Enhancement of Protein-Protein Association Rate by Interaction Potential: Accuracy of Prediction Based on Local Boltzmann Factor

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ABSTRACT Electrostatic interactions are known experimentally to enhance the rate of protein-protein association by three to four orders of magnitude. However, theoretical efforts to quantitatively account for such rate enhancement have been hampered by the need to consider a large number of relative configurations of two associating proteins sampled during their diffusional encounter. Our recent work indicates that a good estimate of the rate enhancement is given by the average Boltzmann factor in the region of configurational space where association can effectively take place. This estimate is tested on a model system consisting of two spherical proteins, each with a "reactive patch." Three different forms of interaction potential are considered. Comparison with exact results for the association rate constant demonstrates that predictions based on the local Boltzmann factor are accurate to within ~50% for realistic sizes of the reactive region and amplitudes of the interaction potential.

INTRODUCTION

The prototypical model for protein-protein association, consisting of two uniformly reactive spherical proteins, predicts that the diffusion-controlled association rate constant is $4\pi DR$, where D is the relative diffusion constant and R is the sum of the radii of the two spheres (Smoluchowski, 1917). Because the product of protein-protein association in general consists of the subunits in a specific relative orientation, a better model is one in which each sphere bears a small reactive patch. If the first (second) patch is restricted to the part of the first (second) spherical surface spanned by the polar angle from 0 to δ_1 (δ_2) (see Fig. 1), the diffusion-controlled association rate constant k^0 for small reactive patches is approximately given by (Berg, 1985; Zhou, 1993a)

$$k^0/4\pi DR = F_1\xi_2\tan(\delta_2/2) + F_2\xi_1\tan(\delta_1/2) \qquad (1)$$

In this expression, $F_i = \sin^2(\delta_i/2)$ are the fractions of surface occupied by the reactive patches, and $\xi_i = [(1 + D_iR^2/D)/2]^{1/2}$, where D_i are the rotational diffusion constants of the spherical proteins. For typical proteins, $D \approx 2 \times 10^{-6}$ cm^2 s^{-1}, $R \approx 4 \times 10^{-7}$ cm, and $D_i = 2 \times 10^7$ s^{-1}; Eq. 1 predicts $k^0 = 6 \times 10^5$ M^{-1} s^{-1} when $\delta_1 = \delta_2 = 5°$. This is more than an order of magnitude larger than what one naively predicts by multiplying the Smoluchowski result $4\pi DR$ by the surface fractions of the reactive patches, F_1 and F_2. The physical reason, as explained previously (Shoup et al., 1981; Berg, 1985), is that the proteins can make repeated attempts to associate during their diffusional encounter.

Experimentally, association rate constants around 5×10^9 M^{-1} s^{-1} have been observed for a number of protein complexes, including hirudin and thrombin (Stones et al., 1989), ferricytochrome c and ferrocytochrome b_5 (Eltis et al., 1991), barnase and barstar (Schreiber and Fersht, 1993, 1996), colicin E9 and its cognate immunity protein Im9 (Wallis et al., 1995), and two peptides forming a leucine zipper (Wendt et al., 1997). The four-order-of-magnitude increase over what is predicted by Eq. 1 is mainly due to favorable electrostatic interactions between the associating proteins, as demonstrated by decrease in the association rate constant to a level predicted by Eq. 1 at very high ionic strengths. There is enormous interest in quantitatively accounting for the electrostatic rate enhancement based on the structures of the associating proteins. For the binding of proteins with small ligands, Brownian dynamics simulation, pioneered by McCammon and co-workers (Northrup et al., 1984; Allison et al., 1985), has been a very successful tool. The major simplification there is that the protein can be treated as immobile and the ligand can be treated as a test charge. As a result, the interaction potential can be obtained from a single calculation of the electrostatic potential of the protein.

