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THE CRITICAL SPHERICAL SHELL IN ENZYMATIC FAST REACTION SYSTEMS

Kuo-chen CHOU, Tse-tsai LI* and Sture FORSÉN
Physical Chemistry 2, Chemical Center, P.O.B. 740, S-220 07 Lund, Sweden

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As we go more deeply into the theoretical analysis and actual calculations for enzymatic fast reaction systems, the concept of the critical shell would naturally be confronted with. In this paper, a formula is presented to estimate the thickness of the critical shell. And on such a basis, we have made calculations and discussed the influences of various factors such as short-range force, long-range force, activation energy and cavity-active site upon the thickness of the critical spherical shell.

As is well known, in the great majority of cases, the enzymatic active site is entirely situated in a concave region termed molecular crevice (fig. 1), or only a part of it is on the surface of enzyme molecule. Therefore, when applying Smoluckowski's diffusion-controlled reaction theory to investigate the enzymatic fast reaction systems, one must meet the non-spherically symmetric problem for which it is difficult to acquire the perfect analytic solution [1] and hence one could not help resorting numerical solutions [2]. In order to simplify the problem, Jönsson and Wennerström [3] confined their discussion about the effects of diffusion-controlled reactions in reversible enzyme reaction systems to the region outside the spherical shell depicted by dotted line in fig. 1. They supposed that outside the shell, the concentrations of substrate and product molecules might be described with spherically symmetric equations. Recently, Chou [4] also assumed that if the diffusion rate of substrate molecules (S) along the surface of enzyme molecule (E) is sufficiently large, then the thickness, δ, of the spherical shell is generally rather thin; from this, he derived an approximate formula to calculate the diffusion-controlled reaction rate k_{lim} of enzyme, and the calculated results are quite in accordance with those obtained through a computer [2].

However, in the general case, how does the thickness of such a shell change with various factors in the reaction system? In what case is the shell very thin, and in what case is it rather thick so that one must consider the effects inside the shell? Since these questions are directly related to the physical picture of enzymatic fast reaction, particularily to the mutual dovetail joint between various rate constants in the reversible enzymatic fast reaction system, it seems to be necessary to make a special discussion. Especially when people are not yet clearly aware of the ice-like structure on the surface of enzyme and of the interactions between the substrate molecule and the various chemical groups of the enzyme molecule, it would have some special significance to consider these questions.

Now, let us call the spherical shell S_{R^+} in fig. 1 the critical spherical shell of E molecule. Actually it is a demarcation surface between two kinds of physical pictures: outside the shell the diffusion picture is of non-spherically symmetry; whereas inside the shell the diffusion picture is of spherical symmetry or of quasi-

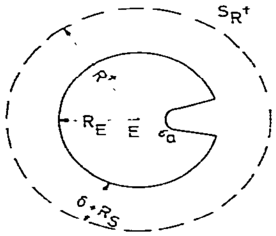

Fig. 1. Enzyme molecule E and its critical spherical shell S_{R^+}. Here σ_a is the active site, δ the thickness of the critical spherical shell. R_E and R_S are respectively the radius of enzyme molecule and that of substrate molecule.

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[*] Present address: Shanghai Institute of Computing Technique.

spherical symmetry. Below, we shall give a formula to estimate the thickness, δ, of the critical spherical shell, and discuss its change tendency in various conditions.

As is well known, in enzyme-substrate fast reaction system the concentration of S molecules C may be described by the following equation [1].

$$\frac{\partial c}{\partial t} = D\,\nabla\cdot(\exp(-U/kT)\,\nabla\exp(U/kT)\,C), \tag{1}$$

where $D = D_E + D_C$ is the sum of the diffusion coefficient of E molecule and that of S molecule, k the Boltzmann constant, T the absolute temperature and U the potential of interaction between E and S molecules. Because the interaction forces between enzyme and substrate are quite complicated, especially within the scope of short region the forces also depend on the orientation of S molecule to E molecule, the potential should be generally expressed as $U(r, \theta, \phi)$. At the steady state, eq. (1) is reduced to

$$\nabla\cdot(\exp(-U(r,\theta,\phi)/kT) \times \nabla\exp(U(r,\theta,\phi)/kT)\,C(r,\theta,\phi)) = 0. \tag{2}$$

