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DIFFUSION-CONTROLLED EFFECTS IN REVERSIBLE ENZYMATIC FAST REACTION SYSTEMS – CRITICAL SPHERICAL SHELL AND PROXIMITY RATE CONSTANT

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In this paper the diffusion-controlled effects in reversible enzyme fast reaction systems have been discussed. The main results are as follows: 1) An expression for the relation between the proximity second-order rate constant and the usual experimental second-order rate constant has been presented. From this expression we can see that the two kinds of rate constants are generally not equal unless the reaction proceeds very slowly (in comparison with the corresponding diffusion limit). 2) A new joint relation has been given between the theoretical calculated results and the experimentally measured ones for the activation energy. It has been pointed out that, for the reaction systems discussed here, it would no longer be valid to adopt the absolute reaction rate theory to calculate the activation energy as done commonly. 3) A formula has been given to calculate the upper limit obtainable possibly by experiments for the second-order rate constants in the reversible enzymatic fast reaction system. According to this formula, the value of such an upper limit is related not only to the diffusion coefficients of reacting molecules, the size of active surface, and the like, but also to the ratio of the concentration of product molecules to that of the substrate molecules at the equilibrium of the reaction system.

Furthermore, the reversible enzymatic fast reaction system with multi-substrate and multi-product has been discussed, and a general equation for calculating the degree of reaction flow derived as well.

1. The non-spherical symmetry of an enzyme molecule and its critical spherical shell

Although enzyme molecules are usually regarded as spherical ones, the active site of enzyme molecule generally occupies only a small part of its surface, or the entire active site may be burried in a concave region termed the molecular crevice. Therefore, in discussing the diffusion-controlled reactions of enzymes, one must meet the non-spherically symmetric problems [1–2]. However, the numerical calculations and analytical results [3–5] indicate that, when there exist van der Waals short-range forces, such a non-spherically symmetric effect is significant only within quite a thin shell (whose thickness may be assumed as δ) along on the surface of an enzyme molecule; whereas outside the shell the concentration distribution of the substrate molecules almost exhibits a state of spherical symmetry. Generally speaking, the stronger the short-range forces between enzyme and substrate are, the thinner the thickness of the shell is, namely, the narrower the scope of the non-spherical symmetry is. Furthermore, as pointed out by Jönsson and Wennerström [6], in this shell there probably exist the effect of rapid surface diffusion as well as some other specific effects known little by now. So, an appropriate approach is to confine our discussion on the diffusion process to the region outside the spherical shell with the radius of $R^+ = (R_E + R_S) + \delta = R_0 + \delta$ (fig. 1). We term such a spherical shell the critical spherical shell of an enzyme molecule. Outside the shell, the equations of diffusion-controlled reactions can be treated as a problem of spherical symmetry or quasi-spherical symmetry. Besides, in this case one needn't know the detailed knowledge about the short-range forces between enzyme and the other reacting molecules.

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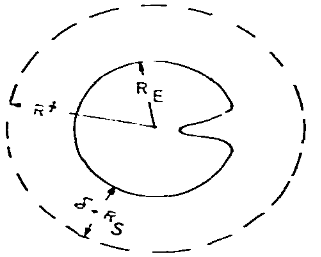
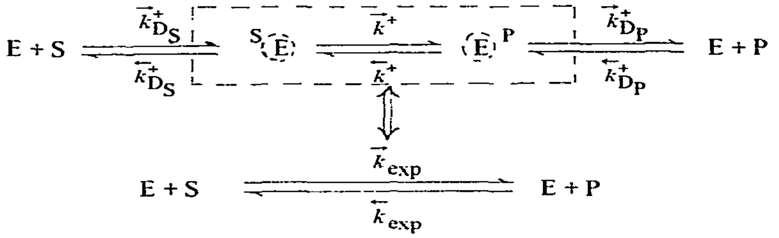


Fig. 1. The enzyme molecule E and its critical spherical shell. R_E and R_S are respectively the radius of the enzyme molecule and that of the substrate molecule, δ the thickness of the critical spherical shell.

2. Mono-substrate and mono-product reaction systems

According to the above discussion, reaction systems of this type can be expressed as



$$\mathrm{E+S} \underset{\overleftarrow{k}^+_{\mathrm{D_S}}}{\overset{\overrightarrow{k}^+_{\mathrm{D_S}}}{\rightleftharpoons}} \mathrm{S(E)} \underset{\overleftarrow{k}^+}{\overset{\overrightarrow{k}^+}{\rightleftharpoons}} \mathrm{(E)P} \underset{\overleftarrow{k}^+_{\mathrm{D_P}}}{\overset{\overrightarrow{k}^+_{\mathrm{D_P}}}{\rightleftharpoons}} \mathrm{E+P} \qquad (1)$$

$$\Updownarrow$$

$$\mathrm{E+S} \underset{\overleftarrow{k}_{\mathrm{exp}}}{\overset{\overrightarrow{k}_{\mathrm{exp}}}{\rightleftharpoons}} \mathrm{E+P} \qquad (2)$$

where S denotes the substrate and P the product; $\overrightarrow{k}^+_{D_S}$ is the second-order rate constant associated with the process of S molecules in the bulk solution diffusing to the critical spherical shell of E molecule; $\overrightarrow{k}^+$ is the rate constant of the S molecule on the critical spherical shell changing into the P molecule of the corresponding state; and so on. It is worthwhile to note that since $\delta = R^+ - R_0$ is usually very small, $\overrightarrow{k}^+$ and $\overleftarrow{k}^+$ are actually the second-order rate constants between E molecules and those reacting molecules that are very close to the E molecules. We shall call such a kind of rate constant the proximity second-order rate constant, which is generally different from the experimental second-order rate constant like $\overrightarrow{k}_{\mathrm{exp}}$ and $\overleftarrow{k}_{\mathrm{exp}}$. Below, we shall find out their relations.

