Role of the Protein Outside Active Site on the Diffusion-Controlled Reaction of Enzyme

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Abstract: Enzymes possess extremely high catalytic rates, but the catalytic reactions occur only when substrate molecules contact with the active site, a quite small part in comparison with the corresponding major protein. Therefore, it is interesting from different perspectives to discuss the functions of the major protein outside the active site. In this paper, from the viewpoint of diffusion-controlled reactions, the role of the major protein is discussed, and on such a basis, it is pointed out in which case the major protein will act like a "hard wall", hindering some part of the substrate molecules from diffusing into the active site, and in which case the major protein will behave as a "promoter", accelerating the flow of substrate molecules around into the active site so as to increase the rate of diffusion-controlled reactions significantly. Calculated results show that these two extremely opposite cases will markedly depend on the size of van der Waals binding energy between substrate molecules and the enzyme protein outside active site.

Smoluchowski[1] first put forward the theory of diffusion-controlled reactions in colloid-coagulation kinetics. Debye[2] developed Smoluchowski's theory 26 years later to cover the effect of Coulomb interaction between reacting molecules. Since then, much attention has been paid to the kinetics of diffusion-controlled reactions. A critical review for the papers up to 1960 in this field was presented by Noyes.[3] More recently, some investigators discussed this subject from rather different approaches, taking into account correlations among individual particles in the system[4,5] and hydrodynamic interaction between pairs of diffusing particles.[6,7] All the above theories are based on a spherically symmetric picture, in which no effects of hetergeneity of surface reactivity are considered.

On the other hand, in enzyme kinetics Alberty and Hammes[8] first introduced the diffusion-controlled reaction theory to estimate the upper limit of the second-order rate constant between enzyme (E) and substrate (S) molecules. Since the active site of an E molecule generally occupies only a small part of its surface (the so-called surface-active site), or the entire active site may be burried in a concave region termed molecular crevice (the so-called cavity-active site), the spherically symmetric diffusion picture can obviously no longer apply. They therefore proposed a model of semispherically symmetric diffusion (hereafter abbreviated as the semispherical model) as illustrated in Figure 1, where it is assumed that the major protein outside the active site acts like a "hard wall", obstructing the flow of S molecules from the left of the wall to the active site, and the active site is modeled as a small semisphere so that a semispherically symmetric diffusion picture is established. Their pioneer work has greatly stimulated the theoretical investigation of enzyme-catalysed fast reactions although such a model is rather simple. In fact, with the research of fast kinetics in molecular biology becoming more advanced, the nonspherically symmetric effects cannot be ignored and the related mathematic problems have to be solved, although which, unfortunately, are much more difficult than those in the spherically symmetric case. Thus, recently, many attempts have been made to develop the nonspherically symmetric difussion-controlled reaction theory, and a series of models put forward. They can be classified as follows.

Classification and Discussion of Models

1. Solc and Stockmayer[9,10]—considering the reaction between two spherical molecules, each of which bears a surface-active site, and taking both translational and rotational movements into account, with no molecular forces between reacting molecules being involved. Numerical calculations are used to obtain the desired results.

2. Schurr and Schmitz[11,12]—studying the reaction between a spherical molecule with a surface-active site and a reaction spot on a plane surface. Both translational and rotational movements of the spherical molecule are counted, but no force field is involved.

3. Chou et al[13-22]—considering the reaction between a big E molecule with one or several[16,17] surface-active sites and a small S molecule of uniform reactivity (Figure 2). Various molecular forces between E and S molecules are taken into consideration, but no rotational movement is involved.

4. Richter and Eigen[23]—discussing the reaction between two spheroidal molecules in order to investigate the surprisingly high association rate of repressor to nonoperator DNA. A model is presented in which unspecific binding to any part of the DNA is assumed together with subsequent diffusion along the chain, but no molecular forces are concerned.

5. Zhou[24]—a simplified model for nonspherically symmetric diffusion-controlled reactions between E and S molecules is described. On such a basis an approximate analytic expression for calculating the upper limit of the rate constant is derived and some critical relations discussed.