Under the action of the force field and the concentration gradient, the flow of S molecules through unit area is [1]

$$i = -D\exp(-U/kT)\,\nabla(\exp(U/kT)\,C). \tag{3}$$

According to the completeness of the spherical functions $Y_l^m(\theta,\phi)$ the solution of eq. (2) can be written as

$$C(r,\theta,\phi) = R_0(r) + \sum_{l=1}^{\infty}\sum_{m=-l}^{l} R_{lm}(r)\,Y_l^m(\theta,\phi), \tag{4}$$

where

$$R_{lm} = \frac{2l+1}{4\pi}\frac{(l-m)!}{(l+m)!}\int_0^{2\pi}\int_0^{\pi} C(r,\theta,\phi)\,Y_l^m(\theta,\phi)\sin\theta\,\mathrm{d}\theta\,\mathrm{d}\phi.$$

Now, define

$$g_\rho(r) = \frac{\int_0^{2\pi}\int_0^{\pi} C^\rho(r,\theta,\phi)\sin\theta\,\mathrm{d}\theta\,\mathrm{d}\phi}{4\pi C_0^\rho}, \quad (\rho = 1, 2, \ldots). \tag{5}$$

Obviously, $g_1(r)$ is none other than the ratio [1] of the average concentration of S molecules on the spherical surface S_r to the concentration C_0 of S molecules in the bulk solution. When $\rho = 1$, substituting eq. (4) into eq. (5) and utilizing the orthonormality relation of spherical harmonics, we immediately get

$$R_0(r) = g_1(r)\,C_0. \tag{6}$$

From this we see that the first term in (4), the spherically symmetry term, is actually the average concentration of S molecules on the surface S_r. From (4), utilizing the orthonormality of spherical harmonics once again we obtain the variance of the concentration distribution deviating from the spherically symmetric one for the S molecules on S_r:

$$\Delta(r) = \sum_{l=1}^{\infty}\sum_{m=-l}^{l}\frac{1}{2l+1}\frac{(l+m)!}{(l-m)!}R_{lm}^2(r)$$

$$= [g_2(r) - g_1^2(r)]\,C_0^2. \tag{7}$$

from which it follows that the mean square deviation

$$\eta(r) = \frac{(\Delta(r))^{1/2}}{\int_0^{2\pi}\int_0^{\pi} C(r,\theta,\phi)\sin\theta\,\mathrm{d}\theta\,\mathrm{d}\phi} = (g_2(r)/g_1^2(r) - 1)^{1/2}. \tag{8}$$

When the mean square deviation $\eta < 0.05$, the corresponding concentration distribution may be regarded as one having approached nearly spherical symmetry, or quasi-spherical symmetry. Consequently, we can define the radius, R^+, of the critical spherical shell by

$$\eta(R^+) = 0.05, \qquad \text{or } R^+ = \eta^{-1}(0.05). \tag{9}$$

And then the thickness of the critical spherical shell should be

$$\delta = R^+ - (R_E + R_S) = R^+ - R_0. \tag{10}$$

Now, we can apply the methods in [2] to calculate $g_1(r)$ and $g_2(r)$ first, and then get the thickness of critical spherical shell by means of eqs. (8)–(10).

In our calculations, the involved potentials and some concrete parameters are considered as follows:

Coulomb long-range force. When the distribution of charges in E molecule is non-spherically symmetric, strictly speaking, the Coulomb potential should be calculated according to eq. (29) in [6], that means, we should consider the action of multipole potentials. However, for convenience, we could also assume that some part of the charges is situated at the centre of E molecule, and the other part concentrates at the centre of its active site (fig. 2). Then, within the scope where Debye-Hückel's limiting law is valid, we have [6]:

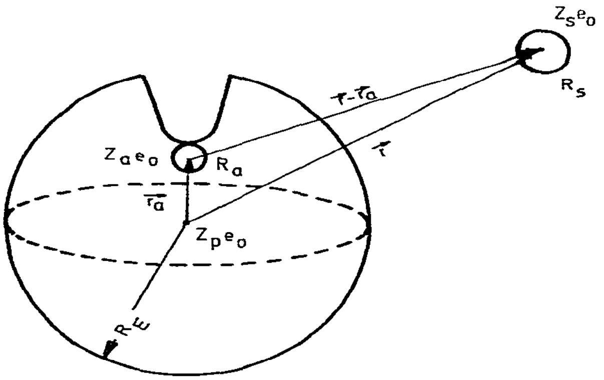

Fig. 2. The Coulomb interaction between enzyme molecule and substrate molecule.

