Enhancement of Association Rates by Nonspecific Binding to DNA and Cell Membranes

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(Received 9 June 2004; published 18 October 2004)

A comprehensive analytic theory is developed for the kinetics of reversible association with specific sites on DNA and receptors on cell membranes in the presence of nonspecific binding to the target surfaces. Nonspecific binding is treated as a short-range attractive potential, which is more fundamental and realistic than the surface sliding model. The presence of a surface potential around the target enhances the rate of specific association and for reversible reactions leads to deviations from single exponential relaxation.

DOI: 10.1103/PhysRevLett.93.178101 PACS numbers: 87.15.Aa, 82.39.–k, 87.15.Rn

Biological processes are under the opposing constraints of specificity and speed. A transcription regulatory protein needs to find a small specific operator site on a long stretch of DNA. Similarly, a signaling protein may need to quickly associate with its cognate receptor on a cell membrane. Nonspecific binding to the target surface can significantly enhance the association rate by reducing the dimensionality of the search space for the specific site [1]. The seminal ideas of Adam and Delbruck [1] have been developed over the years, most notably by Berg and Ehrenberg [2,3], who obtained expressions for the irreversible steady-state diffusion-influenced association rate by generalizing the usual boundary conditions to incorporate surface sliding. Work presented here transcends previous developments in two significant respects: (i) we consider reversible association using a sophisticated formalism [4], and (ii) we treat nonspecific binding in a physically transparent way by introducing a short-range attractive potential for the interaction of the ligand with the entire target surface.

We consider the kinetics of reversible association of a ligand with concentration [L] (assumed in excess) with a specific site on the surface of a macromolecular target:

$$\mathrm{M} + \mathrm{L} \rightleftarrows \mathrm{ML}.$$

A ligand can associate with the target when their mutual diffusion brings the ligand into contact with the specific site. The association reaction at contact is described by an intrinsic forward rate constant κ_f. The ligand is attracted by a short-range potential to the entire target surface. The ML complex can dissociate to form a contact pair with rate constant κ_f. The equilibrium constant $K_{\mathrm{eq}} = \kappa_f/\kappa_r$ is independent of the diffusion coefficients of the reactants and product and is independent of the attraction the ligand feels toward the nonspecific sites on the target, and hence independent of surface diffusion. The kinetics of the reversible association for any initial concentrations can be described by a relaxation function defined by

$$R(t) = \frac{[\mathrm{M}(t)] - [\mathrm{M}]_{\mathrm{eq}}}{[\mathrm{M}(0)] - [\mathrm{M}]_{\mathrm{eq}}} = \frac{[\mathrm{ML}(t)] - [\mathrm{ML}]_{\mathrm{eq}}}{[\mathrm{ML}(0)] - [\mathrm{ML}]_{\mathrm{eq}}},$$

where the equilibrium concentrations are given by

$$\frac{[\mathrm{M}]_{\mathrm{eq}}}{[\mathrm{M}]_{\mathrm{total}}} = \frac{1}{1 + K_{\mathrm{eq}}[\mathrm{L}]} \equiv m_{\mathrm{eq}}$$

and $[\mathrm{M}] + [\mathrm{ML}] = [\mathrm{M}]_{\mathrm{total}}$, the total concentration of the macromolecular target. When diffusion is infinitely fast, one finds $R(t) \to \exp\{-(\kappa_f[\mathrm{L}] + \kappa_r)t\}$.

To obtain $R(t)$ when diffusional effects are significant, we generalize the self-consistent relaxation time formalism of Gopich and Szabo [4] to treat anisotropic reactivity. This formalism gives the exact power-law asymptotic relaxation to equilibrium [5] and is based on a set of coupled reaction-diffusion equations for the M-L and ML-L pair distribution functions. For uniformly reacting spherical ligand and target, the Laplace transform of the relaxation function is

$$\hat{R}(s) = [s + ([\mathrm{L}] + 1/K_{\mathrm{eq}})s\hat{k}_{\mathrm{SG}}(s)]^{-1}, \tag{1a}$$

where

$$\frac{1}{s\hat{k}_{\mathrm{SG}}(s)} = \frac{m_{\mathrm{eq}}}{s\hat{k}(s)} + \frac{1 - m_{\mathrm{eq}}}{(s + k_0)\hat{k}(s + k_0)}. \tag{1b}$$