The stochastic analysis in [7] showed us, for this kind of reaction system, around the E molecules both the concentration of S molecules and that of P molecules would exhibit some statistical gradients so far as the collective effect is concerned, and they can be described respectively by the following equations:

$$\begin{aligned} \partial C_{\mathrm{S}}/\partial t &= D_{\mathrm{S}}\, \nabla \cdot (\exp(-U_{\mathrm{S}}/kT)\, \nabla \exp(U_{\mathrm{S}}/kT) C_{\mathrm{S}}), \\ \partial C_{\mathrm{P}}/\partial t &= D_{\mathrm{P}}\, \nabla \cdot (\exp(-U_{\mathrm{P}}/kT)\, \nabla \exp(U_{\mathrm{P}}/kT) C_{\mathrm{P}}), \end{aligned} \qquad (3)$$

where C_{S} and C_{P} are respectively the concentration of S molecules and that of P molecules, ∇ is the hamilton operator, U_{S} and U_{P} are respectively the potential of interaction between S molecule and E molecule and that between P molecule and E molecule, k is the Boltzmann constant, T the absolute temperature, D_{S} and D_{P} are respectively the sum of the diffusion coefficients of S molecule and E molecule and that of P molecule and E molecule.

If only the region outside the critical spherical shell is discussed, in the steady state eq. (3) can be written as

$$\frac{1}{r^2}\frac{\mathrm{d}}{\mathrm{d}r}\left(r^2 \frac{\mathrm{d}}{\mathrm{d}r} C^*_{\mathrm{S}}\right) - \frac{1}{kT}\left(\frac{\mathrm{d}C^*_{\mathrm{S}}}{\mathrm{d}r}\right)\left(\frac{\mathrm{d}U_{\mathrm{S}}}{\mathrm{d}r}\right) = 0, \quad \frac{1}{r^2}\frac{\mathrm{d}}{\mathrm{d}r}\left(r^2 \frac{\mathrm{d}}{\mathrm{d}r} C^*_{\mathrm{P}}\right) - \frac{1}{kT}\left(\frac{\mathrm{d}C^*_{\mathrm{P}}}{\mathrm{d}r}\right)\left(\frac{\mathrm{d}U_{\mathrm{P}}}{\mathrm{d}r}\right) = 0, \quad (r \geqslant R^+). \qquad (4)$$

where

$$C_S^*(r) = \exp(U_S(r)/kT)\,C_S(r), \qquad C_P^*(r) = \exp(U_P(r)/kT)\,C_P(r), \tag{5}$$

the corresponding boundary conditions are

$$C_S^*\big|_{r\to\infty} = C_S\big|_{r\to\infty} = C_{S0}, \qquad C_P^*\big|_{r\to\infty} = C_P\big|_{r\to\infty} = C_{P0}. \tag{6}$$

Under such boundary conditions the solutions of eq. (4) can be expressed as

$$C_S^*(r) = C_{S0} - B_S \int_r^\infty \frac{\exp(U_S(r)/kT)}{r^2}\,\mathrm{d}r, \qquad C_P^*(r) = C_{P0} - B_P \int_r^\infty \frac{\exp(U_P(r)/kT)}{r^2}\,\mathrm{d}r. \tag{7}$$

We know that the flows of molecules through unit area are [1,7]

$$\boldsymbol{i}_S = -D_S \exp(-U_S(r)/kT)\,\nabla C_S^*, \qquad \boldsymbol{i}_P = -D_P \exp(-U_P(r)/kT)\,\nabla C_P^*. \tag{8}$$

Then from (7) and (8), we obtain the total flows, I_S and I_P, of S molecules and P molecules to the critical spherical shell in unit time:

$$I_S = \iint_{S_{R^+}} \boldsymbol{i}_S \cdot \mathrm{d}\boldsymbol{S} = 4\pi D_S B_S, \qquad I_P = \iint_{S_{R^+}} \boldsymbol{i}_P \cdot \mathrm{d}\boldsymbol{S} = 4\pi D_P B_P. \tag{9}$$

At steady state, according to the conservation of mass, the total flow into the critical spherical shell S_{R^+} in unit time should be equal to the total flow out of the shell; i.e., we have

$$I_S = I_P = I, \tag{10}$$

where I may be called the degree of reaction flow, with which the flow speeds of the various reacting molecules may be reflected.

On the other hand, according to the theory of chemical kinetics, at steady state we have

$$-\mathrm{d}[\mathrm{S}]/\mathrm{d}t = \mathrm{d}[\mathrm{P}]/\mathrm{d}t = \overrightarrow{k}_{\exp} C_{S0}\,[\mathrm{E}] - \overleftarrow{k}_{\exp} C_{P0}\,[\mathrm{E}] = \overrightarrow{k}^+ C_S(R^+)[\mathrm{E}] - \overleftarrow{k}^+ C_P(R^+)\,[\mathrm{E}], \tag{11}$$

which means that the degree of reaction flow can also be expressed as

$$I = \overrightarrow{k}^+ C_S(R^+) - \overleftarrow{k}^+ C_P(R^+), \qquad I = \overrightarrow{k}_{\exp} C_{S0} - \overleftarrow{k}_{\exp} C_{P0}. \tag{12, 13}$$

Substituting eq. (7) into eq. (12), and utilizing (5), (9) and (10), we obtain

$$I\{\overrightarrow{k}^+ \exp(-U_S(R^+)/kT)\,C_{S0} - \overleftarrow{k}^+ \exp(-U_P(R^+)/kT)\,C_{P0}\}$$
$$\times \left[1 + \frac{1}{4\pi R^+}\left(\frac{\overrightarrow{k}^+}{f_S^+ D_S}\exp(-U_S(R^+)/kT) + \frac{\overleftarrow{k}^+}{f_P^+ D_P}\exp(-U_P(R^+)/kT)\right)\right]^{-1}, \tag{14}$$

where the force-range factors are defined as [8]

$$f_S^+ = \left(R^+ \int_{R^+}^\infty \exp(U_S(r)/kT)\frac{\mathrm{d}r}{r^2}\right)^{-1}, \qquad f_P^+ = \left(R^+ \int_{R^+}^\infty \exp(U_P(r)/kT)\frac{\mathrm{d}r}{r^2}\right)^{-1}. \tag{15}$$

Thus, we obtain the following results:

1) The relation between the second-order rate constant obtained usually by experiments and proximity second order rate constant.

