Biophysical Chemistry 8 (1978) 271–280
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ASSOCIATION KINETICS WITH COUPLED DIFFUSION .II. IONIC-STRENGTH DEPENDENCE OF THE LAC REPRESSOR–OPERATOR ASSOCIATION

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Received 21 March 1978

The repressor–operator association is treated in a model where the repressor molecule can find its specific binding site, the operator, on a large DNA chain by performing a one-dimensional diffusion along the chain. The ionic-strength dependence is calculated by introducing a screened electrostatic potential around the DNA chain and coupling the free diffusion of the repressor in this potential to the proposed one-dimensional diffusion along the chain. The main influence on the association rate comes from the competitive binding of ions to the unspecific DNA sites. It is also demonstrated that during the time that the repressor is bound in a global sense, the diffusion along the chain will be made up of a strictly one-dimensional motion over fairly short distances, interspersed with many local dissociations during which the repressor in essence is free in solution.

1. Introduction

The anomalously high association rate [1] of the lac repressor to its specific binding site, the operator, on a long DNA chain can be explained by assuming that the repressor can slide along the unspecific DNA sites in a one-dimensional diffusion. Richter and Eigen [2] showed that the association can be speeded up considerably in this manner by calculating the association rate to a rod-shaped spheroid with a length equal to the mean distance the repressor could diffuse along the chain while unspecifically bound.

In a more detailed treatment of the association process in this model we considered the whole length of the DNA chain, first as a rigid rod [3] and then as a random coil [4], and treated the diffusional motion of the repressor as a coupling between the proposed one-dimensional diffusion along the chain and the three-dimensional one in solution.

For an experimental verification of the model, some interesting questions remained. Namely, how will the association rate depend on the total length of the DNA chain, and how will it be influenced by variations in the experimental conditions. The first of these questions was recently addressed by Schranner and Richter [5] who derived the length dependence, particularly for fairly short chains without appreciable coiling.

In the following we shall confine our interest to long DNA chains (~ phage DNA) in the shape of random coils and derive the ionic-strength dependence of the association process. An interesting length dependence appears also in this case. The calculations are based on mean times which in part are related to the equilibrium unspecific binding constant. In sections 2 and 3 these quantities are recalculated by considering the diffusion in a screened electrostatic potential around the DNA chain. The salt effects come from two sources, the screening length of the potential and the competition from the bound ions to the DNA. The competition effects on the unspecific binding have been derived by Record and coworkers [6–8] in the framework of Mannings polyelectrolyte theory [9,10]. In section 4 we relate the parameters of our results to these theories. In section 5 the predictions are amended by noting that the proposed one-dimensional diffusion along the chain should not be interpreted as a motion strictly along the chain. Instead, the theory of diffusion-controlled association leads to the conclusion that during the time (given by the globa dissociation rate) while the repressor is unspecifically bound, it will in fact be free in solution a great many times. If this process is interpreted as a phenomenological one-dimensional diffusion, it will introduce an ionic-strength dependence of the corresponding diffusion constant. Finally, in section 6 a brief summary and discussion are given.

2. Diffusion in an electrostatic potential; equilibrium effects

The negatively charged DNA chain is in solution surrounded by a screened electrostatic potential $V(\rho)$. In the following it will be assumed that the DNA chain can be taken as a cylinder with radius b and that the potential has cylindrical symmetry determined by the distance ρ from the cylinder axis. A positively charged particle will experience an attractive, i.e. negative potential. The free diffusion of particles outside the chain is given by

$$\frac{\partial c}{\partial t} = D\nabla^2 c + \frac{D}{k_B T}\nabla c \cdot \nabla V + \frac{D}{k_B T} c\nabla^2 V, \tag{2.1}$$

or in cylindrical symmetry

$$\frac{\partial c}{\partial t} = D\frac{1}{\rho}\frac{\partial}{\partial \rho}\left[\rho\frac{\partial c}{\partial \rho} + \frac{1}{k_B T}\rho c(\rho)\frac{\partial V}{\partial \rho}\right], \tag{2.2}$$

where $c(\rho, t)$ is the concentration of particles and D their diffusion constant. $k_B T$ is Boltzmann's constant times the temperature.

In the "closed-cell" approach [3] the chain is considered as lying in the center of a large cylinder with impenetrable walls at $\rho = R$ which gives the boundary condition

$$\left[\frac{\partial c}{\partial \rho} + \frac{1}{k_B T} c \frac{\partial V}{\partial \rho}\right]_{\rho=R} = 0. \tag{2.3}$$

At the chain surface the particle is absorbed with a certain probability which can be expressed by the boundary condition

$$kc(\rho = b) = \left[\frac{\partial c}{\partial \rho} + \frac{1}{k_B T} c \frac{\partial V}{\partial \rho}\right]_{\rho=b} \tag{2.4}$$

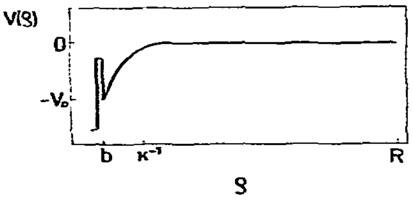

Fig. 1. The screened electrostatic potential with cylindrical symmetry. The size of the barrier at $\rho = b$ is given by the intrinsic reaction rate $2\pi Dbk$. For $\rho > \kappa^{-1}$, the screening length, the potential goes quickly to zero. The distance R is several orders of magnitude larger than b.

