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THE FLEXIBILITY DURING THE JUXTAPOSITION OF REACTING GROUPS AND THE UPPER LIMITS OF ENZYME REACTIONS

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The combinations between enzymes and substrates occur only after their reacting groups are in juxtaposition with each other. This will greatly reduce the probability of their effective encounters. However, the results calculated with the finite element method show that the reaction limits will not decrease substantially if van der Waal's forces and a reasonable flexibility during such a juxtaposition are taken into account.

1. Introduction

When the Smoluchowski theory of colloid-coagulation kinetics is employed to estimate the upper limit of the reaction rate between enzyme (E) and substrate (S) molecules, the size of the "sink" is usually taken as $S_a = 2\pi r_0^2$ with $r_0 = 5$ Å. Obviously, such a size of the "sink" taken for an E–S reaction system like this is rather arbitrary and gross [1] due to oversimplification of the source model. If we adopt the improved model given by Chou and his co-workers [1–3], the size of the "sink", or in their words, the "active surface" should be expressed as

$$S_a = 4\pi R_0^2 \sin^2 \tfrac{1}{2}\theta_a, \tag{1}$$

where $R_0 = R_E + R_S$ is the sum of the radii of an E molecule and an S molecule, and θ_a the maximal derivation angle of the "sink", as illustrated in fig. 1. Based on such a model, the Chou-Jiang formula for calculating the upper limit of the reaction rate between E and S molecules is derived [1] as follows:

$$k_{\lim} = 4\pi DN \Big/ \left(1000 \int_{R_0}^{\infty} e^{U(r)/kT}\, dr/r^2\right) \times \left(1 - g\, e^{U(R_0)/kT} \cos^2 \tfrac{1}{2}\theta_a\right), \tag{2}$$

where $D = D_E + D_S$ is the sum of the diffusion coefficients of an E molecule and an S molecule (which is approximately equal to D_S due to $D_S \gg D_E$), N Avogadro's number, U the interaction potential between the E and S molecules, k the Boltzmann constant, T the absolute temperature, and

$$g = \lim_{\Delta r \to 0} \frac{\int_{R_0}^{R_0+\Delta r} \int_0^{2\pi} \int_{\theta_a}^{\pi} c(r,\theta) r^2 \sin\theta\, d\theta\, d\phi\, dr}{c_0 \int_{R_0}^{R_0+\Delta r} \int_0^{2\pi} \int_{\theta_a}^{\pi} r^2 \sin\theta\, d\theta\, d\phi\, dr} = \int_{\theta_a}^{\pi} c(R_0,\theta) \sin\theta\, d\theta \Big/ \left(2c_0 \cos^2 \tfrac{1}{2}\theta_a\right), \tag{3}$$

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is the ratio of the average concentration of S molecules on S_b (see fig. 1), the accessible surface [4] of the protein outside the active site, to c_0, the bulk concentration of S molecules in solution.

As for understanding the rationality of the "sink" model in enzyme kinetics, the reader may refer to ref. [3], where a stochastic analysis is presented, and the corresponding physical picture described. It is emphasized in this paper that we prefer to use the word "sink" rather than "active surface" as used in the previous papers of Chou et al. [1–3], since the latter is liable to be misunderstood as being the surface whose area is equal to that of the active site of an E molecule. As is well known, combinations between E and S molecules occur only after their reacting groups are in juxtaposition with each other as illustrated in fig. 2. Due to such a requirement, the area of the "sink" should be much smaller than that of the surface formed by the chemical groups at the active site, and actually would be reduced to a geometric point if there were no flexibility at all for the constraint of juxtaposition. Of course such an extreme case will never appear because there always exists some flexibility in biomacromolecules as shown by Chou et al. [5,6]. Nevertheless, the flexibility can enlarge the effective area of the "sink" only a little. In other words, the value of θ_a in fig. 1 should be very small, at least much smaller than 20° as taken in ref. [1]. Therefore, it becomes a critical problem to calculate k_{lim} for the case of small θ_a.