$$
\begin{aligned}
U^{Z_E Z_E}_{\text{Coulomb}} &= U^{Z_P Z_S}_{\text{Coulomb}} + U^{Z_a Z_S}_{\text{Coulomb}} \\
&= \frac{1}{2}\left[\frac{\exp(R_S/\mathbf{R})}{1+1+R_S/\mathbf{R}} + \frac{\exp(R_E/\mathbf{R})}{1+R_E/\mathbf{R}}\right] \\
&\quad \times \frac{Z_P Z_S e_0^2}{\epsilon r}\exp(-r/\mathbf{R}) \\
&\quad + \frac{1}{2}\left[\frac{\exp(R_S/\mathbf{R})}{1+R_S/\mathbf{R}} + \frac{\exp(R_a/\mathbf{R})}{1+R_a/\mathbf{R}}\right] \\
&\quad \times \frac{Z_a Z_S e_0^2}{\epsilon|r-r_a|}\exp\left(-\frac{|r-r_a|}{\mathbf{R}}\right), \qquad (11)
\end{aligned}
$$

where z_P, z_a, z_S are respectively the charge number of the enzymatic protein other than the active site, that of the active site, and that of the S molecule; e_0 is the electronic charge; ϵ the dielectric constant of the intervening medium; R_E and R_S are respectively the radius of E molecule and the effective radius [7] of the active site, and in this paper, they are taken as 18.5 Å and 1.5 Å respectively; $\mathbf{R}$ is the "thickness" of the ion atmosphere given by

$$\mathbf{R} = [1000\ \epsilon kT/8\pi e_0^2 N\mu]^{1/2},$$

in which N is the Avogadro constant, and μ the ionic strength. When $\epsilon = 78.56$, $T = 298$ K, we have

$$\mathbf{R} = (3.044/\sqrt{\mu})\ \text{Å}.$$

Van der Waals short-range force. This is a type of force with the interaction range in only a few Å. The van der Waals potential between a macromolecule and a common small molecule has been discussed in [1,2], and could be expressed as

$$
U_{\text{van}} = \begin{cases} -U_0, & (0 \leqslant d \leqslant a_0), \\ -U_0 a_0(b_0/d - 1)/(b_0 - a_0), & (a_0 < d < b_0), \\ 0, & (d \geqslant b_0). \end{cases} \qquad (12)
$$

where $a_0 = 0.2$ Å, $b_0 = 3$ Å, d is the perpendicular distance between S molecule and the surface of E molecule, and when the S molecule is in the inside of the active cavity, d should be the perpendicular distance to the nearest wall of the cavity.

The distribution state of active site can be considered in the following two cases:

The surface-active site. In this case it is assumed that the active site is situated on a circular patch, S_a, on the surface of an E molecule (fig. 3). In this paper, we take $R_0 = R_E + R_S = 20$ Å, $\theta_a = 20°$ [1]. For the active site of this type, in solving eq. (2), the corresponding boundary conditions are

$$C|_{r,\theta,\phi \in S_a} = 0,$$

$$(n_{S_b} \cdot i)_{r,\theta,\phi \in S_b} = \partial(e^{U/kT} C)/\partial r|_{r,\theta,\phi \in S_b} = 0, \qquad (13)$$

$$C|_{r \to \infty} = C_0.$$

where n_{S_b} is the normal of the surface of the enzymic protein.

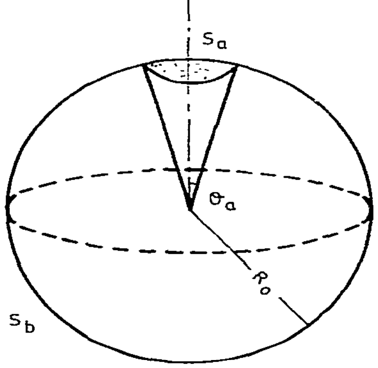

Fig. 3. Surface-active site of enzyme molecule.

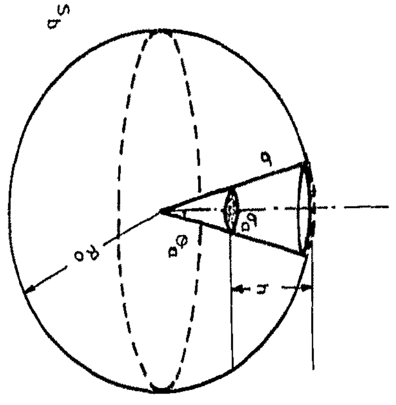

Fig. 4. Cavity-active site of enzyme molecule.