This follows from the assumption that the flux of particles onto the chain

$$\Phi(t) \equiv -\int_b^R 2\pi\rho \frac{\partial c}{\partial t}\, d\rho \tag{2.5}$$

is equal to the intrinsic reaction rate, $2\pi bDk$, times the concentration of free particles at the chain surface.

In this way the association to the chain is described as a two-step process; first the electrostatic attraction in the potential well and then a discrete binding reaction with a certain activation energy (given by k, cf. fig. 1). This two-step division is consistent with the polyelectrolyte theory of Manning [9,10] which divides the counterions bound to DNA into a condensed layer around the chain and a diffuse screening layer outside. The last association step involves the displacement of the condensed ions and, if necessary, the establishment of specific interactions, structural changes etc. Consequently, the chain surface $\rho = b$ should be taken as including the layer of condensed ions.

The equilibrium distribution of particles can be determined after the introduction of the intrinsic dissociation rate λ from the chain. Then detailed balance at the chain surface gives

$$\lambda u_0 = 2\pi bDkc(\rho = b), \tag{2.6}$$

where u_0 is the number of bound particles per unit length of the chain. The general time-independent solution to eq. (2.2) is

$$c(\rho) = \exp[-V(\rho)/k_B T] \times \left\{c_1 + c_2 \int_b^\rho \rho^{-1} \exp[V(\rho)/k_B T]\, d\rho\right\} \tag{2.7}$$

where c_1 and c_2 are the integration constants. At equilibrium

$$c(\rho) = c_1 \exp[-V(\rho)/k_\mathrm{B}T], \tag{2.8}$$

which gives the mean concentration of free particles

$$n = \frac{c_1}{\pi(R^2 - b^2)} \int_b^R 2\pi\rho \exp[-V(\rho)/k_\mathrm{B}T]\, \mathrm{d}\rho.$$

The number of bound particles is from eq. (2.6)

$$2Lu_0 = 2Lc_1 \exp[-V(b)/k_\mathrm{B}T]\, 2\pi bDk/\lambda.$$

Hence, the equilibrium dissociation constant for the chain is

$$K_\mathrm{c} \equiv \frac{n}{2Lu_0} = \frac{\lambda \exp[V(b)/k_\mathrm{B}T]}{4\pi LDbk} \times \frac{2}{R^2 - b^2} \int_b^R \rho \exp[-V(\rho)/k_\mathrm{B}T]\, \mathrm{d}\rho. \tag{2.9}$$

With a potential like that of fig. 1 the largest contribution to the integral comes from the region where $V \approx 0$ and

$$K_\mathrm{c} \approx \lambda \exp[V(b)/k_\mathrm{B}T]/4\pi LDbk.$$

The equilibrium constant for the unspecific binding is more commonly expressed in molar basepairs, as each basepair can be the beginning of a new binding site

$$K_\mathrm{RD} \equiv \frac{2L}{l} K_\mathrm{c} \frac{10^3}{N_\mathrm{A}} = \frac{\lambda \exp[V(b)/k_\mathrm{B}T]}{2\pi Dbk} 5 \times 10^{-14}\ \mathrm{M}, \tag{2.10}$$

where N_A is Avogadro's number and $l \approx 3.4 \times 10^{-8}$ cm is the length of a basepair. It should be stressed that the intrinsic reaction rate, $2\pi Dbk$, is the quantity of primary physical significance which is determined by the molecular properties. It must be independent of D. The parameter combination bk, which later turns out to be more useful, is consequently inversely proportional to the free diffusion constant D.

As argued previously [4], the equilibrium binding must be independent of how the DNA is organized in solution, i.e. like a flexible chain (random coil) and not like a rigid rod as assumed in the derivation above. This argument is still valid as long as the chain is not packed so tightly that the screened potentials overlap.

From the equilibrium constant we can derive the mean time τ_2 for the reassociation to any site on any chain for a particle which has just dissociated [4]

$$\tau_2 = \frac{1}{\lambda} \frac{K_\mathrm{c}}{n_0} = \frac{1}{\lambda} \frac{K_\mathrm{RD}}{D_\mathrm{t}}. \tag{2.11}$$

Here n_0 is the concentrations of chains in solution (number of chains per unit volume) or, when K_RD is used, D_t is the total concentration of unspecific DNA (molar basepairs).