6. Chou[25]—a model for investigating the kinetic characters of the cavity-active site is put forward as illustrated in Figure 3. On such a basis the mathematical formulation, in which the molecular forces between E and S molecules are also involved, for calculating the corresponding diffusion-controlled reaction rate

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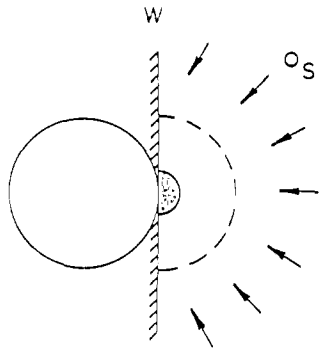

Figure 1. The model of semispherically symmetric diffusion. The dotted part is the active semisphere. W is a "hard wall".

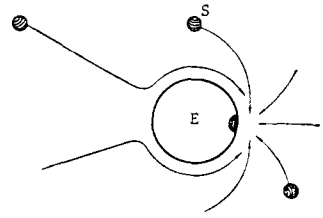

Figure 2. The model describing the reaction between a big E molecule and a small S molecule when the spatial factor and force field factor are taken into account.

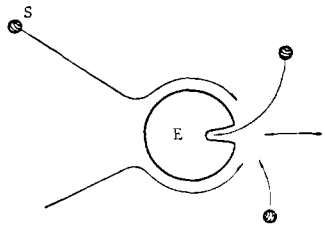

Figure 3. The model for investigating the kinetic characters of cavity-active site as proposed by Chou[25] and Samson and Deutch.[26]

is presented and an actual calculated result is given, which can be well used to explain the experimental results.

7. Samson and Deutch[26]—taking a model basically the same as in class 6 but with an approximate expression presented for calculating the diffusion-controlled reaction rate in assuming that no molecular forces exist.

As is well known, an E molecule can usually be treated as a spherical molecule, and also there generally exist various molecular forces, such as Coulomb force and van der Waals force,[27-29] between E and S molecules. Therefore among the above classification of models, it would be more suitable to take class 3 or class 6 to discuss diffusion-controlled reactions of enzymes. We would prefer to adopt class 3 here not only due to more simplicity but also because the upper limit of reaction rates calculated in both cases is in the same order of magnitude[25] when the van der Waals binding energy is greater than $6kT$. Furthermore, although in the model of class 3, for mathematical convenience, no effect of rotational movements is concerned, such an approximate treatment is rational 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 radius or to the molecular weight, hence the importance of this kind of motion in restoring influence on the rate of reaction will be much different for a biomacromolecule and

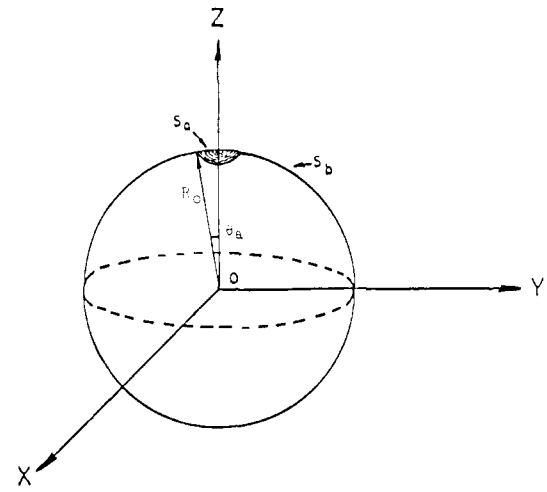

Figure 4. S_a is the surface of the "sink" for an E–S reaction system. S_b is the accessible surface[22] of an S molecule to the major protein outside the active site. $R_0 = R_E + R_S$ is the sum of the radii of an E molecule and an S molecule.

a small substrate. In most E–S reaction systems, the molecular weight of E molecules is two or three order of magnitude larger than that of S molecules,[8,31] so it follows that $D_S^{rot} \gg D_E^{rot}$. As a result, the rotational Brownian motion of an S molecule is so fast that the whole molecule can be treated as a small uniform sphere, while the rotational Brownian motion of the E molecule is so slow that its effect can be neglected. The numerical estimation given by Schurr and Schmitz[11,12] also confirms the above assumption.

Nevertheless, due to its simplicity, the semispherical model is still frequently adopted by many biochemists[30-37] to estimate the upper limit of the second-order rate constants between E and S molecules. Since the upper limit is often used as an important criterion in judging[30,32,37] whether a supposed enzyme-catalyzed mechanism is reasonable or not, it is worthwhile to make a rather careful comparison between the semispherical model and the model of class 3. Especially, as we shall see below, the role of the major protein outside the active site on diffusion-controlled reactions will be obviously revealed through such a comparison.