The cavity-active site. It is assumed that the active site is at the bottom, σ_a, of a circular cone cavity [8,9] (fig. 4). In our calculations, we take the depth of the active cleft $h = 10$ Å, $R_0 = 20$ Å, $\theta_a = 20°$, and the corresponding boundary conditions are

$$C|_{r,\theta,\phi \in \sigma_a} = 0,$$

$$(\boldsymbol{n}_{S_b} \cdot \boldsymbol{i})_{r,\theta,\phi \in S_b} = (\boldsymbol{n}_{S_\sigma} \cdot \boldsymbol{i})_{r,\theta,\phi \in \sigma} = 0,$$

$$C|_{r \to \infty} = C_0, \qquad (14)$$

where $\boldsymbol{n}_{S_b}$ is the normal of the side face of the cavity.

Now, the values calculated under various conditions for the thickness, δ, of the critical spherical shell are listed in table 1, from which we can draw the following points:

1. For the enzyme-catalyzed reaction systems of cavity-active site, δ is generally rather smaller. This is because when the active site is in the molecular crevice; the contact between S molecules and active sites is more difficult, and then the reaction rate will relatively decrease. Consequently, the effects due to such a non-spherically symmetric reaction in driving the distribution of S molecules to deviate from spherical symmetry are less also.

2. The short-range force generally renders δ smaller. And the stronger the short-range is, the more remarkable such an effect is. However, in reaction systems of cavity site, the short-range force expressed by (12) possesses in itself non-spherical symmetry. So, actually, it will affect δ from two opposite directions: on

Table 1
The values (Å) calculated in various cases for the thickness of the critical spherical shell

Type of active site	Without force field	Form of force field								
		Long-range force			Short-range force			Long-range + Short range force		
		$z_p z_S = -24$ $z_a z_S = 0$	$z_p z_S = -22$ $z_a z_S = -2$	$z_p z_S = -20$ $z_a z_S = -4$	$U_0 = 2$ kcal/mole	$U_0 = 4$ kcal/mole	$U_0 = 6$ kcal/mole	$z_p z_S = -24$, $z_a z_S = 0$ $U_0 = 6$ kcal/mole	$z_p z_S = -22$, $z_a z_S = -2$ $U_0 = 6$ kcal/mole	$z_p z_S = -20$, $z_a z_S = -4$ $U_0 = 6$ kcal/mole
Surface-active site	13.79	39.62	43.44	47.26	21.81	13.34	2.67	2.22	2.90	3.35
Cavity-active site	0	36.55	36.80	37.23	3.51	6.82	3.31	3.70	4.05	4.45

The diffusion coefficient $D = D_E + D_S = 7.0 \times 10^{-6}$ cm^2/s, and the ionic strength $\mu = 10^{-2}$.

the one hand the short-range property of the force field will make δ become smaller, on the other the non-spherical symmetry of the force field will cause δ to be larger. Therefore, for the reaction system of cavity-active site, δ does not vary monotonically with the increase of van der Waals force.

3. The long-range force generally renders δ larger, and if the long-range force possesses in itself some non-spherical symmetry, then such an effect will be more remarkable. Therefore, in this case, we have to consider the effects inside the critical spherical shell in order for calculating the proximity rate constant [5].

It should be pointed out that in the above calculation we have supposed that the concentration of S molecules on the active surface keeps equal to zero. The reaction rate thus calculated is corresponding to the diffusion-limit. It is readily conceivable that under such a condition the effects due to non-spherically symmetric reaction in driving the surrounding S molecules towards non-spherically symmetric distribution are greatest. So, such a consideration is also most typical and significant. In fact, for the reaction systems of surface-active site, if taking the activation energy ϵ_a into consideration, we can use [10]

$$\frac{\partial(\exp(U/kT)C}{\partial r} = \frac{1}{D}\left(\frac{kT}{2\pi m^*}\right)^{1/2} \exp(-\epsilon_a/kT)$$

$$\times \exp(U/kT)C \qquad (15)$$

to replace the first equation in (13). Here, $m^* = m_E m_S/(m_E + m_S)$ is the reduced mass of E and S molecules. Accordingly, we are able to calculate the relation between δ and ϵ_a. The calculated results show that when the activation energy ϵ_a is larger, since the reaction proceeds more slowly, the effects due to chemical reactions in causing the concentration distribution to be non-spherically symmetric are smaller, and hence so are the values of δ.

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