3. Mean-time calculations

It was shown previously [3,4] that the mean time of association to a specific site (the operator) on the chain can be expressed as

$$\tau = N(\tau_2 + 1/\lambda) = N\tau_2(1 + n_0/K_\mathrm{c}). \tag{3.1}$$

Here a term due to the unspecific association time has been neglected; it will be important only for short chains. As $1/\lambda$ is the mean residence time at an unspecific site and τ_2 from eq. (2.11) is the mean reassociation time, N can be interpreted as the mean number of visits to the chain before the specific site is found. It is this quantity which contains all detailed information about the association process.

For the case of a long DNA chain in the shape of a random coil, N can be calculated by separating the reassociation flux for a particle, which has just dissociated from an unspecific site on the chain, into two components [4]: $\varphi(t)$ giving the reassociation to a site close (as measured along the chain) to the original site, and $\psi(t)$ describing the reassociation to an uncorrelated site i.e. when the particle has diffused through the coil or to another coil. These fluxes are coupled through the requirements:

$$1 = \int_0^\infty [\varphi(t) + \psi(t)]\, \mathrm{d}t = \tilde{\varphi}(0) + \tilde{\psi}(0), \tag{3.2}$$

i.e. the particle is reassociated with certainty at some time; and

$$\tau_2 = \int_0^\infty t\, [\varphi(t) + \psi(t)]\, \mathrm{d}t = -[\tilde{\varphi}'(0) + \tilde{\psi}'(0)], \tag{3.3}$$

i.e. the mean time for reassociation is that given by the equilibrium binding, eq. (2.11). $\tilde{\varphi}(s)$ and $\tilde{\psi}(s)$ are the Laplace transforms of $\varphi(t)$ and $\psi(t)$ which are easier to handle for mean-time calculations.

In the appendix of ref. [4] it was shown that

$$N = [1-\tilde{\varphi}(0)]^{-1}\left[\left\{\frac{\lambda}{D_1} L^2[1-\tilde{\varphi}(0)]\right\}^{1/2} \times \coth\left\{\frac{\lambda}{D_1} L^2[1-\tilde{\varphi}(0)]\right\}^{1/2} - 1\right], \tag{3.4}$$

where $2L$ is the total length of the chain and D_1 is the diffusion constant for the proposed one-dimensional diffusion along the chain. Thus, it suffices to calculate $\tilde{\varphi}(0) = \int_0^\infty \varphi(t)\mathrm{d}t$ which simply is the fraction of the total reassociation flux which goes to sites close to the original one. Actually, the approximation leading to eq. (3.4) is equivalent to the assumption that these "close" sites are lumped together at the site of the original one. The implications of this assumption will be discussed further in section 5.

Assuming that the particle starts at the distance ρ' from the chain axis, $\tilde{\varphi}(s)$ can be calculated from the Laplace transformed version of the diffusion equation (2.2)

$$s\tilde{c} - \frac{1}{2\pi\rho'}\,\delta(\rho-\rho') = D\frac{1}{\rho}\frac{\partial}{\partial\rho}\left[\rho\frac{\partial\tilde{c}}{\partial\rho} + \frac{1}{k_\mathrm{B}T}\rho\tilde{c}\frac{\partial V}{\partial\rho}\right]. \tag{3.5}$$

with the boundary condition at the chain surface, eq. (2.4)

$$k\tilde{c}(\rho=b) = \left[\frac{\partial\tilde{c}}{\partial\rho} + \frac{1}{k_\mathrm{B}T}c\frac{\partial V}{\partial\rho}\right]_{\rho=b} \tag{3.6}$$

To get rid of the particles that stray too far from the chain and should be included in the flux $\psi(t)$, one can use an absorbing boundary condition at the distance $\rho = R_\mathrm{c}$

$$\tilde{c}(\rho=R_\mathrm{c}) = 0. \tag{3.7}$$

R_c is a measure of the compactness of the coil (cf. ref. [4] where it was called R) and should not be confused with the quantity R of the preceding section which is a measure of the overall concentration of DNA in solution [3].

Now, the reassociation to correlated sites is given by

$$\tilde{\varphi}(s) = \lim_{\substack{\rho\to b\\ \rho'\to b}} 2\pi Dbk\,\tilde{c}(\rho, s) \tag{3.8}$$

As we are primarily interested in $\tilde{\varphi}(0)$ we can let $s \to 0$ in eqs. (3.5)–(3.8). The solution is then simple. It is the Green's function

$$\tilde{c}(\rho,0) \equiv g(\rho,\rho'), \tag{3.9}$$

which in the limit $\rho' \to b$ takes the form

$$g(\rho,b) = (1/2\pi D) \times \frac{\exp[-V(\rho)/k_\mathrm{B}T]\int_\rho^{R_\mathrm{c}}\rho^{-1}\exp[V(\rho)/k_\mathrm{B}T]\mathrm{d}\rho}{bk\exp[-V(b)/k_\mathrm{B}T]\int_b^{R_\mathrm{c}}\rho^{-1}\exp[V(\rho)/k_\mathrm{B}T]\mathrm{d}\rho + 1} \tag{3.10}$$