In the case of protein-protein association, it is difficult to rationalize treating one of the proteins as a set of test charges, and indeed it has been established that such a treatment results in significant errors in the interaction energy (Zhou, 1993a). Even with this treatment, Brownian dynamics simulation of protein-protein association is a challenging problem, owing to the need to check whether each trial move leads to overlapping between the proteins and the need to generate a large number of trajectories to obtain statistically significant results. Several groups (Northrup et al., 1987, 1993; Nambi et al., 1991; Kozack et al., 1995) have courageously tackled this problem. Significant progress has also been made toward efficiently and accurately calculating the electrostatic interaction energy between two proteins (Zhou, 1993b; Gabdoulline and Wade,

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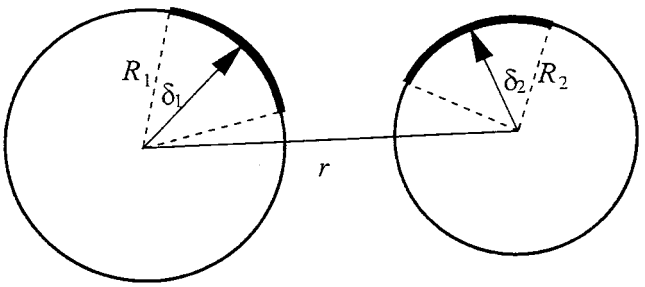

FIGURE 1 A model system for protein-protein association. The polar axis of each spherical protein is indicated by an arrow. The reactive patch on each sphere is the part of the surface spanned by the polar angle from 0 to δ_1 or δ_2. The closest center-center distance between the two spheres is $r = R_1 + R_2 \equiv R$, where R_1 and R_2 are the radii of the two spheres, respectively. In the diffusion-controlled limit, association occurs as soon as the two reactive patches come into contact. In the constant-flux approximation, this absorbing boundary condition is satisfied only as an average over (i.e., not on every point of) the absorbing boundary. In the meantime, the flux across the absorbing boundary is assumed to be uniform.

1996, 1997). This undoubtedly will improve results for electrostatic rate enhancement obtained from Brownian dynamics simulations in the future.

Recently we have derived an analytic result for rate enhancement that promises to circumvent much of the difficulty of Brownian dynamics simulation (Zhou, 1996). When the region of configurational space where association can effectively take place is small, we showed

$$k/k^0 = \langle \exp(-\beta U) \rangle \tag{2}$$

where k and k^0 are the association rate constants in the presence and absence of an interaction potential U, and the averaging of the Boltzmann factor is over the association or "reactive" region. If this region is viewed as defining a "transition state," then Eq. 2 superficially resembles the transition-state theory. However, it should be emphasized that Eq. 2 is valid both when the association process is reaction controlled and when it is diffusion controlled.

This equation was actually derived for the binding of a protein with a small ligand (more precisely, a pointlike particle). Its predictions have been found to compare very favorably with results from Brownian dynamics simulations of acetylcholinesterase and its substrate (Zhou et al., 1996). It was argued previously that, relative to the case of the binding of a protein with a small ligand, the reactive region in the case of protein-protein association is more restricted; hence Eq. 2 should be even more accurate for the latter case (Zhou, 1996). The purpose of this paper is to test the accuracy of Eq. 2 for predicting the enhancement of protein-protein association rate by interaction potentials.

THEORY

We have chosen the model system shown in Fig. 1 to test the accuracy of Eq. 2. A general expression for the diffusion-controlled association rate constant of this system under the influence of a centrosymmetrical interaction potential $U(r)$ has been derived previously (Zhou, 1993a). This is based on the constant-flux approximation (Shoup et al., 1981) and is given by

$$\frac{k}{4\pi DR \exp[-\beta U(R)]} = \frac{[c_1(0)c_2(0)]^2}{\sum_{l,l_1,l_2=0}^{\infty} \frac{f_l(R;\mu_{l_1l_2})}{-Rf_l'(R;\mu_{l_1l_2})}[c_1(l_1)c_2(l_2)]^2 C_{l_1l_2}} \tag{3}$$

where $\mu_{l_1l_2} = [(l_1(l_1+1)D_1 + l_2(l_2+1)D_2)/D]^{1/2}$, $f_l(r;\mu)$ are the regular solutions of the equations

$$\left\{\frac{\exp[\beta U(r)]}{r^2}\frac{d}{dr}r^2\exp[-\beta U(r)]\frac{d}{dr} - \left[\mu^2 + \frac{l(l+1)}{r^2}\right]\right\} f_l = 0 \tag{4}$$

