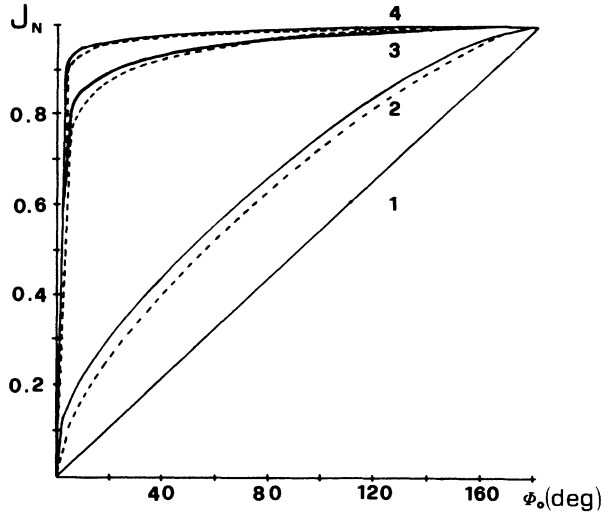

FIG. 5. Flux (normalized to its maximum value) vs the width of the reactant capture angle ϕ_0 (degrees) for a spherical enzyme with a uniformly reactive surface. Solid lines have been calculated by the exact numerical procedure developed in I, dashed lines have been obtained according to the present jump-diffusion model [Eq. (B4)]. All the curves were calculated setting $D_T=10^{-6}$ cm^2 sec^{-1} and the enzyme radius equal to 10^2 Å, and were plotted for different values of D_R. Curve 1, $D_R=0$; curve 2; $D_R=10^6$; curve 3, $D_R=10^9$; curve 4, $D_R=10^{10}$ (units are rad^2 cm^{-1}).

where: $\lambda^2\equiv(1/D_T)(k_{+\rightarrow-}+k_{-\rightarrow+})$.

According to Eqs. (A7) and (A8) the interconversion constants $k_{+\rightarrow-}$ and $k_{-\rightarrow+}$ have been related to the rotational-diffusion coefficient D_R. The numerical results are shown in Fig. 5, where we report the normalized flux versus the width of the correct orientational angles ϕ_0. The curves were calculated for different values of D_R, whereas the enzyme radius a and the translational diffusion coefficient D_T were kept constant and set equal to 100 Å and 10^{-6} cm^2 sec^{-1}, respectively. For the sake of comparison, we report also the values obtained by the exact numerical procedure developed in paper I. The agreement between the two methods is excellent, apart from a small underestimation of the flux by the jump-diffusion model which is less severe for higher values of D_R.

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