Based on the semispherical model (Figure 1), the following formula for calculating the upper limit of the second-order rate constant between E and S molecules is naturally obtained[8,31] according to the Smoluchowski–Debye theory:[1,2]

$$k_{semi} = \frac{2\pi DN}{1000 \int_{r_o}^{\infty} \exp[U(l)/kT] \frac{dl}{l^2}} \qquad (1)$$

where D is the sum of the diffusion coefficients of S and E molecules, N the Avogadro constant, $U(l)$ the interaction potential between the S molecule and the active site of the E molecule, k the Boltzmann constant, T the absolute temperature, and r_o = 5 Å is the sum of the radii of the active semisphere and an S molecule.

As mentioned above, in the model of Figure 1, the major protein outside the active site is assumed as a "hard wall", which excludes all the effects coming from the left side of this wall, such as the flow of S molecules and the molecular forces between S molecules and the major protein. To take these effects into account, we have to solve the following nonspherically symmetric equations given by Chou and his co-workers[13-17] (Figure 4):

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$$\begin{aligned} &\vec{\nabla}[\exp(-U/kT)\vec{\nabla}C^*] = 0 \\ &C^*|_{r=R_0} = 0 \qquad (0 \le \theta \le \theta_a) \\ &(\partial C^*/\partial r)|_{r=R_0} = 0 \qquad (\theta_a < \theta \le \pi) \\ &C^*|_{r\to\infty} = C_0 \qquad (0 \le \theta \le \pi) \end{aligned} \tag{2}$$

where

$$C^* = e^{U/kT}C \tag{3}$$

while C is the concentration of S molecules, C_0 is the bulk concentration, $\vec{\nabla}$ is the Hamilton operator, and

$$U = U(r, \theta, \phi) \tag{4}$$

which means now the potential is not only dependent on r, the distance between the centers of E and S molecules, but also on the spherical coordinates θ and ϕ. θ_a is the maximal deviation angle of the "sink" in the E–S fast reaction systems. The dependence of θ_a on the surface area of the "sink" is given by

$$S_a = 4\pi R_0^2 \sin^2(\theta_a/2) \tag{5}$$

where R_0 is the sum of the radii of one E molecule and one S molecule. As for how to understand the rationality of the "sink" model in enzyme kinetics, the reader may refer to ref. 9 and 16, where a stochastic analysis is presented, and the corresponding physical picture described. For the comparison to be made in as similar conditions as possible, S_a should be given the same area as that of the active semisphere of Figure 1. We thus have

$$\theta_a = 2\sin^{-1}\sqrt{S_a/4\pi R_0^2} = 2\sin^{-1}(r_0/\sqrt{2}R_0) \tag{6}$$

The corresponding upper limit of the diffusion-controlled reaction rate can be expressed as[13,14,16]

$$k_{lim} = \frac{I}{C_0} = \frac{N}{1000C_0}\int\int_{S_a}[-D\exp(-U/kT)\vec{\nabla}C^*]\,d\vec{S} = \frac{DN}{1000C_0}\int\int_{S_a} e^{-U/kT}\frac{\partial C^*}{\partial r}\,dS = \frac{DN}{1000C_0}\int\int_{S_{R_0}} e^{-U/kT}\frac{\partial C^*}{\partial r}\,dS \tag{7}$$

where I is the total amount of S molecules which, governed by the concentration gradient and force field, diffuse to an E molecule in unit time, and $S_{R_0} = S_a + S_b$. When $U(r,\theta,\phi) = U(r)$, eq 7 can be reduced to

$$k_{lim} = \frac{4\pi DN}{1000\int_{R_0}^{\infty} e^{U(r)/kT}\frac{dr}{r^2}}\left[1 - ge^{U(R_0)/kT}\cos^2\frac{\theta_a}{2}\right] \tag{8}$$