$c_i(l_i)$ are given by Legendre polynomials,

$$c_i(l_i) = P_{l_i-1}(\cos\delta_i) - P_{l_i+1}(\cos\delta_i) \tag{5}$$

and $C_{l_1l_2}$ are given by 3-j symbols,

$$C_{l_1l_2} = \frac{2l+1}{(2l_1+1)(2l_2+1)}\begin{pmatrix} l_1 & l_2 & l \\ 0 & 0 & 0 \end{pmatrix}^2 \tag{6}$$

When sphere 2 is uniformly reactive (i.e., $\delta_2 = \pi$), Eq. 3 simplifies to

$$\frac{k}{4\pi DR \exp[-\beta U(R)]} = \frac{[c_1(0)]^2}{\sum_{l=0}^{\infty} \frac{f_l(R;\mu_l)}{-Rf_l'(R;\mu_l)}[c_1(l)]^2/(2l+1)} \tag{7}$$

where $\mu_l = [l(l+1)D_1/D]^{1/2}$.

If no interaction potential is present, then $f_l(r;\mu) = (\pi/2\mu r)^{1/2}K_{l+1/2}(\mu r)$, the modified spherical Bessel functions of the third kind (Abramowitz and Stegun, 1964; Press et al., 1986). The corresponding rate constant is

$$\frac{k^0}{4\pi DR} = \frac{[c_1(0)c_2(0)]^2}{\sum_{l,l_1,l_2=0}^{\infty} \frac{[c_1(l_1)c_2(l_2)]^2 C_{l_1l_2}}{h_l(x)}} \tag{8}$$

where $x = \mu R$ and $h_l = l + 1 + xK_{l-1/2}(x)/K_{l+1/2}(x)$. For small δ_1 and δ_2, this is well approximated by Eq. 1 (Zhou, 1993a). If an interaction potential is present, in general it is not possible to solve Eq. 4 and obtain $f_l(r;\mu)$ analytically. We have found two forms of $U(r)$ that allow for the solution of Eq. 4. These are

$$\beta U(r) = -2\ln(1 + r_0/r) \tag{9a}$$

and

$$\begin{aligned} \beta U(r) &= -V, & R < r < R' \\ &= 0, & r > R' \end{aligned} \tag{9b}$$

For the potential given by Eq. 9a, by making the variable change $f_l(r;\mu) = g_l(r;\mu)/(1 + r_0/r)$ (Belyi, 1984), one can easily show that $g_l(r;\mu) = (\pi/2\mu r)^{1/2}K_{l+1/2}(\mu r)$. Hence

$$\frac{-Rf_l'(R;\mu)}{f_l(R;\mu)} = h_l(x) - \frac{r_0}{R + r_0} \tag{10a}$$

For the potential given by Eq. 9b, one finds

$$\begin{aligned} &\frac{-Rf_l'(R;\mu)}{f_l(R;\mu)} \\ &\quad = h_l(x) - (1 - e^{-V})h_l(x')/\{[K_{l+1/2}(x)/K_{l+1/2}(x')]^2 \\ &\qquad + (1 - e^{-V})h(x')[I_{l+1/2}(x)K_{l+1/2}(x') \\ &\qquad - I_{l+1/2}(x')K_{l+1/2}(x)]K_{l+1/2}(x)/K_{l+1/2}(x')\} \end{aligned} \tag{10b}$$

where $x' = \mu R'$ and $(\pi/2x)^{1/2}I_{l+1/2}(x)$ are the modified spherical Bessel functions of the first kind.