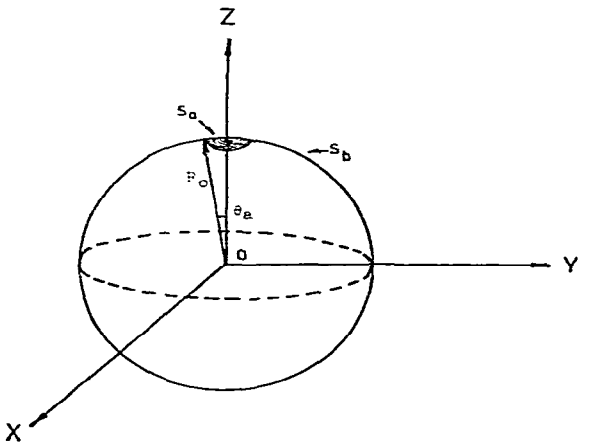

Fig. 1. S_a is the surface of the "sink" for an E-S reaction system. S_b is the accessible surface [4] between E and S molecules outside the "sink". $R_0 = R_E + R_S$ is the sum of the radii of an E molecule and an S molecule.

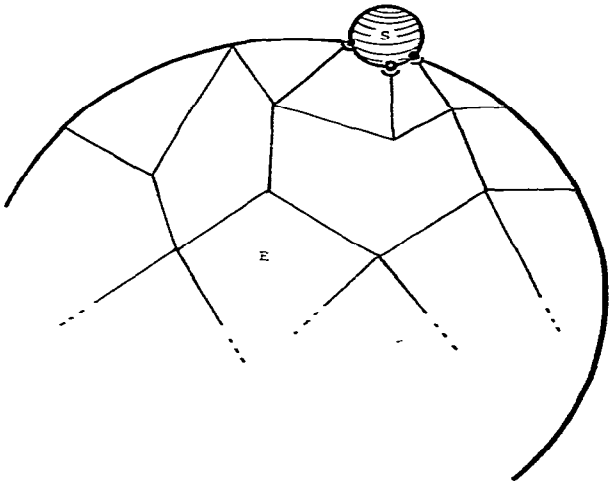

Fig. 2. The juxtaposition of the reacting groups of an S molecule with those in an E molecule. From this schematic drawing it is easy to realize that, if no flexibility is allowed for the juxtaposition, θ_a will reduce to zero and even the small S molecule can be treated as a spherically symmetric sphere due to the very fast rotational brownian motion (see eq. (14)].

Unfortunately, in actual numerical calculations [2], it is very difficult to obtain reliable numerical solutions for small θ_a. To overcome this here we adopt the finite element method. From the calculated results we are able not only to see distinctly how the diffusion-controlled reaction rates vary with θ_a even in the region of very small angles, but also to discover that the bimolecular reaction limits will not decrease substantially if van der Waal's forces and a reasonable flexibility for the juxtaposition are taken into consideration.

2. Calculated equations

First of all, we are confronted with the following equation [1]

$$\vec{\nabla} \cdot (e^{-U/kT} \vec{\nabla} c^*) = 0. \qquad (4)$$

with the boundary conditions (fig. 1):

$$c^*|_{r=R_0} = 0 \qquad (0 \leqslant \theta < \theta_a), \tag{5}$$

$$(\partial c^*/\partial r)_{r=R_0} = 0 \qquad (\theta_a < \theta \leqslant \pi), \tag{6}$$

$$c^*|_{r=\infty} = c_0 \qquad (0 \leqslant \theta \leqslant \pi). \tag{7}$$

where $\vec{\nabla}$ is the Hamilton operator, and c^* has the following relation to c, the concentration of S molecules:

$$c = e^{-U/kT} c^*. \tag{8}$$

The upper limit of the corresponding second-order rate constant can thus be written as [1,2]

$$k_{\lim} = I/c_0 = \frac{D}{c_0} \iint_{S_a} e^{-U/kT} \frac{\partial c^*}{\partial r}\, dS = \frac{D}{c_0} \iint_{S_{R_0}} e^{-U/kT} \frac{\partial c^*}{\partial r}\, dS, \tag{9}$$

where I is the total amount of S molecules which diffuse to an E molecule in unit time, and $S_{R_0} = S_a + S_b$.

Now the concentration ratio g in eq. (3) can be written as

$$g = \frac{1}{c_0 S_b} \iint_{S_b} e^{-U/kT} c^*\, dS, \tag{10}$$

which is a useful index to describe the outline of the concentration distribution of S molecules in the closest proximity of an E molecule.