where

$$g = \lim_{\Delta r\to 0}\frac{\int_{R_0}^{R_0+\Delta r}\int_0^{2\pi}\int_{\theta_a}^{\pi} C(r,\theta)r^2\sin\theta\,d\theta\,d\phi\,dr}{C_0\int_{R_0}^{R_0+\Delta r}\int_0^{2\pi}\int_{\theta_a}^{\pi} r^2\sin\theta\,d\theta\,d\phi\,dr} = \frac{\int_{\theta_a}^{\pi} C(R_0,\theta)\sin\theta\,d\theta}{2C_0\cos^2\frac{\theta_a}{2}} \tag{9}$$

is the ratio of the average concentration of S molecules on S_b (see Figure 4), the accessible surface of the protein outside the active site, to C_0, the bulk concentration of S molecules in solution. In the general case, however, we have

$$g = \frac{1}{C_0 S_b}\int\int_{S_b} e^{-U(R_0,\theta,\phi)}C^*\,dS \tag{10}$$

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

Discussion of the Interaction Potentials

Generally speaking, the potential between an E and an S molecule can be expressed as

$$U = U_{van} + U_{Coulomb} \tag{11}$$

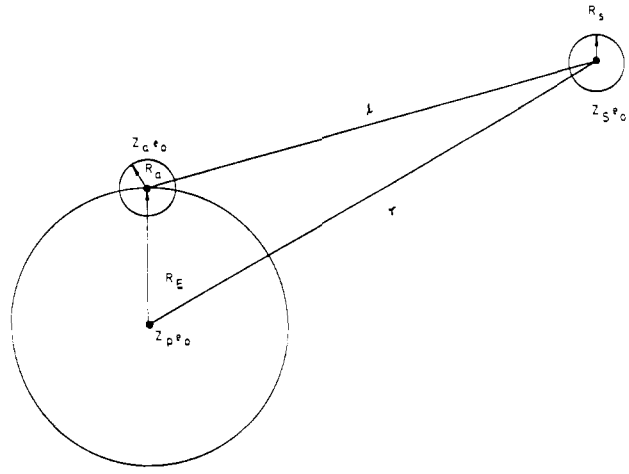

Figure 5. An illustration of the Coulomb interaction between an E molecule and an S molecule.

Lifshitz[38] has proposed a theory to derive the van der Waals potential between two macroscopic bodies (here "macroscopic" means large in comparison with atomic dimensions). On the basis of the Lifshitz's theory, Langbein[39] and Mitchell and Ninhan[40] calculated the van der Waals potential between two spherical macromolecules, respectively. From their calculations, the van der Waals potential between two spherical macromolecules (or a macromolecule and a small one) can be expressed as:[14,18]

$$\begin{aligned} &U_{van} = -U_0 \qquad (0 \le d \le a_0) \\ &U_{van} = -U_0 a_0[(b_0/d) - 1]/(b_0 - a_0) \qquad (a_0 < d < b_0) \\ &U_{van} \sim -1/d^6 \qquad (d \gg b_0) \end{aligned} \tag{12}$$

where $a_0 = 0.2$ Å, $b_0 = 3$ Å, and d is the closest approach between the S molecule and the E molecule. Such an expression is consistent with the experimental reports[27-29] that the van der Waals binding potential of small neutral molecules to protein molecules is about a few kilocalories per mole, and the force range is about a few angstroms. In actual calculations, the interaction for $d \ge b_0$ can be neglected. Although U_{van} is a short-range interaction with a force range of only a few angstroms, below we shall see that the contribution of U_{van} to the upper limit of the second-roder reaction rate between E and S molecules can by no means be ignored. But in the models of classes 1, 2, 4, and 7, no force field is taken into account, and in the semispherical model the effect of van der Waals force could not be adequately taken into account since the major protein of the E molecule is cut off from the active site by the "hard wall" as illustrated in Figure 1. Therefore, d in eq 12 can only denote the distance of the closest approach between an S molecule and the active semisphere, although the functional form of U_{van} could assume the same. As for the reaction system with a cavity-active site, expression 12 for van der Waals potential can also be used. But when the S molecule enters into the active cavity, d should be counted as the closest approach to the nearest wall or bottom of the cavity.[25]