Making a comparison between (13) and (14), we get

$$\begin{aligned}\overrightarrow{k}_{\mathrm{exp}} &= \overrightarrow{k}^{+} \exp(-U_{\mathrm{S}}(R^{+})/kT) \left[1 + \frac{1}{4\pi R^{+}}\left(\frac{\overrightarrow{k}^{+}}{f_{\mathrm{S}}^{+}D_{\mathrm{S}}} \exp(-U_{\mathrm{S}}(R^{+})/kT) + \frac{\overleftarrow{k}^{+}}{f_{\mathrm{P}}^{+}D_{\mathrm{P}}} \exp(-U_{\mathrm{P}}(R^{+})/kT)\right)\right]^{-1}, \\ \overleftarrow{k}_{\mathrm{exp}} &= \overleftarrow{k}^{+} \exp(-U_{\mathrm{P}}(R^{+})/kT) \left[1 + \frac{1}{4\pi R^{+}}\left(\frac{\overrightarrow{k}^{+}}{f_{\mathrm{S}}^{+}D_{\mathrm{S}}} \exp(-U_{\mathrm{S}}(R^{+})/kT) + \frac{\overleftarrow{k}^{+}}{f_{\mathrm{P}}^{+}D_{\mathrm{P}}} \exp(-U_{\mathrm{P}}(R^{+})/kT)\right)\right]^{-1}.\end{aligned} \tag{16}$$

$$K = \frac{\overrightarrow{k}_{\mathrm{exp}}}{\overleftarrow{k}_{\mathrm{exp}}} = \frac{\overrightarrow{k}^{+}}{\overleftarrow{k}^{+}} \exp\left(\frac{U_{\mathrm{P}}(R^{+}) - U_{\mathrm{S}}(R^{+})}{kT}\right) \tag{17}$$

$$\begin{aligned}\overrightarrow{k}^{+} &= \overrightarrow{k}_{\mathrm{exp}} \exp(U_{\mathrm{S}}(R^{+})/kT) \left[1 - \frac{1}{4\pi R^{+}}\left(\frac{\overrightarrow{k}_{\mathrm{exp}}}{f_{\mathrm{S}}^{+}D_{\mathrm{S}}} + \frac{\overleftarrow{k}_{\mathrm{exp}}}{f_{\mathrm{P}}^{+}D_{\mathrm{P}}}\right)\right]^{-1}, \\ \overleftarrow{k}^{+} &= \overleftarrow{k}_{\mathrm{exp}} \exp(U_{\mathrm{P}}(R^{+})/kT) \left[1 - \frac{1}{4\pi R^{+}}\left(\frac{\overrightarrow{k}_{\mathrm{exp}}}{f_{\mathrm{S}}^{+}D_{\mathrm{S}}} + \frac{\overleftarrow{k}_{\mathrm{exp}}}{f_{\mathrm{P}}^{+}D_{\mathrm{P}}}\right)\right]^{-1}\end{aligned} \tag{18}$$

2) The relation between the concentration distributions and the rate constants.
From eq. (13) or eq. (14), and with the help of (5), (7), (9) and (10), we obtain

$$\begin{aligned}C_{\mathrm{S}}(r) &= \left[C_{\mathrm{S0}} - \frac{\overrightarrow{k}_{\mathrm{exp}} C_{\mathrm{S0}} - \overleftarrow{k}_{\mathrm{exp}} C_{\mathrm{P0}}}{4\pi D_{\mathrm{S}}} \int_{r}^{\infty} \frac{\exp(U_{\mathrm{S}}(r)/kT)}{r^{2}}\,\mathrm{d}r\right] \exp(-U_{\mathrm{S}}(r)/kT), \\ C_{\mathrm{P}}(r) &= \left[C_{\mathrm{P0}} + \frac{\overrightarrow{k}_{\mathrm{exp}} C_{\mathrm{S0}} - \overleftarrow{k}_{\mathrm{exp}} C_{\mathrm{P0}}}{4\pi D_{\mathrm{P}}} \int_{r}^{\infty} \frac{\exp(U_{\mathrm{P}}(r)/kT)}{r^{2}}\,\mathrm{d}r\right] \exp(-U_{\mathrm{P}}(r)/kT),\end{aligned} \tag{19}$$

or

$$\begin{aligned}C_{\mathrm{S}}(r) &= \left\{C_{\mathrm{S0}} - \left[\frac{\overrightarrow{k}^{+} \exp(-U_{\mathrm{S}}(R^{+})/kT)\, C_{\mathrm{S0}} - \overleftarrow{k}^{+} \exp(-U_{\mathrm{P}}(R^{+})/kT)\, C_{\mathrm{P0}}}{1 + (1/4\pi R^{+})\,(\overrightarrow{k}^{+} \exp(-U_{\mathrm{S}}(R^{+})/kT)/f_{\mathrm{S}}^{+}D_{\mathrm{S}} + \overleftarrow{k}^{+} \exp(-U_{\mathrm{P}}(R^{+})/kT)/f_{\mathrm{P}}^{+}D_{\mathrm{P}})}\right]\left(\frac{1}{4\pi D_{\mathrm{S}}}\right)\right. \\ &\quad \left. \times \int_{r}^{\infty} \frac{\exp(U_{\mathrm{S}}(r)/kT)}{r^{2}}\,\mathrm{d}r\right\} \exp(-U_{\mathrm{S}}(r)/kT), \\ C_{\mathrm{P}}(r) &= \left\{C_{\mathrm{P0}} + \left[\frac{\overrightarrow{k}^{+} \exp(-U_{\mathrm{S}}(R^{+})/kT)\, C_{\mathrm{S0}} - \overleftarrow{k}^{+} \exp(-U_{\mathrm{P}}(R^{+})/kT)\, C_{\mathrm{P0}}}{1 + (1/4\pi R^{+})\,(\overrightarrow{k}^{+} \exp(-U_{\mathrm{S}}(R^{+})/kT)/f_{\mathrm{S}}^{+}D_{\mathrm{S}} + \overleftarrow{k}^{+} \exp(-U_{\mathrm{P}}(R^{+})/kT)/f_{\mathrm{P}}^{+}D_{\mathrm{P}})}\right]\left(\frac{1}{4\pi D_{\mathrm{P}}}\right)\right. \\ &\quad \left. \times \int_{r}^{\infty} \frac{\exp(U_{\mathrm{P}}(r)/kT)}{r^{2}}\,\mathrm{d}r\right\} \exp(-U_{\mathrm{P}}(r)/kT).\end{aligned} \tag{20}$$