In the last equation, $\hat{k}(s)$ is the Laplace transform of the Smoluchowski-Collins-Kimball time-dependent rate coefficient for the irreversible association and k_0 is to be determined by requiring that the area under the relaxation function is k_0^{-1}, i.e., $k_0^{-1} = \hat{R}(0)$.

At first sight, the problem of generalizing this formalism to anisotropic reactivity in the presence of a surface potential seems formidable since one must solve coupled reaction-diffusion equations subject to mixed boundary conditions (e.g., radiation on the specific site and reflection on the rest of the target surface). However, it has been pointed out [4] that these equations for nonlocal reactivity can be solved within the framework of the Wilemski-Fixman approximation. The final result for $\hat{R}(s)$ is the

same as Eq. (1) when the irreversible association rate coefficient is obtained within the same approximation. In addition, we have shown previously [6] that the Wilemski-Fixman approximation is equivalent to the constant-flux approximation [7] when reactivity is treated as a radiation boundary condition.

To conclude, we can use Eq. (1) to calculate $\hat{R}(s)$ for anisotropic reactivity when we use the constant-flux approximation to obtain the irreversible association rate coefficient. In this approximation, the radiation boundary condition over the specific site is replaced by a uniform diffusive flux. The approximation has been shown to be quite accurate. For example, for a circular disk (with radius a) on a planar target in the absence of a potential, it predicts the irreversible steady-state diffusion-controlled association rate constant as $3\pi^2 Da/8 \approx 3.7Da$ [7], compared to the exact result of $4Da$ [8]. With this approximation, the Laplace transform of the time-dependent rate coefficient for irreversible association has the form [6]

$$\frac{1}{s\hat{k}(s)} = \frac{1}{\kappa_f} + \frac{1}{s\hat{k}_D(s)}, \tag{2}$$

where $k_D(t)$ is the irreversible rate coefficient in the diffusion-controlled limit. With Eq. (2), the self-consistent condition for determining k_0 becomes

$$\frac{1}{m_{\rm eq}K_{\rm eq}k_0} = \frac{1}{\kappa_f} + \frac{m_{\rm eq}}{k_D(\infty)} + \frac{1-m_{\rm eq}}{k_0\hat{k}_D(k_0)},$$

which can be iterated to convergence starting from the steady-state relaxation rate

$$k_0^{\rm ss} = \frac{(\kappa_f[{\rm L}] + \kappa_r)k_D(\infty)}{\kappa_f + k_D(\infty)}.$$

Our model for a receptor on a cell membrane is a reactive circular disk with radius a on an infinite plane [Fig. 1(a)]. The ligand is modeled as a uniformly reactive sphere and the relative diffusion constant is D. In the absence of a potential, $\hat{k}(s)$ has been obtained previously with the constant-flux approximation [9]. To treat nonspecific binding to the cell membrane, we introduce a potential, $U(z)$, that depends on the coordinate perpendicular to the plane. Cell membranes [10] and DNA have surface charges that may provide the surface potential for the ligand. For example, the interaction potential of a test charge near an infinite plane with a smeared charge distribution is $U(z) = -U_0\exp(-z/\lambda)$, where λ is the Debye-Huckel ion screening length. Short-range interactions can also be modeled by a square-well potential, $U(z) = -U_0$ for $0 < z < \varepsilon$ and zero elsewhere. With the constant-flux approximation, it can be shown that

$$\frac{1}{s\hat{k}_D(s)} = \frac{2e^{\beta U(0)}}{\pi Da}\int_0^\infty d\nu \frac{J_1^2(\nu)}{\nu}\frac{f(0)}{-af'(0)}, \tag{3}$$