It is worthwhile to point out that, if one uses eq. (2) to calculate $k_{\lim}$, the interaction potential is restricted in the form of $U(r)$, which means the potential depends only on r, the distance between the centers of an E molecule and an S molecule [1,2]. If we calculate directly from eq. (4), however, such a restriction can be removed, i.e., the interaction potential can also be written as

$$U = U(r, \theta, \phi), \tag{11}$$

if necessary. For example, for the enzyme-charged substrate reaction system, the coulomb interaction potential between E and S molecules can be expressed as [7]

$$U_{\text{coulomb}} = \frac{z_S z_E e_0^2}{\varepsilon r} + \frac{z_S e_0}{\varepsilon} \sum_{l=1}^{\infty} \frac{L_l(\theta, \phi)}{r^{l+1}}, \tag{12}$$

where

$$L_l(\theta, \phi) = \sum_{m=-l}^{l} \frac{(l-m)!}{(l+m)!} P_l^m(\cos\theta)\, e^{im\phi} \times \int_0^{R_E} \int_0^{\pi} \int_0^{2\pi} \rho(r', \theta', \phi') r'^l \times P_l^m(\cos\theta')\, e^{-im\phi'}\, d\tau', \tag{13}$$

is the lth-order multipole moment, and the corresponding potential due to it is termed the lth-order multipole potential. In eqs. (12) and (13), z_E and z_S are the number of net charges on an E and an S molecule, respectively, e_0 the electronic charge, ε the dielectric constant of the intervening medium, $\rho(r', \theta', \phi')$ the density distribution of charges on an E molecule, and $P_l^m(\cos\theta)$ the associated Legendre function. When the distribution of charges on an E molecule is spherically symmetric, ρ is independent of θ' and ϕ', we have $L_l = 0$ ($l = 1, 2, \ldots$) according to the orthonormality of Legendre functions, and then $U_{\text{coulomb}} = z_E z_S e_0^2/\varepsilon r$. When the distribution of the charges is non-spherically symmetric, if the density distribution ρ is known, then the coulomb effects on the diffusion-controlled reactions can be calculated in arbitrary-order approximation by means of eqs. (4), (12) and (13). If ρ is not known, we can take the zero-order approximation of eq. (12). As pointed out by Chou et al. in ref. [7] the effect of coulomb long-range forces on diffusion-controlled reactions is mainly a successive acceleration over a long distance. (On this point, the van der Waal's short-range force is much different; its role in the diffusion-controlled reactions [8–10] is in generating a high concentration gradient of S molecules around the E molecule so as to increase the reaction rate, as will be further discussed in section 3.) However, except within quite a short distance (small r), all the multipole potentials are trifling in comparison with the first term of eq. (12). Therefore, when ρ is not known, taking the zero-order approximation of eq. (12) can also give a reasonable estimation for the effect of coulomb forces on the diffusion-controlled reaction rate.

Also it should be pointed out that, for mathematical convenience, in his original theory [1,3] Chou did not consider the restoring influence of

rotational diffusion on the rate of reactions. Such an approximate treatment is reasonable at least for reaction systems discussed here due to the following reason. As is well known, the relaxation time for rotational brownian motion of a sphere is proportional to the third power of the radius or to the molecular weight, hence the importance of this motion will be very different for a biomacromolecule and a small substrate. In our case, the molecular weight of E molecules is two or three orders of magnitude larger than that of S molecules [1–3], so we have

$$D_S^{rot} \gg D_E^{rot}. \quad (14)$$

As a result, the rotational brownian motion of an S molecule is so fast that the whole molecule can be treated as a small symmetric sphere; while the rotational brownian motion of the E molecule is so slow that its effects can be neglected. The numerical estimation given by Schurr and Schmitz [11,12] also confirms the above assumption.

3. Results and discussion

The results calculated with the finite element method are given in table 1, from which the following points are discussed:

(1) As mentioned above, the reactions between E and S molecules occur only after their reacting groups come into juxtaposition, and hence the value of θ_a, which, to some degree, reflects the flexibility during the juxtaposition, should be quite small. Nevertheless, table 1 indicates that, when θ_a is as small as 0.3°, the value of k_{lim} can also attain the magnitude of 10^{10} M^{-1} s^{-1}. This means that the geometric restriction in the mutual juxtaposition will not markedly decrease the upper limit of the diffusion-controlled reaction rate if only a little flexibility is allowed during the juxtaposition and the van der Waal's forces between E and S molecules are taken into consideration.