It should be pointed out that the above equations were derived in the concentration unit of (number of molecules)/cm^3, and if we take the moles/dm^3 as the unit of concentration, then into some places of the above equations we have to add the coefficient $N_a/1000$. Here, N_a is the Avogadro constant. For instance, in this case, eq. (18) should be rewritten as

$$\vec{k}^+ = \vec{k}_{\mathrm{exp}} \exp(U_{\mathrm{S}}(R^+)/kT)\left[1 - \frac{1}{4\pi R^+(N_{\mathrm{a}}/1000)}\left(\frac{\vec{k}_{\mathrm{exp}}}{f_{\mathrm{S}}^+D_{\mathrm{S}}} + \frac{\overleftarrow{k}_{\mathrm{exp}}}{f_{\mathrm{P}}^+D_{\mathrm{P}}}\right)\right]^{-1},$$

$$\overleftarrow{k}^+ = \overleftarrow{k}_{\mathrm{exp}} \exp(U_{\mathrm{P}}(R^+)/kT)\left[1 - \frac{1}{4\pi R^+(N_{\mathrm{a}}/1000)}\left(\frac{\vec{k}_{\mathrm{exp}}}{f_{\mathrm{S}}^+D_{\mathrm{S}}} + \frac{\overleftarrow{k}_{\mathrm{exp}}}{f_{\mathrm{P}}^+D_{\mathrm{P}}}\right)\right]^{-1}. \tag{21}$$

And the rest of the above equations (except eq. (17)) should also be revised likewise.

3. The joint relations between various kinds of rate constants

By means of eq. (18), we are able to calculate the proximity second-order rate constants from the values of the experimental second-order rate constants. eq. (18) also tells us that only when $\vec{k}_{\mathrm{exp}} \ll 4\pi D_{\mathrm{S}} f_{\mathrm{S}}^+ R^+$ and $\overleftarrow{k}_{\mathrm{exp}} \ll 4\pi D_{\mathrm{P}} f_{\mathrm{P}}^+ R^+$, can we have $\vec{k}^+ = \vec{k}_{\mathrm{exp}} \exp(U_{\mathrm{S}}(R^+)/kT)$ and $\overleftarrow{k}^+ = \overleftarrow{k}_{\mathrm{exp}} \exp(U_{\mathrm{P}}(R^+)/kT)$. In other words, only when the experimental second-order rate constants are far less than their corresponding diffusion-limits [1], would there be such a simple Boltzmann relation between the proximity second-order rate constant and the corresponding experimental one. However, for the common liquid phase fast reaction systems, especially the enzymatic fast reaction system, such a condition doesn't seem to hold. In this case, due to the existance of solvent molecules the diffusive transport of reactant molecules is greatly slowed down. So, when the reactions proceed more rapidly, there would appear such a picture: the tendency due to the chemical reactions in driving the spatial distribution of reactant molecules to deviate from the equilibrium state will cover the tendency due to the diffusive movements in restoring the spatial distribution towards the equilibrium. In other words, the diffusive movements of reactant molecules are not fast enough to be able to keep the spatial distribution of the system being in equilibrium state. For such reaction systems, the second-order rate constants obtained by experiments will be sharply restrained by diffusion coefficients, and hence explicitly different from the proximity second-order rate constant. What should be pointed out particularly is that, for reaction systems of this type, since the whole spatial distribution is no longer subject to the equilibrium statistical laws, we could not apply the absolute reaction rate theory to calculate $\vec{k}_{\mathrm{exp}}$ and $\overleftarrow{k}_{\mathrm{exp}}$. Then, there would naturally appear a problem how to compare the theoretical calculations with the experimental results. Below, we shall see, if the thickness, δ, of the critical spherical shell is small, we can use eq. (8) to resolve this difficulty, and establish a new and more general corresponding relation between theoretical calculation and experimental measurements.

At first, let us further decompose the part framed with the dotted line in (1) into:

$$^{\mathrm{S}}(\mathrm{E}) \underset{\overleftarrow{k}^{\delta}_{\mathrm{D_S}}}{\overset{\vec{k}^{\delta}_{\mathrm{D_S}}}{\rightleftharpoons}} \mathrm{S} \,\vdots\, \mathrm{E} \underset{\overleftarrow{k}}{\overset{\vec{k}}{\rightleftharpoons}} \mathrm{E} \,\vdots\, \mathrm{P} \underset{\overleftarrow{k}^{\delta}_{\mathrm{D_P}}}{\overset{\vec{k}^{\delta}_{\mathrm{D_P}}}{\rightleftharpoons}} (\mathrm{E})^{\mathrm{P}} \tag{22}$$