The Coulomb potential in eq 11 is given by[18] (Figure 5)

$$U_{Coulomb} = U_{Coulomb}^{Z_pZ_S} + U_{Coulomb}^{Z_aZ_s} = \frac{1}{2}\left\{\frac{e^{R_s/\mathcal{R}}}{1 + R_s/\mathcal{R}} + \frac{e^{R_E/\mathcal{R}}}{1 + R_E/\mathcal{R}}\right\}\frac{Z_pZ_Se_o^2}{\epsilon r}\exp\left(-\frac{r}{\mathcal{R}}\right) + \frac{1}{2}\left\{\frac{e^{R_s/\mathcal{R}}}{1 + R_s/\mathcal{R}} + \frac{e^{R_a/\mathcal{R}}}{1 + R_a/\mathcal{R}}\right\}\frac{Z_aZ_se_o^2}{\epsilon l}\exp\left(-\frac{l}{\mathcal{R}}\right) \tag{13}$$

where Z_a, Z_p, and Z_S are the charge numbers of the active site, the remaining part of the enzyme, and the substrate molecule,

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Table I. The Values of k_{semi}, k_{lim}, and σ Calculated under Different U_0[a]

	U_0					
	0	$2kT$	$3kT$	$5kT$	$7kT$	$10kT$
k_{semi} (10^{10}/Ms)	0.130	0.147	0.152	0.159	0 164	0.170
k_{lim} (10^{10}/Ms)	0.177	0.257	0.350	0.727	1.07	1.17
σ	0.265	0.428	0.566	0.781	0.847	0.855

[a] $R_0 = 20$ Å, $\theta_a = 20°$, $D = 7 \times 10^{-6}$ cm²/s, $Z_pZ_S = Z_aZ_S = 0$, and $T = 298$ K.

Table II. The Values of k_{semi}, k_{lim}, and σ Calculated under Different Z_aZ_S[a]

	Z_aZ_S				
	−8	−4	0	4	8
k_{semi} (10^{10}/Ms)	0.704	0.467	0.170	0.018	0.00072
k_{lim} (10^{10}/Ms)	1.752	1.471	1.137	0.911	0.621
σ	0.598	0.683	0.851	0.980	0.999

[a] $R_0 = 20$ Å, $R_E = 18$ Å, $R_S = 2$ Å, $R_a = 3$ Å, $\theta_a = 20°$, $D = 7 \times 10^{-6}$ cm²/s, $U_0 = 10kT$, $\epsilon = 78.56$, $T = 298$ K, and $\mu = 0.01$.

respectively; e_0 is the electronic charge; ϵ the dielectric constant of the intervening medium; R_a the effective radius[18] of the active site; and $\mathcal{R}$ the "thickness" of the ion atmosphere given by

$$\mathcal{R} = \left\{\frac{1000\epsilon kT}{8\pi e_0^2 N\mu}\right\}^{1/2} \tag{14}$$

in which μ is the ionic strength. However, it is assumed in semispherical model that the Coulomb interaction exists only between the active site and S molecules while not between the rest of the protein molecule and S molecules.[8,31] That means the net charge of an E molecule is wholly concentrated at its active site. Such an assumption is not true of course, but the semispherically symmetric picture of Figure 1 will otherwise be violated. In other words, when using eq 1 to calculate k_{semi}, one could not help but suppose $Z_p = 0$. For the comparison to be favored, here in calculating k_{lim} with eq 7 we also put $Z_p = 0$ although it is not necessary as far as the latter model itself is concerned. Moreover, put $R_a + R_S = r_0 = 5$ Å to make the comparison in as analogous conditions as possible.

Discussion of the Results

Define the relative deviation

$$\sigma = \frac{|k_{lim} - k_{semi}|}{k_{lim}} \tag{15}$$

which can be used to reflect the percentage of the flow excluded by the hard wall. The method for calculating k_{lim} is the same as in ref 14 and 17. Tables I and II give the values of k_{semi}, k_{lim}, and σ calculated under different values of U_0 and Z_aZ_S, respectively.

From Table I we can see that when U_0 is greater than $3kT$, the values of σ are already larger than 0.5. That means more than 50% of the total flow is excluded by the "hard wall", and hence eq 1 will no longer give a good approximation. It is well known that the van der Waals binding energy of a small neutral molecule to a protein molecule in solution is generally from $-kT$ to $-16kT$. If according to ref 27 the van der Waals binding energy is −6.0 kcal/mol at 25 °C (i.e., $U_0 \simeq 10kT$), then the results listed in Table II tell us σ is always larger than 0.5 even though the Coulomb interaction is taken the same in calculating both k_{semi} and k_{lim}. And in this case Table I tells us k_{lim} is one order of magnitude larger than k_{semi}.