where $J_1(\nu)$ is the Bessel function and $f(z)$ satisfies

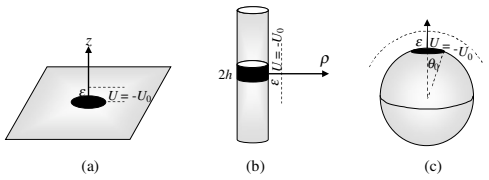

FIG. 1. Models of macromolecular targets with a specific binding site: (a) a planar target, modeling a cell membrane with a receptor for binding a signaling protein, (b) a cylindrical target, modeling a DNA with an operator site for binding a regulatory protein, and (c) a spherical target, modeling a ligand-binding protein.

$$e^{\beta U(z)}\frac{d}{dz}e^{-\beta U(z)}\frac{df}{dz} - \frac{\mu^2}{a^2}f = 0,$$

with $\mu = (\nu^2 + sa^2/D)^{1/2}$. Here and later a prime signifies derivative. For $U(z) = -U_0\exp(-z/\lambda)$, by making the variable changes $-\beta U(z) = x$ and $f(z) = \exp(-x/2)x^{-1/2}g(x)$, we find

$$\frac{f(0)}{-af'(0)} = \frac{1}{\mu}\frac{M_{-1/2,\lambda\mu/a}(\beta U_0)}{M_{1/2,\lambda\mu/a}(\beta U_0)}, \tag{4a}$$

where $M_{\gamma,\mu}(x)$ are Whittaker functions. For the square-well potential we find

$$\frac{f(0)}{-af'(0)} = \frac{1}{\mu}\frac{e^{\beta U_0} + \tanh(\mu\varepsilon/a)}{1 + e^{\beta U_0}\tanh(\mu\varepsilon/a)}. \tag{4b}$$

If the ligand is considered nonspecifically bound whenever it comes inside the potential well, we may define the nonspecific binding constant per unit surface area as

$$K_{\rm ns} = \int_0^\infty dz[e^{-\beta U(z)} - 1]. \tag{5}$$

For the square-well potential Eq. (5) becomes $K_{\rm ns} = \varepsilon(e^{\beta U_0} - 1)$. When the potential is short ranged and strongly attractive around the target surface, both Eqs. (4a) and (4b) lead to the same general result,

$$\frac{1}{s\hat{k}_D(s)} = \frac{2}{\pi Da}\int_0^\infty d\nu \frac{J_1^2(\nu)}{\nu\mu(1 + \mu K_{\rm ns}/a)}. \tag{6}$$

This expression also reduces correctly when $U_0 = 0$, leading to the steady-state ($s \to 0$) value $3\pi^2 Da/8$ cited earlier. For strong nonspecific binding (i.e., $K_{\rm ns}/a \gg 1$), the steady-state rate constant is well approximated by

$$k_D(\infty) = 2\pi DK_{\rm ns}/\ln(K_{\rm ns}e^{2\gamma}/2a), \tag{7}$$

where $\gamma = 0.5772\ldots$ is Euler's constant. Remarkably this asymptotic result is nearly identical to that obtained by Berg [3] using a phenomenological surface sliding model, in which $K_{\rm ns}$ appears as an empirical parameter.

Our model for a specific site on a DNA is a reactive strip with height $2h$ on an infinite cylinder with radius R [Fig. 1(b)]. In the presence of an axially symmetric potential $U(\rho)$, it can be shown that

$$\frac{1}{s\hat{k}_D(s)} = \frac{e^{\beta U(R)}}{2\pi^2 DR}\int_0^\infty d\nu \frac{\sin^2\nu_1}{\nu_1^2}\frac{f(R)}{-Rf'(R)}, \tag{8}$$

where $\nu_1 = \nu h/R$ and $f(\rho)$ satisfies

$$\frac{e^{\beta U(\rho)}}{\rho}\frac{d}{d\rho}\rho e^{-\beta U(\rho)}\frac{df}{d\rho} - \frac{\mu^2}{R^2}f = 0,$$