where S $\vdots$ E and E $\vdots$ P respectively denote the states in which an S molecule and a P molecule have encountered the active surface of an E molecule (merely the physical contact) but have not yet combined with it (even for the initial stage of the combination reaction) [7], namely, they are still at the bimolecular stage; $\vec{k}^{\delta}_{\mathrm{D_S}}$ denotes the second-order rate constant with which S molecules on the critical spherical shell of an E molecule diffuse to its active surface; $\vec{k}$ is the rate constant of the S molecule on the enzymatic active surface changing into the P molecule of the corresponding state, so it is actually the contact rate constant; and so on. At the steady state, we then have [9]

$$\vec{k}^+ = \vec{k}^{\delta}_{\mathrm{D_S}}\,\vec{k}\,\vec{k}^{\delta}_{\mathrm{D_P}}/(\vec{k}\,\vec{k}^{\delta}_{\mathrm{D_P}} + \overleftarrow{k}^{\delta}_{\mathrm{D_S}}\,\vec{k}^{\delta}_{\mathrm{D_P}} + \overleftarrow{k}\,\overleftarrow{k}^{\delta}_{\mathrm{D_S}}), \qquad \overleftarrow{k}^+ = \overleftarrow{k}^{\delta}_{\mathrm{D_P}}\,\overleftarrow{k}\,\overleftarrow{k}^{\delta}_{\mathrm{D_S}}/(\vec{k}\,\vec{k}^{\delta}_{\mathrm{D_P}} + \overleftarrow{k}^{\delta}_{\mathrm{D_S}}\,\vec{k}^{\delta}_{\mathrm{D_P}} + \overleftarrow{k}\,\overleftarrow{k}^{\delta}_{\mathrm{D_S}}). \tag{23}$$

As mentioned above, the thickness, δ, of the critical spherical shell of an E molecule is generally small, so the diffusive distance, $\Delta l \sim \delta$, from the spot on the shell to the active site is a trifling quantity, too. It could be proved that when the path of diffusion is very short, we have the corresponding diffusion-controlled rate constant $k_{\mathrm{D}}^{\Delta l} \sim 1/\Delta l$ (see Appendix). Consequently, when the thickness of the critical spherical shell is sufficiently thin, we have

$$\overrightarrow{k}_{\mathrm{D_S}}^{\delta} \sim \overleftarrow{k}_{\mathrm{D_S}}^{\delta} \sim \overrightarrow{k}_{\mathrm{D_P}}^{\delta} \sim \overleftarrow{k}_{\mathrm{D_P}}^{\delta} \sim 1/\Delta l \sim 1/\delta \gg \overrightarrow{k} \text{ and } \overleftarrow{k}. \tag{24}$$

On the other hand, according to the method of derivation in [7], it follows readily that

$$\overrightarrow{k}_{\mathrm{D_S}}^{\delta}/\overleftarrow{k}_{\mathrm{D_S}}^{\delta} = \exp\left(\frac{U_{\mathrm{S}}(R^{+}) - U_{\mathrm{S}}^{\mathrm{a}}}{kT}\right), \qquad \overrightarrow{k}_{\mathrm{D_P}}^{\delta}/\overleftarrow{k}_{\mathrm{D_P}}^{\delta} = \exp\left(\frac{U_{\mathrm{P}}(R^{+}) - U_{\mathrm{P}}^{\mathrm{a}}}{kT}\right). \tag{25}$$

where $U_{\mathrm{S}}^{\mathrm{a}}$ and $U_{\mathrm{P}}^{\mathrm{a}}$ respectively denote the potentials of an S molecule and a P molecule being in contact (physical contact) with the active site of an E molecule. Substituting (24) and (25) into (23), we obtain

$$\overrightarrow{k}^{+} = \exp\left(\frac{U_{\mathrm{S}}(R^{+}) - U_{\mathrm{S}}^{\mathrm{a}}}{kT}\right)\overrightarrow{k}, \qquad \overleftarrow{k}^{+} = \exp\left(\frac{U_{\mathrm{P}}(R^{+}) - U_{\mathrm{P}}^{\mathrm{a}}}{kT}\right)\overleftarrow{k}. \tag{26}$$

We know, for this kind of fast reaction system, although the ubiquitous solvent molecules decrease the diffusive speed of reactants considerably, and hence destroy the equilibrium state of the spatial distribution of reacting molecules, on the other hand, however, the existance of the solvent molecules does accelerate the energy-exchange so much *as to ensure that the energy level distributions of solute molecules in the reaction system are still governed by the equilibrium statistical laws. Then, in such a system, S ¦ E and its activated complex are always in thermodynamic equilibrium with each other. Therefore, we could still apply the absolute reaction rate theory to calculate the contact rate constant $\overrightarrow{k}$. Likewise, we have the same conclusion for $\overleftarrow{k}$. We thus have

$$\overrightarrow{k} = (kT/h)\exp(-\overrightarrow{\Delta G}^{\ddagger}/kT), \qquad \overleftarrow{k} = (kT/h)\exp(-\overleftarrow{\Delta G}^{\ddagger}/kT). \tag{27}$$

But in applying the above formulae in actual calculations, we should take such a state as the starting point of the energy where an S molecule or a P molecule is in contact with the active site of an E molecule.

Substituting (26) and (27) into (18), we obtain

$$\begin{aligned} \Delta\overrightarrow{G}^{\ddagger} &= -kT\ln\left[\frac{(h/kT)\,\overrightarrow{k}_{\mathrm{exp}}}{1-(1/4\pi R^{+})\,(\overrightarrow{k}_{\mathrm{exp}}/f_{\mathrm{S}}^{+}D_{\mathrm{S}} + \overleftarrow{k}_{\mathrm{exp}}/f_{\mathrm{P}}^{+}D_{\mathrm{P}})}\right] + U_{\mathrm{S}}(R^{+}) - U_{\mathrm{S}}^{\mathrm{a}}, \\ \Delta\overleftarrow{G}^{\ddagger} &= -kT\ln\left[\frac{(h/kT)\,\overleftarrow{k}_{\mathrm{exp}}}{1-(1/4\pi R^{+})\,(\overrightarrow{k}_{\mathrm{exp}}/f_{\mathrm{S}}^{+}D_{\mathrm{S}} + \overleftarrow{k}_{\mathrm{exp}}/f_{\mathrm{P}}^{+}D_{\mathrm{P}})}\right] + U_{\mathrm{P}}(R^{+}) - U_{\mathrm{P}}^{\mathrm{a}}. \end{aligned} \tag{28}$$

This provides a joint relation between the second-order rate constant measured by experiments and the activation energy calculated with the absolute reaction rate theory for the reversible enzymatic fast reaction systems.