In the limit $V = 0$ we recover the previous result [4]

$$\tilde{\varphi}(0) = bk\ln(R_\mathrm{c}/b)/[bk\ln(R_\mathrm{c}/b) + 1]\,. \tag{3.11}$$

In a similar manner we can also calculate the mean time for the correlated reassociation. The normalized flux is $\tilde{\varphi}(s)/\tilde{\varphi}(0)$. Hence, the mean time for each dissociation which does not lead to an uncorrelated site is

$$\tau_\mathrm{c} = -\tilde{\varphi}'(0)/\tilde{\varphi}(0). \tag{3.12}$$

This can also be calculated without the full solution of $\tilde{\varphi}(s)$ as follows. Taking the derivative of the equations (3.5)–(3.8) with respect to s and letting $s \to 0$ one gets a set of equations for $\tilde{c}'_0(\rho) \equiv \lim_{s\to 0}\mathrm{d}\tilde{c}/\mathrm{d}s$, where $-\tilde{c}_0(\rho) \equiv -\tilde{c}(\rho,0)$ appears similarly to an initial condition. As the Green's function solution, $g(\rho,\rho')$, is known one gets

$$\tilde{c}'_0(\rho) = -\int_b^{R_\mathrm{c}} 2\pi\rho\,\tilde{c}_0(\rho')g(\rho,\rho')\,\mathrm{d}\rho'. \tag{3.13}$$

Hence, from (3.8), (3.9), (3.13) and the fundamental property of the Green's function $g(\rho,\rho') = g(\rho',\rho)$ one gets

$$\tilde{\varphi}'(0) = 2\pi Dbk\,\tilde{c}'_0(\rho=b) = -2\pi Dbk\int_b^{R_\mathrm{c}} 2\pi\rho[g(\rho,b)]^2\mathrm{d}\rho. \tag{3.14}$$

In the limit $V(\rho) = 0$ we find

$$\tau_\mathrm{c} \approx \frac{R_\mathrm{c}^2}{4D}\,\frac{1}{\ln(R_\mathrm{c}/b)[bk\ln(R_\mathrm{c}/b) + 1]}\,. \tag{3.15}$$

The approximation is only the assumption $R_\mathrm{c} \gg b$. The meaning of τ_c will be discussed further in section 5.

4. Ionic-strength effects

The ionic-strength dependence of the unspecific binding has been treated in depth by Record and coworkers [6–8] in the framework of Manning's polyelectrolyte theory [9,10]. Below we shall relate the parameters of the previous sections to this theory.

According to Manning [9] the number of positive ions condensed on the DNA chain is independent of the ionic strength which instead determines the screening length κ^{-1} of the potential. In the cylindrical symmetry the electrostatic potential for a positive test charge can be taken as [9]

$$V(\rho)/k_{\mathrm{B}}T = -2K_0(\kappa\rho),$$

K_0 is a modified Bessel function of the second kind which quickly goes to zero when $\rho > \kappa^{-1}$. If it is assumed that the repressor attraction can be described by N positive charges on its DNA-binding site, this expression should be multiplied by N. When a bulky molecule, like the repressor, approaches the chain it will displace the screening ions. As this requires work, a positive term should also be added to the potential. Hence, for the repressor

$$V(\rho)/k_{\mathrm{B}}T = -2NK_0(\kappa\rho) + V_{\mathrm{s}}(\rho), \tag{4.1}$$

where V_{s} is the contribution from the displacement of the screening ions. There may be other contributions, such as the interaction from negative charges on the repressor. For the present analysis, however, the exact form of the potential is not needed.

Record et al. [6–8] have shown that the ionic-strength dependence of the unspecific binding constant, is determined primarily by the release from the chain of the bound ions. In the absence of divalent ions, which complicate the binding, the dissociation constant is proportional to the ionic strength, I, to the power of the number of ions released in the association, $K_{\mathrm{c}} \propto I^{10}$.

The intrinsic dissociation rate, λ, can be assumed independent of ionic strength as it describes a dissociation in the unscreened electrostatic potential just far enough to give the counterions space to recondense. Thus, in our picture, eqs. (2.9)–(2.10), the competition effect will enter through the factor $2\pi Dbk \exp[-V(b)/k_{\mathrm{B}}T] \propto K_{\mathrm{c}}^{-1} \propto I^{-10}$. This factor has the same division between condensed ions and screening ions as used by Record et al. [6] since the intrinsic reaction rate $2\pi Dbk$ is concerned only with the condensed ions; from eq. (4.1) the factor $\exp[-V(b)/k_{\mathrm{B}}T]$ carries the dependence on the number of screening ions displaced. Although the variability is introduced solely in the *intrinsic association* rate, it should be stressed that for the commonly measured global rates it will show up in the *global dissociation* rate as long as the association is diffusion controlled (i.e. $bk \gg 1$). Cf. eq. (4.3) below where the global dissociation rate Λ has the same ionic-strength dependence as the equilibrium constant when $bk \gg 1$. See also ref. [11] for a more thorough discussion of the intrinsic rates.