From these results we can obtain the following conclusion: the van der Waals force between E and S molecules plays a key role in deciding whether the semispherical model is valid or not. In other words, the upper limit of the reactions estimated by eq 1 is reliable only when the van der Waals force is very weak ($U_0 < 3kT$). It seems at first surprising that the van der Waals force, whose action range is as short as only a few angstroms, should play so significant a role in raising the diffusion-controlled reaction limit. This phenomenon may be understood as follows. Due to the van der Waals interaction between E and S molecules, the concentration of S molecules on the surface of the major protein outside the active site is much higher than that of the bulk solution. (If there were no reaction, the concentration of S molecules on the surface of the protein molecule would be $\sim\exp\{-U(R_0)/kT\}$ according to Boltzmann statistics.) As a consequence, the diffusion flow of S molecules to the "sink" (on whose surface $C = 0$) around the E molecule will be sped up significantly. Note that, due to the existance of the force field, rather than $\vec{i} = -D\vec{\nabla}C$, the flow should be expressed as[16]

$$\vec{i} = -De^{-U/kT}\vec{\nabla}C^* = -De^{-U/kT}\vec{\nabla}\{e^{U/kT}C\} \tag{16}$$

So even the concentration C on the surface of the major protein is higher than C_0, the bulk soltuion; still there is a diffusion flow driven by the force field from bulk solution toward the E molecule to maintain such a very steep concentration gradient around the active site. However, if the concentration of S molecules on the surface of the major protein is too high, that will be unfavorable for the surrounding S molecules to come up. Therefore, when a force field (no matter if it is a long range force or short one) results in

$$1 \ll g \ll \exp\{-U(R_0)/kT\} \tag{17}$$

which can be deemed as the optimal condition for the diffusion-controlled reaction of enzymes, we obtain from eq 8 that

$$k_{lim} \simeq \frac{4\pi DN}{1000\int_{R_0}^{\infty} e^{U(r)/kT}\,\frac{dr}{r^2}} \tag{18}$$

In this case, the whole surface of an E molecule can be equivalently regarded as a big "sink". Such a phenomenon does really exist in some biological processes as discussed by Adam and Delbruck[41] and Richter and Eigen,[23] who, however, only gave a presumption but not the physical mechanism.

Consequently, when the van der Waals is taken into consideration, the major protein outside the active site will play the role of a "promoter", and give rise to a fast flow of S molecules around the E molecule to its active site. But in the semispherical model, this part of the flow is completely blocked by the "hard wall". If the van der Waals binding energy is very small ($U_0 < 3kT$), the concentration of S molecules in the proximity of an E molecule will not be much higher than that in the bulk solution, then the flow of S molecules around the E molecule to its active site will be trifling. In this case, the major protein outside the active site will itself play role of a "hard wall" rather than a "promoter" during the diffusion-controlled reactions.

In summary, therefore, the binding energy between E and S molecules will play a key role in deciding whether the major protein outside the active site acts as a "hard wall" or as a "promoter".

Finally, three points should be mentioned here.

First, 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 bulk. A similar difficulty also appears in the Richter–Eigen model.[23] Although such a problem still remains to be solved, it will not influence our essential results owing to the following: (a) Unlike the Richter–Eigen picture[23] 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[13,16] there is no such a constraint, that S molecules have to keep on the surface all the time, even during the diffusion process along the surface of a protein molecule, 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

(41) G. Adam and M. Delbruck, in "Structure *Chemistry* and Molecular *Biology*", W. H. Freeman, San Francisco, Calif., 1974, 198–215.

of repressors to the DNA follow a "one-dimensional" diffusion along the chain, in Chou's picture most of the flow of S molecules to the active site comes from the "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 van der Waals force.[18] With the knowledge about the interfacial diffusion coefficient, especially about the complicated molecular forces and the detailed structure (such as the so-called "icelike" structure) on the surface of an E molecule, not yet sufficiently known, the present calculations and discussions based on Chou's model are rational at least in a sense of approximation.