with $\mu = (\nu^2 + sR^2/D)^{1/2}$. The interaction potential of a test charge around a cylinder with a smeared surface charge distribution is $U(\rho) = -U_0 K_0(\rho/\lambda)/K_0(R/\lambda)$. For the square-well potential $U(\rho) = -U_0$ for $R < \rho < R + \varepsilon$ and zero elsewhere, it can be readily shown that

$$\frac{f(R)}{-Rf'(R)} = \frac{1}{\mu}\frac{K_0(\mu) + AI_0(\mu)}{K_1(\mu) - AI_1(\mu)},$$

where $I_n(\mu)$ and $K_n(\mu)$ are modified Bessel functions, and

$$A = \frac{(e^{\beta U_0} - 1)K_0(\mu_+)K_1(\mu_+)}{e^{\beta U_0}K_0(\mu_+)I_1(\mu_+) + K_1(\mu_+)I_0(\mu_+)},$$

with $\mu_+ = \mu(1 + \varepsilon/R)$. For a narrow deep well [$\varepsilon/R \to 0$ and $\beta U_0 \to \infty$ but $\varepsilon(e^{\beta U_0} - 1) \equiv K_{\rm ns}$ finite], the result in Eq. (8) becomes

$$\frac{1}{s\hat{k}_D(s)} = \frac{1}{2\pi^2 DR}\int_0^\infty d\nu \frac{\sin^2\nu_1}{\nu_1^2\mu[K_1(\mu)/K_0(\mu) + \mu K_{\rm ns}/R]}. \tag{9}$$

This expression is also valid for $U_0 = 0$. For an arbitrary axially symmetric potential $U(\rho)$ we may define the nonspecific binding constant per unit surface area as

$$K_{\rm ns} = \int_R^\infty d\rho\rho[e^{-\beta U(\rho)} - 1]/R, \tag{10}$$

and expect Eq. (9) to provide a good approximation if the potential is short ranged and strongly attractive. When h/R is less than or about 1 and $K_{\rm ns}/R \gg 1$, the steady-state rate constant calculated from Eq. (9) is well approximated by

$$k_D(\infty) = 2\pi^2 D[RK_{\rm ns}/2^{1/2}\ln(K_{\rm ns}e^{4\gamma}/R)]^{1/2}. \tag{11}$$

This result has the same functional dependence on $K_{\rm ns}$ as that previously found by Berg and Ehrenberg [2,3] using a surface sliding formalism in which $K_{\rm ns}$ entered as a parameter in the boundary conditions. Interestingly the above result is independent of the reactive strip height.

The effect of the finite size of a cell can be investigated on a model with a reactive circular patch (spanning polar angles up to θ_0) on the surface of a large spherical target (with radius R) [Fig. 1(c)]. Without any potential, $\hat{k}(s)$ was obtained previously [9]. With a centrosymmetric potential $U(r)$, it has been found that [11]

$$\frac{1}{s\hat{k}_D(s)} = \frac{e^{\beta U(R)}}{4\pi DR}\sum_{l=0}^\infty \frac{[P_{l-1}(\cos\theta_0) - P_{l+1}(\cos\theta_0)]^2}{(2l+1)(1-\cos\theta_0)^2} \times \frac{f_l(R)}{-Rf_l'(R)}, \tag{12}$$

where $P_l(x)$ are Legendre polynomials and $f_l(r)$ satisfies

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

with $\mu = (s/D)^{1/2}R$. For the square-well potential $U(r) = -U_0$ for $R < r < R + \varepsilon \equiv R_+$ and zero elsewhere, it has been shown that [12]

$$\frac{f_l(R)}{Rf_l'(R)} = \frac{1}{\mu}\frac{k_{l+1/2}(\mu) + A_l i_{l+1/2}(\mu)}{k'_{l+1/2}(\mu) + A_l i'_{l+1/2}(\mu)},$$

where $i_{l+1/2}(\mu)$ and $k_{l+1/2}(\mu)$ are modified spherical Bessel functions, and