The association rate to the specific operator site can now be expressed as (eqs. (2.11), (3.1), (3.4) and (3.10))

$$k_{\mathrm{a}} \equiv \frac{1}{n_0\tau} = \frac{1}{1+n_0/K_{\mathrm{c}}}\,\frac{\lambda}{NK_{\mathrm{c}}} = \frac{1}{1+n_0/K_{\mathrm{c}}}\,\frac{\lambda}{K_{\mathrm{c}}} \times \left[(\Lambda L^2/D_1)^{1/2}\coth(\Lambda L^2/D_1)^{1/2} - 1\right]^{-1} \ \mathrm{cm}^3\mathrm{s}^{-1} \tag{4.2}$$

where

$$\Lambda \equiv \lambda[1-\tilde{\varphi}(0)] = \lambda\left\{bk\exp[-V(b)/k_{\mathrm{B}}T] \times \int_b^{R_C} \rho^{-1}\exp[V(\rho)/k_{\mathrm{B}}T]\,\mathrm{d}\rho + 1\right\}. \tag{4.3}$$

Λ is the "global dissociation rate" from the unspecific sites in the sense that Λ/λ is the fraction of all dissociations which goes to uncorrelated chain segments as described above. The expression (4.2) may look different from our previous result [4] also in the limit $V \to 0$ when they should be identical. This is because the previous treatment was concerned only with the experimental data of Riggs et al. [1] for which the approximations spelled out in the appendix of ref. [4] were justified; i.e. $\Lambda L^2/D_1 \gg 1$. For the present purpose the full expression above is needed.

The ionic-strength dependence of the association rate k_{a} from eq. (4.2) comes predominantly from the competitive binding of ions through the factor $bk\exp[-V(b)/k_{\mathrm{B}}T] \propto K_{\mathrm{c}}^{-1}$ as discussed above. This influence can be taken into account simply by using the experimentally determined ionic-strength dependence of the unspecific binding. In this way it is not important that $V(b)$ from eq. (4.1) is not adequately determined.

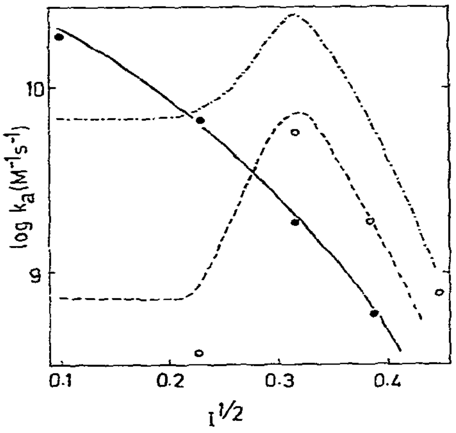

Fig. 2. Predicted ionic-strength dependence of k_a. The relation between the parameters of eqs. (4.2)–(4.3) and the ionic strength is given by the experimentally determined dependence of the unspecific binding and eq. (2.10), as discussed in the text. The data used are: $2L = 1.6 \times 10^{-3}$ cm, $D = 5 \times 10^{-7}$ cm^2 s^{-1}, $\ln(R_c/b) = 3$ and the operator concentration = 1.1 10^{-12} M. (a) ——, result in the presence of Mg^{2+}. To provide a good fit with the data points ● of Riggs et al. [1], the parameters have been chosen $\lambda = 10^3$ s^{-1} and $D_1 = 1.7 \times 10^{-9}$ cm^2 s^{-1}. From eq. (2.10) and ref. [12] $I = [\log(\lambda/bk)/9.8]^2$. (The agreement at $I = 0.01$ M may be fortuitous as this point was measured [1] in a buffer with lower Mg^{2+} content). (b) –.–.–., result in the absence of Mg^{2+}. λ and D_1 chosen the same as in case (a) above. From ref. [13] and eq. (2.10) $I = 0.1\ (0.27\ \lambda/bk)^{0.1}$. (c) – – –, the same as in case (b) above, only D_1 chosen a factor of 10 smaller, $D_1 = 1.7 \times 10^{-10}$ cm^2 s^{-1}. The data points ○ are from M.D. Barkley (unpublished results) under ionic conditions similar to ref. [13].