If the contact rate constant can be expressed through the collision activation energy [7]; i.e.

$$\overrightarrow{k} = (kT/2\pi m_{\mathrm{S}}^{*})^{1/2}\,S_{\mathrm{a}}\exp(-\overrightarrow{E}_{\mathrm{a}}/kT), \qquad \overleftarrow{k} = (kT/2\pi m_{\mathrm{P}}^{*})^{1/2}\,S_{\mathrm{a}}\exp(-\overleftarrow{E}_{\mathrm{a}}/kT) \tag{29}$$

where m_{S}^{*} is the reduced mass of S molecule and E molecule, m_{P}^{*} the reduced mass of P molecule and E molecule, S_{a} the area of the active surface. Substituting (26) and (29) into (18), we then have

$$\begin{aligned} \overrightarrow{E}_{\mathrm{a}} &= kT\ln\left\{\frac{(kT/2\pi m_{\mathrm{S}}^{*})^{1/2}\,S_{\mathrm{a}}[1-(1/4\pi R^{+})\,(\overrightarrow{k}_{\mathrm{exp}}/f_{\mathrm{S}}^{+}D_{\mathrm{S}} + \overleftarrow{k}_{\mathrm{exp}}/f_{\mathrm{P}}^{+}D_{\mathrm{P}})]}{\overrightarrow{k}_{\mathrm{exp}}}\right\} + U_{\mathrm{S}}(R^{+}) - U_{\mathrm{S}}^{\mathrm{a}}. \\ \overleftarrow{E}_{\mathrm{a}} &= kT\ln\left\{\frac{(kT/2\pi m_{\mathrm{P}}^{*})^{1/2}\,S_{\mathrm{a}}[1-(1/4\pi R^{+})\,(\overrightarrow{k}_{\mathrm{exp}}/f_{\mathrm{S}}^{+}D_{\mathrm{S}} + \overleftarrow{k}_{\mathrm{exp}}/f_{\mathrm{P}}^{+}D_{\mathrm{P}})]}{\overleftarrow{k}_{\mathrm{exp}}}\right\} + U_{\mathrm{P}}(R^{+}) - U_{\mathrm{P}}^{\mathrm{a}}. \end{aligned} \tag{30}$$

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* For a solute molecule with diameter of a few Å, the average number of collisions between such a molecule and the neighbouring solvent molecules runs into 10^{14} times per second.

which presents a new relation for more precisely obtaining the collision activation energy through experiments.

From eq. (17) we see that $\overrightarrow{k}_{\mathrm{exp}}/\overleftarrow{k}_{\mathrm{exp}} \neq \overrightarrow{k}^+/\overleftarrow{k}^+$ unless $U_{\mathrm{P}}(R^+) = U_{\mathrm{S}}(R^+)$.

And from eq. (18) we see that, since $\overrightarrow{k}^+$ and $\overleftarrow{k}^+$ must not be negative, it follows

$$(1/4\pi R^+)\,(\overrightarrow{k}_{\mathrm{exp}}/f^+_{\mathrm{S}}D_{\mathrm{S}} + \overleftarrow{k}_{\mathrm{exp}}/f^+_{\mathrm{P}}D_{\mathrm{P}}) < 1$$

namely,

$$\overrightarrow{k}_{\mathrm{exp}} < 4\pi D_{\mathrm{S}} f^+_{\mathrm{S}} R^+/(1 + D_{\mathrm{S}} f^+_{\mathrm{S}}/K D_{\mathrm{P}} f^+_{\mathrm{P}}), \qquad \overleftarrow{k}_{\mathrm{exp}} < 4\pi D_{\mathrm{P}} f^+_{\mathrm{P}} R^+/(1 + K D_{\mathrm{S}} f^+_{\mathrm{S}}/D_{\mathrm{P}} f^+_{\mathrm{P}}). \tag{31}$$

When the inequality in the above equations is replaced by equality, $\overrightarrow{k}^+$ and $\overleftarrow{k}^+$ will become infinite, which is of course impossible. So, we must find another route to estimate the upper limit of $\overrightarrow{k}_{\mathrm{exp}}$ and $\overleftarrow{k}_{\mathrm{exp}}$. One reasonable way is to proceed from eq. (29). As the maximal values of $\overrightarrow{k}$ and $\overleftarrow{k}$ should be corresponding to the case of the collision activation energy being equal to zero, we then have from (26)

$$\mathrm{Max}\ \overrightarrow{k}^+ = \left(\frac{kT}{2\pi m^*_{\mathrm{S}}}\right)^{1/2} S_{\mathrm{a}} \exp\left(\frac{U_{\mathrm{S}}(R^+) - U^{\mathrm{a}}_{\mathrm{S}}}{kT}\right), \qquad \mathrm{Max}\ \overleftarrow{k}^+ = \left(\frac{kT}{2\pi m^*_{\mathrm{P}}}\right)^{1/2} S_{\mathrm{a}} \exp\left(\frac{U_{\mathrm{P}}(R^+) - U^{\mathrm{a}}_{\mathrm{P}}}{kT}\right) \tag{32, 33}$$