$$A_l = \frac{-(e^{\beta U_0} - 1)k_{l+1/2}(\mu_+)k'_{l+1/2}(\mu_+)}{e^{\beta U_0}k_{l+1/2}(\mu_+)i'_{l+1/2}(\mu_+) - k'_{l+1/2}(\mu_+)i_{l+1/2}(\mu_+)},$$

with $\mu_+ = \mu(1 + \varepsilon/R)$. It can be checked that the values of k_D from Eq. (3) for a disk with radius a and from Eq. (12) for a patch with the same radius are nearly identical when $a/R \ll 1$. The spherical target may also be used to model ligand-binding proteins (e.g., in dealing with substrate channeling).

In Fig. 2 we show the effect of a short-range square-well potential on the irreversible steady-state diffusion-controlled association rate constant for planar, cylindrical, and spherical targets. The increase of $k_D(\infty)/k_D^{U_0=0}(\infty)$ with the magnitude of U_0 is the steepest for a planar target. For a spherical target, $k_D(\infty)$ reaches the limiting value $4\pi DR_+$ as $\beta U_0 \to \infty$. The approximate formulas, Eqs. (6) and (9), involving the equilibrium constant for nonspecific binding are found to be very accurate. For a planar target with $\varepsilon/a = 0.1$, the maximal difference between Eq. (3) and its approximation by Eq. (6) is just 4% (at $\beta U_0 = 1.6$). The maximal difference between Eq. (8) for a cylindrical target, with $\varepsilon/R = 0.1$ and $h/R = 0.1$, and its approximation by Eq. (9) is 5% (also occurring around $\beta U_0 = 1.6$). The asymptotic for-

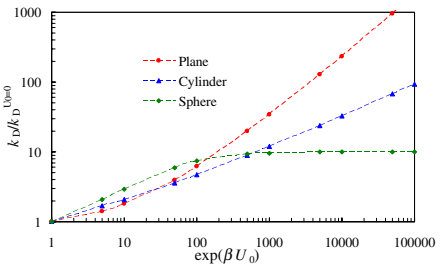

FIG. 2 (color online). The increase of the irreversible steady-state diffusion-controlled association rate constant by a surface potential around planar, cylindrical, and spherical targets. The width of the square-well potential is at $\varepsilon/a = 0.1$ for the planar target, and $\varepsilon/R = 0.1$ for the cylindrical and spherical targets. The height of the absorbing strip on the cylindrical target is at $h/R = 0.1$. The absorbing disk on the spherical target spans polar angles from 0 to $\theta_0 = 5.7°$. Note that the radius of this disk is given by $a/R = [2(1 - \cos\theta_0)]^{1/2} = 0.1$; therefore, for it $\varepsilon/a = 1$ and the potential well appears tenfold wider than for the disk present on the planar target.