Apart from this competition, which is an equilibrium effect, there is also the kinetic effect from the screened potential on the free diffusion. This screening effect is expressed by the integral in eq. (4.3) which takes its maximum value for $V \to 0$ or $\kappa \to \infty$ when it is

$$\int_b^{R_c} \rho^{-1} \exp[V(\rho)/k_B T]\, d\rho \to \ln(R_c/b). \qquad (4.4)$$

The error in using eq. (4.4) as an approximation is $\sim \ln(\kappa b)$. As $\kappa^{-1} \approx 3I^{-1/2}$ Å [10], the integral can have a value appreciably smaller than $\ln(R_c/b)$ only at very low ionic strength ($I < 0.01$ M). Numerically it can also be shown that the variation of the integral with ionic strength is very slow. Eq. (4.4) consequently gives a good approximation for the experimentally studied situations and will be used in the following. At lower ionic strength, the increase in the screening length would serve to further increase the specific association rate. This is due not so much to an increase in the effective reaction radius but rather to a more efficient reflection; i.e. a particle in the potential well will have a much higher probability of returning to the chain in the more slowly varying potential, thus providing a more efficient channelling along the chain.

In principle, also the one-dimensional diffusion constant D_1 could depend on the ionic strength. The condensed ions, however, are very mobile along the chain [10], and should not be much of a hindrance for the repressor movement. Thus, D_1 will be assumed constant in the following.

In fig. 2 the predicted ionic-strength dependence of k_a is shown under some different conditions. In the presence of Mg^{2+}, $\log K_c$ can be represented as a linear function of the square root of the ionic strength $I^{1/2}$ [12]. From eqs. (4.2)–(4.3) it is seen that $k_a \propto K_c^{-1/2}$ when $bk \gg 1$ (i.e. a diffusion-controlled unspecific association where $\Lambda \propto K_c$) and $\Lambda L^2/D_1 \gg 1$. This is the case discussed previously [3,4]. The fit with the experimental data of Riggs et al. [1] can be improved when it is observed that k_a appears to behave proportionally to K_c^{-1} for $I \gtrsim 0.15$ M. This would correspond to the limit when the unspecific association becomes reaction controlled, i.e. when Λ of eq. (4.3) approaches λ. This behaviour provides a possibility of separately determining the intrinsic parameters λ and bk which otherwise always appear in the combination $\lambda/bk \propto K_c$. A choice of $\lambda = 10^3$ s^{-1} produces the full line in fig. 2. The data are insecure, however, and on this point no definite conclusions should be drawn.

The much stronger ionic-strength dependence of the unspecific binding in the absence of Mg^{2+} [8] produces a quite different behaviour of the association rate for this case (the broken curves of fig. 2). The predicted levelling-off at low ionic strength is due to the very strong unspecific binding making the argument of the coth-function in eq. (4.2) very small. This means that the whole length of the chain serves as an extended target for the association. In fact, when $bk \to \infty$ in eq. (4.2) one finds

$$k_a = 3D_1/n_0 L^2, \qquad (4.5)$$

i.e. inversely proportional to the operator concentration and the square of the chain length. Thus, in this limit the association rate is determined by the speed of the one-dimensional diffusion. Also, the screening effects of eq. (4.4), which in principle would be most important at low ionic strength, disappear completely from k_a in this limit.

A fairly good agreement between the predictions of the model and the experimental data (M.D. Barkley, unpublished results) can be achieved if it is assumed that D_1 is an order of magnitude slower in the absence of Mg^{2+} (curve c of fig. 2). This agreement is encouraging though it is still premature to draw any definite conclusions as more detailed experiments are needed; i.e. the levelling-off at low ionic strength should be confirmed as well as the apparent first-order nature of the reaction in this limit, eq. (4.5). In particular, the unspecific binding constant under exactly the same experimental conditions needs to be determined as the result is quite sensitive to variations here.

That the one-dimensional diffusion may be an order of magnitude slower in the absence of Mg^{2+} is very interesting and could possibly be interpreted as a structural effect of DNA. As the Mg^{2+} ions stabilize the helix, it is conceivable that a bound repressor will find neighbouring DNA segments in a more suitable configuration for binding and consequently it would be more likely to diffuse along the chain in the presence of Mg^{2+}. This should also strengthen the intrinsic binding such that Mg^{2+} would make λ smaller.

It should be stressed that the curves are very insensitive to variations in λ, even for changes in the order of magnitude. Only the tails of the curves at high ionic strength are pushed slightly upwards when $\lambda \to \infty$ (the completely diffusion-controlled case). An increase in D_1, however, lifts the curves proportionally to $D_1^{1/2}$, except at low ionic strength in the absence of Mg^{2+} when they are lifted proportionally to D_1 (cf. curves b and c of fig. 2). Thus D_1 is the important independent parameter of this model.