The constant-flux approximation, which is used for deriving Eq. 3, and its extension to the case of nonlocal reactivity have been found to be quite accurate (Zhou and Szabo, 1996). For example, when $\delta_2 = \pi$ and $\delta_1 \to 0$, the rate constant in the absence of any interaction potential predicted by Eq. 8 is $k^0 = (3\pi^2/8)DR\delta_1$, whereas the exact result is $k^0 = 4DR\delta_1$ (Shoup et al., 1981; Doktorov and Lukzen, 1981). The difference is only 7.5%. A simple way to improve the accuracy is to scale the result in Eq. 8 by $32/3\pi^2 \approx 1.081$. The scaled result is accurate to within 0.5% up to $\delta_1 = 45°$ (see Table 1).

The same scaling factor, $32/3\pi^2$, also makes the constant-flux prediction in the presence of an interaction potential with a moderate amplitude almost exact for small δ_1. This can be illustrated by the results for the Coulombic potential $\beta U(r) = -Q/r$. When $D_1R^2/D \to 0$, the constant-flux approximation gives (Zhou, 1993a)

$$\frac{k}{4\pi DR\exp(Q/R)} = \frac{[c_l(0)]^2}{\sum_{l=0}^{\infty}\dfrac{[c_l(l)]^2/(2l+1)}{l + 1 - q + |q|I_{l+3/2}(|q|)/I_{l+1/2}(|q|)}} \tag{11}$$

TABLE 1 $k^0/4\pi DR$ **for** $\delta_2 = \pi$ **and small to moderate** δ_1

δ_1 (degree)	Eq. 8 scaled by $32/3\pi^2$	Exact
5	3.044×10^{-2}	3.055×10^{-2}
10	6.462×10^{-2}	6.493×10^{-2}
15	0.1017	0.1022
20	0.1411	0.1418
25	0.1826	0.1833
30	0.2258	0.2263
35	0.2702	0.2704
40	0.3157	0.3153
45	0.3620	0.3607

where $q = Q/2R$. The exact result for $k/4\pi DR\exp(Q/R) \equiv X_0$ is determined by (Traytak and Tachiya, 1995)

$$X_l - \sum_{m=0}^{\infty} B_{lm}b_mX_m = B_{l0}/2 \tag{12}$$

with $B_{bn} = \{\sin[(l + m + 1)\delta_1]/(l + m + 1) + (1 - \delta_{bn})\sin[(l - m)\delta_1]/(l - m) + \delta_{bn}\delta_1\}/\pi$, and $b_l = [1/2 - q + |q|I_{l+3/2}(|q|)/I_{l+1/2}(|q|)][l + 1 - q + |q|I_{l+3/2}(|q|)/I_{l+1/2}(|q|)]$. When $Q = 0$ (i.e., potential is absent), $b_l = 1/2(l + 1)$, and the result for the rate constant has been listed in Table 1. Comparison of Eq. 11 scaled by $32/3\pi^2$ with the exact result for k is shown in Table 2. For $0 < Q/R < 3$ and $\delta_1 < 10°$, the difference is less than 1.5%.

Because the constant-flux results for both k^0 and k can be corrected by the same scaling factor $32/3\pi^2$, and in any event the corrections involved are quite small, these results can be taken to be exact for the purpose of testing the accuracy of Eq. 2.

Accuracy of Eq. 2

We are particularly interested in the difference in the accuracy of Eq. 2 between the case of the binding of a protein with a small ligand and the case of protein-protein association. The former can be modeled by $\delta_2 = \pi$ and $D_1R^2/D \to 0$. For the latter, the translational and rotational diffusion constants are assumed to be given by the Stokes-Einstein relations. The logarithmic and step-function potentials introduced in the previous section have no direct relevance to protein-ligand binding or protein-protein association, but they should serve the purpose of testing the accuracy of Eq. 2.