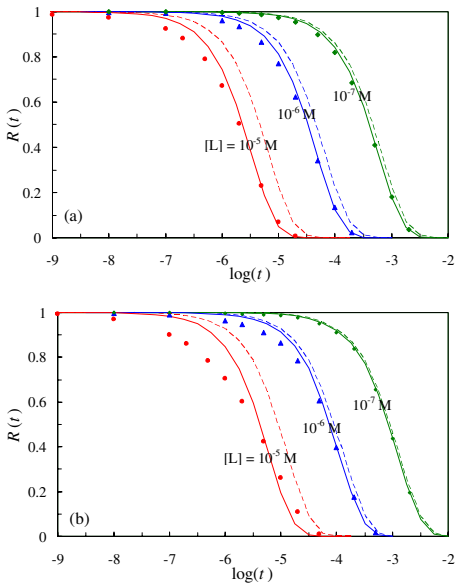

FIG. 3 (color online). Time dependence of the relaxation function. Symbols represent the results of Eq. (1a), with the inverse Laplace transform calculated by the algorithm of Stehfest [14] using eight terms. Solid and dashed curves represent the single exponential functions $\exp(-k_0 t)$ and $\exp(-k_0^{ss} t)$, respectively. Model parameters are $\exp(\beta U_0) = 10^3$, $K_{eq} = 10^9\ \mathrm{M}^{-1}$, and $D = 10^{-6}\ \mathrm{cm}^2/\mathrm{s}$. (a) Association with a circular disk (radius $a = 20$ Å) on a planar target in the presence of a surface potential (width $\varepsilon = 2$ Å). The relevant rate constants are $k_D(\infty) = 15 \times 10^9\ \mathrm{M}^{-1}\,\mathrm{s}^{-1}$, $\kappa_f = 2.5 \times 10^{12}\ \mathrm{M}^{-1}\,\mathrm{s}^{-1}$, and $\kappa_f = 2.5 \times 10^3\ \mathrm{s}^{-1}$. (b) Association with a strip (half height $h = 2$ Å) on a cylindrical target (radius $R = 20$ Å) in the presence of a surface potential (width $\varepsilon = 2$ Å). The rate constants are $k_D(\infty) = 7.7 \times 10^9\ \mathrm{M}^{-1}\,\mathrm{s}^{-1}$, $\kappa_f = 10^{12}\ \mathrm{M}^{-1}\,\mathrm{s}^{-1}$, and $\kappa_f = 10^3\ \mathrm{s}^{-1}$.

mulas given by Eqs. (7) and (10) are also very accurate for $\beta U_0 > 4$, with differences from the full integral results less than 3% and 6%, respectively. These conclusions are validated by results for a planar target with the potential $U(z) = -U_0 \exp(-z/\lambda)$ at small λ/a.

What happens when the range of the potential is large relative to the size of the reactive patch on the target? In this case it has been shown that $k_D(\infty) \rightarrow k_D^{U_0=0}(\infty)\exp(\beta U_0)$ [11,12]. This result is confirmed for the potentials considered here. For example, for a planar target with the potential $U(z) = -U_0 \exp(-z/\lambda)$ at $\exp(\beta U_0) = 100$, $k_D(\infty)/k_D^{U_0=0}(\infty)\exp(\beta U_0)$ increases from 0.08 to 0.35, 0.49, and 0.63, respectively, when λ/a is extended from 0.5 to 5, 10, and 20. Clearly a surface potential enhances the rate much less than a long-ranged one with the same contact value. Thus the intrinsic rate constant κ_f is more likely to exceed $k_D(\infty)$ when the potential is confined to the surface, making ligand association diffusion controlled.

The relaxation function for association with sites on planar and cylindrical targets is shown in Fig. 3. At low ligand concentrations, the difference between k_0 and k_0^{ss} is small and the relaxation function agrees well with the single exponential function $\exp(-k_0 t)$. At higher ligand concentrations, k_0 becomes much greater than k_0^{ss} and the relaxation function deviates significantly from $\exp(-k_0 t)$. As noted earlier, a surface potential tends to make ligand association diffusion controlled and therefore hasten the deviation of the relaxation function from $\exp(-k_0 t)$.

A surface potential does not confine the ligand to the surface of the target; rather it increases the probability of the ligand around the target. A ligand may make many excursions into and out of the surface region before association with the specific site. Therefore it is not just diffusion along the target surface that accounts for rate enhancement, as the surface sliding model would suggest. The contribution of diffusion in the space outside the surface can be illustrated by the cylinder model [Fig. 1(b)] with anisotropic diffusion in the potential well, with components D perpendicular to the surface but $D_\parallel$ parallel to the surface. When $D_\parallel = D$, $k_D(\infty)/k_D^{U_0=0}(\infty) = 12$ at $\exp(\beta U_0) = 10^3$ (see Fig. 2). However, even when $D_\parallel/D$ is reduced by tenfold, it can be shown that $k_D(\infty)/k_D^{U_0=0}(\infty) = 5$ for the same surface potential. The implicated importance of diffusion in the space outside the surface is corroborated by recent experiments comparing the roles of nonspecific DNA either collinear with or linked by catenation to a specific site [13].

This work was supported in part by Grant No. GM 58187 from the National Institutes of Health.

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