5. Implications and extension of the model

Our calculations [3,4] were intended to give a more detailed picture of the Richter and Eigen model [2]. In a recent contribution Schranner and Richter have also extended this model using a steady-state assumption. The similarities and differences between the approaches are now fairly obvious. There is no fundamental difference between a mean-time and a steady-state calculation which is also indicated by the convergence of our results [4,5], when due account is taken of the competition effects from the unspecific binding which Schranner and Richter have neglected. There is one significant difference in the underlying assumptions, however. We use an intrinsic dissociation rate, λ, which gives a physical significance also to the very shortlived dissociations which do not contribute to the global rate Λ. These rates are related as

$$\Lambda = \lambda[1 - \tilde{\varphi}(0)] \xrightarrow[\kappa\to\infty]{} \lambda/[bk \ln(R_c/b) + 1], \qquad (5.1)$$

as $1 - \tilde{\varphi}(0)$ from eq. (3.11) is the total fraction of dissociations leading to a global dissociation. It is this rate Λ which should be identified with the dissociation rate λ used by Schranner and Richter [5].

The general theoretical justification for and implications of this intrinsic rate have been discussed elsewhere [11]. As long as the association is diffusion controlled, $\Lambda/\lambda \ll 1$, there will be a great many local dissociations (given by λ) to each global one (given by Λ). During each local dissociation, the particle will be free in solution and able to move with (almost) the free diffusion constant D. This possibility is neglected in the steady-state approach of Schranner and Richter. The fact that our results are still convergent depends on the approximation leading to eq. (3.4) which was equivalent to the assumption that the local dissociations did not involve any movement along the chain. Thus, while our calculations [4] did contain all the information about the correlated motion, in the final step it was approximated away. While this approximation gave a consistent estimate of D_1 it was dependent on the assumption of a strongly diffusion-controlled unspecific association. This may not be the case for higher ionic strength when the competition effects will lower the intrinsic reaction rate. Below we shall give an estimate of the influence from the local dissociations which have been neglected previously.

During each visit to the chain, the particle will travel the mean distance

$$l_1 = (2D_1/\lambda)^{1/2}. \qquad (5.2)$$

Neglecting, for simplicity, the effects of the electrostatic potential well, we can use τ_c of eq. (3.15) to

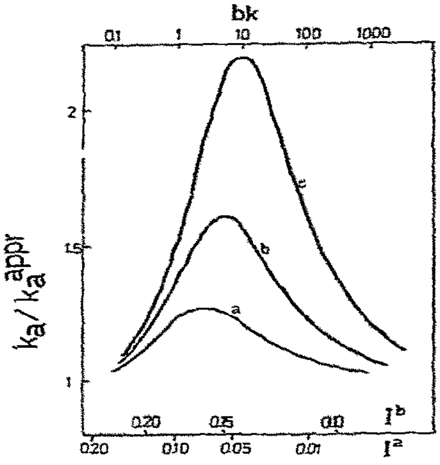

Fig. 3. The ratio between the specific association rates as given by the exact formula eq. (5.5) and the approximative one, eq. (3.4) as a function of bk. Also the corresponding ionic strength I^a or I^b is given, according to the data of cases (a) or case (b) of fig. 2. All curves are with $\ln(R_c/b) = 2.3$. (a) $\lambda L^2/\pi^2 D_1 = 10^5$, (b) $\lambda L^2/\pi^2 D_1 = 10^6$, (c) $\lambda L^2/\pi^2 D_1 = 10^7$. These curves may still contain approximation errors of (at most) 10%.

estimate the mean distance along the chain that the particle will travel during each local dissociation

$$l_2 = (2D\tau_c)^{1/2} = R_c\{2\ln(R_c/b) \times [1 + bk\ln(R_c/b)]\}^{-1/2}. \tag{5.3}$$

Hence,

$$l_1/l_2 = [4\ln(R_c/b)D_1/\Lambda]^{1/2}/R_c \tag{5.4}$$

$(D_1/\Lambda)^{1/2}$ is essentially the effective range of the operator [2] which for low ionic strength is of the order of 10^3 Å, for higher ionic strength it must be smaller. R_c is also $\sim 10^3$ Å [4]. Consequently $l_2 \gtrsim l_1$ and at least at high ionic strength the local dissociations may contribute significantly. Their effect is not as easily evaluated as that given by the strict one-dimensional diffusion since the repressor cannot test all binding sites within l_2 during a local dissociation.

The discussion above can only give a very crude estimate of this effect. Going back to the full expression for N from ref. [4] we have

$$N = 2\frac{\lambda}{D_1}\left(\frac{L}{\pi}\right)^2 \sum_{n=1}^{\infty}\left[n^2 + \frac{\lambda}{D_1}\left(\frac{L}{\pi}\right)^2(1-\varphi_n)\right]^{-1}, \tag{5.5}$$

where $\varphi_n \equiv \tilde{\varphi}(Dn^2\pi^2/L^2)$ and

$$(1-\varphi_n)^{-1} = 1 + \frac{bk}{n\beta}\frac{I_0(n\alpha)K_0(n\beta) - K_0(n\alpha)I_0(n\beta)}{I_0(n\alpha)K_1(n\beta) - K_0(n\alpha)I_1(n\beta)}, \tag{5.6}$$

with $\alpha \equiv \pi R_c/L$ and $\beta \equiv \pi b/L$.