Substituting (32) into (16), and utilizing (17), we obtain

$$\mathrm{Max}\ \overrightarrow{k}_{\mathrm{exp}} = \frac{(kT/2\pi m^*_{\mathrm{S}})^{1/2}\, S_{\mathrm{a}} \exp(-U^{\mathrm{a}}_{\mathrm{S}}/kT)}{1 + (kT/2\pi m^*_{\mathrm{S}})^{1/2}\,(S_{\mathrm{a}}/4\pi R^+)\,(\exp(-U^{\mathrm{a}}_{\mathrm{S}}/kT)/f^+_{\mathrm{S}}D_{\mathrm{S}} + \exp(-U^{\mathrm{a}}_{\mathrm{P}}/kT)/K f^+_{\mathrm{P}}D_{\mathrm{P}})}. \tag{34}$$

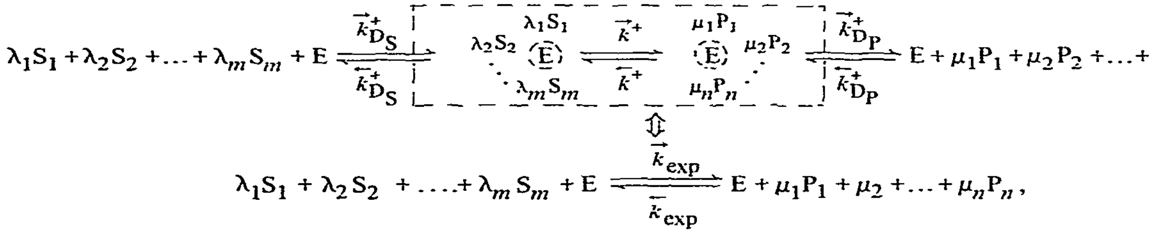
On substituting (33) into (16), and utilizing (17), we obtain

$$\mathrm{Max}\ \overleftarrow{k}_{\mathrm{exp}} = \frac{(kT/2\pi m^*_{\mathrm{P}})^{1/2}\, S_{\mathrm{a}} \exp(-U^{\mathrm{a}}_{\mathrm{P}}/kT)}{1 + (kT/2\pi m^*_{\mathrm{P}})^{1/2}\,(S_{\mathrm{a}}/4\pi R^+)\,(K \exp(-U^{\mathrm{a}}_{\mathrm{S}}/kT)/f^+_{\mathrm{S}}D_{\mathrm{S}} + \exp(-U^{\mathrm{a}}_{\mathrm{P}}/kT)/f^+_{\mathrm{P}}D_{\mathrm{P}})}. \tag{35}$$

We thus see that the upper limit of the second-order rate constant measured by experiments is not only related to the diffusion coefficients, the size of the active surface, and the like, but also related to the proportional constant K, which is none other than the ratio of the concentration of P molecules to that of S molecules when the reaction system attains the equilibrium.

4. Multi-substrate and multi-product reaction system

Here we extend the above discussion to the general case, the case of multi-substrate and multi-product reaction systems. Now, eq. (1) should be rewritten as



$$\begin{gathered}\lambda_1 S_1 + \lambda_2 S_2 + \ldots + \lambda_m S_m + E \underset{\overleftarrow{k}^+_{D_S}}{\overset{\overrightarrow{k}^+_{D_S}}{\rightleftharpoons}} (\lambda_1 S_1, \lambda_2 S_2, \ldots, \lambda_m S_m)E \underset{\overleftarrow{k}^+}{\overset{\overrightarrow{k}^+}{\rightleftharpoons}} E(\mu_1 P_1, \mu_2 P_2, \ldots, \mu_n P_n) \underset{\overleftarrow{k}^+_{D_P}}{\overset{\overrightarrow{k}^+_{D_P}}{\rightleftharpoons}} E + \mu_1 P_1 + \mu_2 P_2 + \ldots + \mu_n P_n \\ \Updownarrow \\ \lambda_1 S_1 + \lambda_2 S_2 + \ldots + \lambda_m S_m + E \underset{\overleftarrow{k}_{\mathrm{exp}}}{\overset{\overrightarrow{k}_{\mathrm{exp}}}{\rightleftharpoons}} E + \mu_1 P_1 + \mu_2 + \ldots + \mu_n P_n ,\end{gathered} \tag{36}$$

where $\lambda_1, \lambda_2, \ldots, \lambda_m$ and $\mu_1, \mu_2, \ldots, \mu_m$ are respectively the stoichiometric coefficients of the substrates and the products. At steady state, the concentrations, $C_{\mathrm{S}_1}, C_{\mathrm{S}_2}, \ldots, C_{\mathrm{S}_m}$, of the various substrates and the concentrations, $C_{\mathrm{P}_1}, C_{\mathrm{P}_2}, \ldots, C_{\mathrm{P}_n}$, of the various products should respectively satisfy the following equations [7]

$$\nabla\cdot(\exp(-U_{S_i}(r)/kT)\,\nabla C^*_{S_i})=0\quad(i=1,2,\ldots,m),\qquad \nabla\cdot(\exp(-U_{P_j}(r)/kT)\,\nabla C^*_{P_j})=0\quad(j=1,2,\ldots,n). \tag{37}$$

where

$$C^*_{S_i}(r)=\exp(U_{S_i}(r)/kT)\,C_{S_i}(r),\qquad C^*_{P_j}(r)=\exp(U_{P_j}(r)/kT)\,C_{P_j}(r), \tag{38}$$

and $U_{S_i}(r)$ and $U_{P_j}(r)$ respectively denote the potential of an E molecule with an S_i molecule and that of an E molecule with a P_j molecule. The corresponding boundary conditions are

$$C^*_{S_i}(r)\big|_{r\to\infty}=C_{S_i}(r)\big|_{r\to\infty}=C_{S_i0},\quad(i=1,2,\ldots,m),\qquad C^*_{P_j}(r)\big|_{r\to\infty}=C_{P_j}(r)\big|_{r\to\infty}=C_{P_j0},\quad(j=1,2,\ldots,n). \tag{39}$$