Logarithmic potential

For protein–small ligand binding under the influence of the logarithmic potential given by Eq. 9a, the comparison of the actual value of k given by Eq. 7 and the prediction of Eq. 2 is shown in Table 3. For small reactive patches on the protein, Eq. 2 gives reasonable results for k up to a contact potential of $3k_BT$. If $D = 10^{-5}$ cm^2 s^{-1} and $R = 2 \times 10^{-7}$ cm, then $k \approx 10^{10}$ M^{-1} s^{-1} at $\delta_1 = 10°$ and $\beta U(R) = -3$, for which Eq. 2 is accurate to within 40%.

If the second reactant is not uniformly reactive but is a protein with a small reactive patch, the accuracy of Eq. 2 increases significantly. Table 4 shows that, at $\delta_1 = \delta_2 = 10°$

TABLE 2 $k/4\pi DR\exp[-\beta U(R)]$ **for** $\delta_2 = \pi$, **small** δ_1, **and** $\beta U(r) = -Q/r$

δ_1 (degree)	$\beta U(R)$	Eq. 11 scaled by $32/3\ \pi^2$	Exact
5	−1	2.808×10^{-2}	2.809×10^{-2}
	−2	2.540×10^{-2}	2.530×10^{-2}
	−3	2.222×10^{-2}	2.200×10^{-2}
10	−1	5.664×10^{-2}	5.669×10^{-2}
	−2	4.810×10^{-2}	4.786×10^{-2}
	−3	3.881×10^{-2}	3.827×10^{-2}

TABLE 3 $k/4\pi DR\exp[-\beta U(R)]$ for $\delta_2 = \pi$, small δ_1, and $\beta U(r) = -2 \ln(1 + r_0/r)$

δ_1 (degree)	$\beta U(R)$	Eq. 7	Eq. 2
5	−1	2.631×10^{-2}	2.817×10^{-2}
	−2	2.475×10^{-2}	
	−3	2.321×10^{-2}	
10	−1	5.343×10^{-2}	5.979×10^{-2}
	−2	4.810×10^{-2}	
	−3	4.298×10^{-2}	

TABLE 5 $k/4\pi DR\exp[-\beta U(R)]$ for $\delta_2 = \pi$, small δ_1, and $\beta U(r) = -V$ in $R < r < 2R$ and 0 in $r > R$

δ_1 (degree)	$\beta U(R)$	Eq. 7	Eq. 2
5	−1	2.729×10^{-2}	2.817×10^{-2}
	−2	2.548×10^{-2}	
	−3	2.186×10^{-2}	
10	−1	5.598×10^{-2}	5.979×10^{-2}
	−2	4.889×10^{-2}	
	−3	3.710×10^{-2}	

and $\beta U(R) = -3$, Eq. 2 is now accurate to within 6%. For this size of the reactive patches, even at $\beta U(R) = -7$, the error of Eq. 2 is less than 11%. There the magnitude of k is $\sim 8 \times 10^9$ M^{-1} s^{-1} if $D = 2 \times 10^{-6}$ cm^2 s^{-1} and $R = 4 \times 10^{-7}$ cm.

Step-function potential

The performance of Eq. 2 for the step-function potential given by Eq. 9b is very similar to that for the logarithmic potential. The potential now is described by two parameters: V determines the amplitude of the potential and R'/R determines the "smoothness" of the potential around the reactive region. For a given V, the accuracy of Eq. 2 increases as R'/R increases. Comparisons of actual and predicted values of k at $R'/R = 2$ for the cases of protein–small ligand binding and protein-protein association are shown in Tables 5 and 6, respectively. Again, the prediction of Eq. 2 improves significantly by limiting the size of the reactive patch on the second reactant. However, the errors at large amplitudes of the potential are much more pronounced relative to those under a logarithmic potential. If the amplitude of the step-function potential is extremely high, the rate constant approaches $4\pi DR'$ and loses its dependence on the potential.