A numerical investigation of the sum in eq. (5.5) shows that the previously used approximation ($\varphi_n \approx \varphi_0 = \tilde{\varphi}(0)$) is good for all values of the parameter bk as long as $\lambda L^2/D_1 \lesssim 10^6$. For larger λ the approximation underestimates k_a, e.g. with a factor $\frac{1}{2}$ for $\lambda L^2/D_1 = 10^8$ and $bk = 10$ (cf. fig. 3). This effect is obviously small and can be interpreted as a slightly erratic ionic-strength dependence of D_1 in the approximation of eq. (4.2).

The curves of fig. 2 are not much affected as they were drawn for $\lambda L^2/D_1 \lesssim 10^6$. The fit of curve c with the data points can be greatly improved, however, if λ is chosen larger and the effect discussed above is included. A choice of a larger λ would also be consistent with the observation in the previous section that the intrinsic binding could be weaker in the absence of Mg^{2+}. More detailed experimental data is needed, however.

The screening effects have been neglected in the discussion of the local dissociations above. Using τ_c as a measure of their influence, it can be shown from eq. (3.14) that only when the screening length κ^{-1} is much larger than the reaction radius b will there be a significant departure from τ_c of eq. (3.15); i.e. again for $I < 0.01$ M which is outside the range of existing experiments.

While the effect of the local dissociations may not give a significant contribution to the association rate, it is important to point out that the one-dimensional diffusion along the chain is not an uninterrupted process during the time Λ^{-1} while the repressor is bound in a global sense. The distance travelled in each local disociation is actually larger than the uninterrupted diffusion length $(2D_1/\lambda)^{1/2}$, which can be a fairly small quantity. Although the whole process can be described by a phenomenological diffusion constant D_1, not much different from the D_1 of the strictly one-dimensional motion, these distinctions must be borne in mind if experiments are designed to observe the diffusion along the chain and measure D_1.

6. Discussion

The ionic-strength dependence of the lac repressor-operator association has been derived for the sliding model. As it turns out, the effects from the screened potential on the free diffusion are negligible for all but the lowest ionic strengths for which there are no experimental data. The important contribution comes from the competitive binding of ions to the unspecific DNA sites. The result is dependent on the intrinsic reaction rates $2\pi Dbk$ and λ which are not yet adequately known. They can be determined from the extent of diffusion control exhibited by the unspecific binding. So far, only the combination $\lambda/2\pi Dbk \propto K_c$ is independently known. A good qualitative agreement between the predictions of the model and the experimental data can be achieved without a knowledge of the intrinsic rates, however, and they will be important only for the finer details of the curves.

The need for these intrinsic reaction rates follows directly from the theory of diffusion-controlled association [11]. They also give rise to a more curious effect: the enhancement due to the local dissociations as discussed in section 5. Fortunately this effect is very small for the parameter values which appear reasonable in the lac repressor case.

The result indicates that the repressor movement along the DNA chain is an order of magnitude slower ($D_1 \sim 10^{-10}$ cm^2 s^{-1}) in the absence of Mg^{2+}. If this interpretation is correct, also the intrinsic binding should be weakened such that λ would be larger in the absence of Mg^{2+} than in their presence. On the global scale, however, this effect is masked by the fact that Mg^{2+} is a competitor to the repressor for the DNA sites.

Due to the complicated geometry of the DNA chain in solution, we have to work with unspecific dissociation rates at three different levels. First, the intrinsic rate λ describing a local dissociation which can be followed by a more or less immediate reassociation. Then there is the rate Λ for the dissociations leading to reassociation to an uncorrelated chain segment. The dissociation rate measured in a temperature-jump experiment, however, is again another entity, $k_D \equiv K_c k_A$. Here k_A is the unspecific association rate determined by the size of the DNA coils in solution [4].

In response to the critisism of Schranner and Richter [5] of our usage of mean times, it should be stressed that this represents no fundamental difference from their steady-state approach. Mathematically, both methods correspond to the limit $s \to 0$ in a Laplace-transformed solution and, as long as there is a dominant relaxation time in the system, both methods should pick it out accurately. The mean-time calculations, however, employ well-defined boundary conditions without the introduction of an ad hoc surface where the concentration is constant. The essential difference between our methods lies in the different constraints used for the coupling between the one- and three-dimensional diffusion. We consider our usage of intrinsic reaction rates theoretically better founded and intuitively more clear than the division of flows used by Schranner and Richter.

Acknowledgement

We are very grateful to Dr. M.D. Barkley for the permission to use her unpublished results as well as for the advance information of preliminary results which prompted this study. We also thank Drs. T.M. Lohman and G.S. Manning for valuable comments on the manuscript. This work has been supported by the Swedish Natural Science Research Council.

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