Then, within the quasi-spherically symmetric scope, the region of $r\geqslant R^+$, the solutions of eq. (37) can be expressed as

$$C^*_{S_i}=C_{S_i0}-B_{S_i}\int_r^\infty\frac{\exp(U_{S_i}(r)/kT)}{r^2}\,\mathrm{d}r,\qquad C^*_{P_j}=C_{P_j0}-B_{P_j}\int_r^\infty\frac{\exp(U_{P_j}(r)/kT)}{r^2}\,\mathrm{d}r. \tag{40}$$

The flows of molecules through unit area are

$$i_{S_i}=-D_{S_i}\exp(-U_{S_i}(r)/kT)\,\nabla C^*_{S_i}(r),\qquad i_{S_j}=-D_{P_j}\exp(-U_{P_j}(r)/kT)\,\nabla C^*_{P_j}(r). \tag{41}$$

The total flows of S_i molecules and P_j molecules towards the critical spherical shell of an E molecule in unit time will respectively be

$$I_{S_i}=\iint_{S_{R^+}} i_{S_i}\cdot\mathrm{d}\boldsymbol{S}=4\pi D_{S_i}B_{S_i},\quad(i=1,2,\ldots,m)\qquad I_{P_j}=\iint_{S_{R^+}} i_{P_j}\cdot\mathrm{d}\boldsymbol{S}=4\pi D_{P_j}B_{P_j},\quad(j=1,2,\ldots,n). \tag{42}$$

At steady state, according to the conservation of mass, we have

$$I_{S_i}/\lambda_i=-I_{P_j}/\mu_j=I,\qquad(i=1,2,\ldots,m;\ j=1,2,\ldots,n). \tag{43}$$

On the other hand, since

$$-\frac{1}{\lambda_i}\frac{\mathrm{d}[S_i]}{\mathrm{d}t}=\frac{1}{\mu_j}\frac{\mathrm{d}[P_j]}{\mathrm{d}t}=\overrightarrow{k}_{\exp}C^{\lambda_1}_{S_10}C^{\lambda_2}_{S_20}\cdots C^{\lambda_m}_{S_m0}[E]-\overleftarrow{k}_{\exp}C^{\mu_1}_{P_10}C^{\mu_2}_{P_20}\cdots C^{\mu_n}_{P_n0}[E]$$

$$=\overrightarrow{k}^+[C_{S_1}(R^+)]^{\lambda_1}[C_{S_2}(R^+)]^{\lambda_2}\cdots[C_{S_m}(R^+)]^{\lambda_m}[E]-\overleftarrow{k}^+[C_{P_1}(R^+)]^{\mu_1}[C_{P_2}(R^+)]^{\mu_2}\cdots[C_{P_n}(R^+)]^{\mu_n}[E],$$

the degree of reaction flow can be expressed as

$$I=\overrightarrow{k}^+\prod_{i=1}^m[C_{S_i}(R^+)]^{\lambda_i}-\overleftarrow{k}^+\prod_{j=1}^n[C_{P_j}(R^+)]^{\mu_j}, \tag{44}$$

$$I=\overrightarrow{k}_{\exp}\prod_{i=1}^m C^{\lambda_i}_{S_i0}-\overleftarrow{k}_{\exp}\prod_{j=1}^n C^{\mu_j}_{P_j0}. \tag{45}$$

From (44), and by means of (40), (42) and (43), we obtain

$$\begin{aligned}I=\;&\overrightarrow{k}^+\prod_{i=1}^m\left(C_{S_i0}-\frac{\lambda_iI}{4\pi D_{S_i}R^+f^+_{S_i}}\right)^{\lambda_i}\exp(-\lambda_iU_{S_i}(R^+)/kT)\\&-\overleftarrow{k}^+\prod_{j=1}^n\left(C_{P_j0}+\frac{\mu_jI}{4\pi D_{P_j}R^+f^+_{P_j}}\right)^{\mu_j}\exp(-\mu_jU_{P_j}(R^+)/kT),\end{aligned} \tag{46}$$

where

$$f^{+}_{S_i} = \left(R^{+}\int_{R^{+}}^{\infty}\exp(U_{S_i}(r)/kT)\frac{\mathrm{d}r}{r^2}\right)^{-1}, \qquad f^{+}_{P_j} = \left(R^{+}\int_{R^{+}}^{\infty}\exp(U_{P_j}(r)/kT)\frac{\mathrm{d}r}{r^2}\right)^{-1}. \tag{47}$$

Then, for a given stoichiometric equation, we can always first apply (46) to seek out I, the degree of reaction flow, and then utilize (40), (42), (43) and (45) to acquire the concentration distributions, the relations between various rate constants, and so on. So, generally speaking, there are no difficulties in principle for this kind of problems.

Appendix

Just for simplicity, we consider the case of spherical symmetry and without force field. Obviously, the conclusion drawn herefrom can be extrapolated to cover the general case. When in spherical symmetry and without field, the diffusion equation of steady state may be written as

$$\frac{1}{r^2}\,\frac{\mathrm{d}}{\mathrm{d}r}\left(r\frac{\mathrm{d}}{\mathrm{d}r}C\right) = 0. \tag{A.1}$$

In order to calculate the rate constant corresponding to short diffusive distance, we put the boundary conditions as

$$C\big|_{r=R_0} = 0, \qquad C\big|_{r=R_0+\delta} = C_0. \tag{A.2}$$

Then, the solution of eq. (A.1) is

$$C(r) = \frac{(R_0+\delta)\,C_0}{\delta} - \frac{(R_0+\delta)\,R_0C_0}{\delta}\left(\frac{1}{r}\right). \tag{A.3}$$

We thus obtain

$$I = \iint_{S_{R_0}} \mathbf{i}\cdot\mathrm{d}\mathbf{S} = \iint_{S_{R_0}} (-D\nabla C)\cdot\mathrm{d}\mathbf{S} = 4\pi D\,\frac{(R_0+\delta)\,R_0}{\delta}\,C_0, \tag{A.4}$$

namely, we have

$$k^{\delta}_{D} = 4\pi D\,\frac{(R_0+\delta)\,R_0}{\delta} \sim \frac{1}{\delta}. \tag{A.5}$$

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