Second, there will be a reduction (between 25% and 60%) in the rate of diffusion-controlled reaction if the hydrodynamic effect[6,7] is taken into account. But, in comparison with the role of the van der Waals force that gives one order of magnitude in raising the rate from that obtained by the semispherical model, the role of the hydrodynamic effect is relatively small. Besides, what we are interested in here is to compare the semispherical model and the Chou's model so as to disicuss the role of the major protein outside the active site. While the hydrodynamic effect will exert analogous influence on both cases, the principal points discussed here are still valid even without taking the hydrodynamic effect into account.

Third, as regards how to take into consideration diffusion of product (P) molecules away from an E molecule, the reader may refer to the paper by Chou and Forsen.[42] There the diffusion-controlled effects in reversible enzymatic fast reaction systems are discussed, and also it is pointed out that, in such a case, the diffusion-controlled reaction rate is related not only to the diffusion coefficient, the force field, the size of an active site, and so on, but also to the ratio of the concentration of the P molecules to that of the S molecules when the reaction system attains equilibrium. Of course, such an effect will exert the same influence on both the semispherical model and Chou's model, too.

Acknowledgment. Valuable discussions with Dr. Theodoor A. J. Payens, Dr. Athel Cornish-Bowden, Professor Xie Si-Dong, and Professor Bjorn Lindman are gratefully acknowledged.

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Model Studies of Terpene Biosynthesis. Intermolecular 1′-2 Electrophilic Condensation of 3-Methyl-2-butenyl Acetate[1,2]

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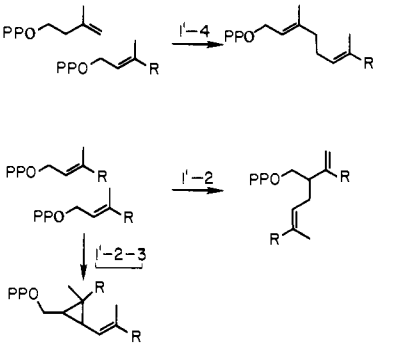
Abstract: The stereochemistry of the carbon–carbon bond-forming step in the electrophilic intermolecular 1′–2 condensation of 3-methyl-2-butenyl acetate (**1**-OAc) to yield lavandulyl acetate (**2**-OAc) was studied. Treatment of (1*S*)-[1-^{2}H]3-methyl-2-butenyl acetate ((1*S*)-[1-^{2}H]**1**-OAc) with aluminum trichloride in ethyl acetate gave labeled lavandulyl acetate ([1,3-$^2H_2$]**2**-OAc) (30%) and isoprene (65%) as the major products. The configurations at C(1), C(2), and C(3) and the relative abundances of the diastereomers of [1,3-$^2H_2$]**1**-OAc were determined by converting the mixture to [2,4-$^2H_2$]3-(2′-propyl)-butyrolactone ([2,4-$^2H_2$]**3**). The intensities of ^{1}H resonances characteristic of each diastereomer were measured with the aid of Pirkle's chiral shift reagent, (*S*)-(+)-2,2,2-trifluoro-1-(9-anthryl)ethanol. The analysis showed that equal amounts of the (1*S*,2*S*,3*R*), (1*S*,2*R*,3*R*), (1*S*,2*S*,3*S*), and (1*S*,2*R*,3*S*) diastereomers of [1,3-$^2H_2$]**2**-OAc were obtained, signifying that the 1′–2 condensation was stereorandom at C(1) of the electrophilic isoprene unit.

Allylic cations are thought to play important roles in the condensation reactions that constitute the major bond-forming reactions in the terpene biosynthetic pathway. Examples include the 1′–4 coupling reaction used to attach isoprenoid residues in a sequential fashion to a growing allylic terpene chain,[4] the 1′–2 coupling reaction which produces the nonhead-to-tail fusion of residues found in some irregular monoterpenes[5] and carotenoids,[6] the 1′–2–3 coupling reaction recently discovered in the sterol[7,8] and carotenoid pathways,[9] and numerous intramolecular cyclizations.[10] The transformations can all be rationalized as electrophilic condensations, and in some instances experiments have confirmed the electrophilic character of the enzyme-catalyzed reactions.[4,11-14]



(1) Taken from the dissertation of C. H. R. King, submitted in partial fulfillment of the requirements for a Ph.D. degree at the University of Utah, 1980.

(2) We wish to acknowledge the National Institutes of Health, GM 21328, for support of this research.

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