(2) Also we can see that the values of g increase with the decrease in θ_a. It should be emphasized that $\theta_a = 0$ here should be understood as signifying no flexibility at all for the process of juxtaposition between E and S molecules. Such a case will never actually appear according to the flexibility of bio-

Table 1
Values of k_{lim} and g calculated for different θ_a [a]

θ_a (°)	k_{lim} (M^{-1} s^{-1})	g
20	1.1426×10^{10}	1.1264×10^{2}
10	1.1387×10^{10}	2.4258×10^{2}
5	1.1213×10^{10}	4.1598×10^{2}
1	1.0905×10^{10}	8.0416×10^{2}
0.3	1.0308×10^{10}	1.8678×10^{3}
0.1	7.6538×10^{9}	6.5743×10^{3}
0.03	2.0252×10^{9}	1.7346×10^{4}
0.01	1.8323×10^{8}	2.1342×10^{4}
⋮	⋮	⋮
0	0	e^{10}

[a] $R_0 = 20$ Å [1,2], $D = 7\times10^{-6}$ cm^2/s [1,2], $T = 298$ K, $U = U_{van}$ whose functional form is the same as in ref. [2] and $U_{van}(R_0) = -10kT$ [13].

macromolecules [5,6] and the induced-fit theory [14] unless there is no active site. In that case, there are simply no reactions, and g will reach its maximum value. Table 1 also tells us that, due to the van der Waal's attraction between E and S molecules, the concentration of S molecules on the surface of an E molecule is much higher than that in the bulk solution. As a consequence, the diffusion flow of S molecules, which is directly proportional to the concentration gradient [1], to the "sink" around the E molecule will be accelerated significantly so as to compensate for the reduction of the probability of the effective encounters between E and S molecules due to the restriction in juxtaposition.

At first sight, the question might be raised as to whether the enormous surface crowding is possible if volume effects are taken into account. But an actual estimation given below will remove such suspicion. Suppose the bulk concentration is 1 mM, which corresponds to $10^{-3}\times6.023\times10^{23}/10^3\times(10^8)^3$ Å^3 $\approx 6\times10^{-7}$ Å^{-3}. Hence, even when $g = 10^4$, the corresponding concentration of S molecules is $\approx 6\times10^{-3}$ Å^{-3}. This means that, even in the region of $g = 10^4$, an S molecule on the average occupies at least a space of ≈ 166 Å^3, which is still larger than its van der Waal's volume (if the van der Waal's radius of a small S molecule is 1.5–2.5 Å). As a matter of fact, the upper limit of the surface crowding of S molecules on an E molecule is given by Boltzmann statistics, i.e., the

maximal $g = \exp[-U(R_0)/kT]$, which is equal to $e^{10} \approx 2.2 \times 10^4$ when $U(R_0) = 10kT$.

(3) It is interesting to discuss the relation between the Richter-Eigen approach [15] and ours. Essentially, Richter and Eigen attribute the surprisingly high association rate to the unspecific binding of repressor to non-operator DNA with subsequent diffusion along the chain. Therefore, the apparent result of the Richter-Eigen treatment seems to be the same as ours, i.e., the whole surface of the biomacromolecules concerned could be equivalently regarded as a sink. But Richter and Eigen did not give the physical mechanism of how the subsequent diffusion along the chain can be fast enough to ensure that the whole surface of the DNA behaves like a "sink", while in our approach, such a physical mechanism has been presented. It is the van der Waal's short-range force and the enormous concentration gradient formed therewith around the proximity of an E molecule that produce a fast flow [16,17] of S molecules around the E molecule to its active site.

Finally, it should be mentioned that, when S molecules diffuse along the surface of an E molecule, the diffusion coefficient (the so-called interfacial diffusion coefficient) should be different from that in the bulk. A similar difficulty also appears in the Richter-Eigen approach. Although this problem still remains to be solved, it will not influence our essential results owing to the following reasons: (a) Unlike the Richter-Eigen picture [15] where there is such an assumption that a repressor will enter into a "one-dimensional" diffusion along the chain once it binds to any part of DNA, in Chou's picture [1] there is no such constraint, even during the diffusion process along the surface of an E molecule, that S molecules have to remain on the surface all the time; in other words, this kind of S molecule still undergoes a "three-dimensional" movement. (b) Unlike the Richter-Eigen picture where nearly all the associations of repressors to the DNA follow a "one-dimensional" diffusion along the chain, in the Chou picture, however, most of the flow of S molecules to the active site comes from "three-dimensional" diffusion around the E molecule in a spherical shell whose thickness is about the same as, or a little larger than, the range of the van der Waal's forces [8,16–18]. Therefore, when knowledge about the interfacial diffusion coefficient, especially about the complicated molecular forces and the detailed structure (such as the so-called "ice-like" structure) on the surface of an E molecule, has not yet been developed sufficiently, the present calculations and discussions based on the Chou picture are rational at least in a sense of approximation.

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