Noncentrosymmetrical potential

In earlier work (Zhou, 1993a) we have studied the influence of a noncentrosymmetrical potential on the association rate constant for the model system shown in Fig. 1. The radii of the two spherical proteins were 21 and 14 Å, respectively. Each protein contained a 10° reactive patch, and three point charges were embedded along the symmetry axis. The interaction potential was given by the electrostatic interaction energy between the proteins. This was obtained by modeling the solvent by the Poisson-Boltzmann equation and the proteins as low dielectric regions.

The average Boltzamnn factor in the reactive region at ionic strengths of 0.25, 0.16, and 0.1 M was found to be 18, 45, and 140, respectively. The diffusion-controlled association rate constant in the absence of any interaction potential given by Eq. 8 is 0.01×10^9 M^{-1} s^{-1}; hence Eq. 2 predicts the rate constant to be 0.18×10^9, 0.45×10^9, and 1.4×10^9 M^{-1} s^{-1}, respectively, at the three ionic strengths. In comparison, the results obtained from Brownian dynamics simulations were 0.14×10^9, 0.32×10^9, and 0.95×10^9 M^{-1} s^{-1}, respectively. The errors of Eq. 2 ranged from 29% to 47%. If the association is only partially controlled by diffusion, Eq. 2 will work even better.

Equation 2 was derived under the condition that the interaction potential around the reactive region is smooth. The variation of the potential increases as the amplitude of the potential increases. This explains the deterioration of the accuracy of Eq. 2 at high amplitudes of the interaction potential.

CONCLUSION

We have tested the accuracy of a simple equation for predicting the enhancement of protein-protein association by interaction potentials. According to this equation, the

TABLE 4 $k/4\pi DR\exp[-\beta U(R)]$ for small δ_1 and δ_2, and $\beta U(r) = -2 \ln(1 + r_0/r)$

$\delta_1 = \delta_1$ (degree)	$\beta U(R)$	Eq. 3	Eq. 2
5	−1	1.689×10^{-4}	1.711×10^{-4}
	−2	1.676×10^{-4}	
	−3	1.667×10^{-4}	
	−5	1.657×10^{-4}	
	−7	1.648×10^{-4}	
	−9	1.632×10^{-4}	
10	−1	1.351×10^{-3}	1.385×10^{-3}
	−2	1.328×10^{-3}	
	−3	1.313×10^{-3}	
	−5	1.288×10^{-3}	
	−7	1.250×10^{-3}	

TABLE 6 $k/4\pi DR\exp[-\beta U(R)]$ for small δ_1 and δ_2, and $\beta U(r) = -V$ in $R < r < 2R$ and 0 in $r > R$

$\delta_1 = \delta_1$ (degree)	$\beta U(R)$	Eq. 3	Eq. 2
5	−1	1.710×10^{-4}	1.711×10^{-4}
	−2	1.709×10^{-4}	
	−3	1.707×10^{-4}	
	−5	1.689×10^{-4}	
	−7	1.563×10^{-4}	
	−9	1.010×10^{-4}	
10	−1	1.382×10^{-3}	1.385×10^{-3}
	−2	1.375×10^{-3}	
	−3	1.362×10^{-3}	
	−5	1.252×10^{-3}	
	−7	0.786×10^{-3}	

rate enhancement is given by the average Boltzmann factor in the reactive region. Analytical and simulation results on a well-known model system demonstrate that rate enhancement predicted from the local Boltzmann factor is accurate to within ~50% for realistic sizes of the reactive region and amplitudes of the interaction potential.

A stable protein-protein complex is formed by two subunits, usually oriented in a very specific fashion; hence it is natural to surmise that the reactive region for association is very restricted. In addition, electrostatic interactions are relatively long-ranged. These considerations lead us to believe that Eq. 2 will be quite accurate for predicting electrostatic rate enhancement for actual protein-protein association processes. Further testing of Eq. 2 will be carried out in the future by using the effective charge model of Gabdoulline and Wade (1996) for calculating the interaction energy between two proteins. Application of Eq. 2 to the association of barnase and barstar, hirudin and thrombin, and other protein systems that have been the subject of recent experimental investigations is expected to yield valuable structural and mechanistic insight.

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