Biophysical Chemistry 8 (1978) 135–150

RATE ENHANCEMENT BY GUIDED DIFFUSION. CHAIN LENGTH DEPENDENCE OF REPRESSOR-OPERATOR ASSOCIATION RATES

Rudi SCHRANNER and Peter H. RICHTER*
Max-Planck-Institut für biophysikalische Chemie,
D-34 Göttingen-Nikolausberg, Germany

Received 5 December 1977

Association rates are calculated for cases where one reaction partner belongs to a chain that has an unspecific affinity to the other. Provided that the unspecific attachment does not completely suppress diffusion along the chain, this channeling may considerably speed up the association. Explicit formulae are derived to show how this effect depends on the chain length and other parameters. The influence of electrostatic forces and reaction barriers is discussed. Time dependent solutions of the diffusion equations are analyzed in order to test the usual steady state assumptions. Experiments on the repressor-operator system seem to be in good agreement with our theory.

1. Introduction

It is a well-known fact that diffusion in one- or two-dimensional space is far more effective in finding a target than it is in three dimensions. Thus, there is no surprise that nature has used this principle in order to enhance reaction rates. As an appealing example thereof, Adam and Delbrück [1] discussed how insects increase their sensitivity for pheromons by means of antennae which do not by themselves possess receptors but guide the pheromons on their random walk. The same idea has successfully been applied to explain the high association rates of repressor-operator binding [2,3] as well as of certain reactions involving membrane bound enzymes [4].

The picture that we use for the repressor-operator association [5], and possibly for the finding of promotor sites by RNA-polymerase, or of other specific sites on a DNA strand by their recognizing enzymes, depends on two prerequisites:

(i) the repressor has an unspecific affinity to any kind of DNA so that it may be trapped not only by the operator itself but also on adjacent parts along the strand. That such an unspecific binding exists has been established by Lin and Riggs [6]. Its nature is primarily electrostatic attraction as shown by its strong dependence on ionic strength.

(ii) the unspecific association to DNA does not completely immobilize the repressor but allows for diffusional motion along the chain. Thus the binding should not be too tight, and therefore electrostatic attraction is particularly appropriate. No direct experimental evidence is so far available to support this assumption, the technical difficulties being aggravated by the expected low value ($\lesssim 10^{-9}$ cm^2/s) of the diffusion constant for one-dimensional motion.

The implications of (i) and (ii) are intuitively obvious. If D_1 is the diffusion constant for the unspecifically bound repressor and λ its dissociation rate, then $\sqrt{D_1/\lambda} = l$ is the length a repressor molecule will cover along the DNA each time it is bound. This may greatly exceed the linear dimensions of the operator itself and therefore increase the specific association rate accordingly.

In ref. [3] the specific association rate k_{ass} was calculated as the flow of particles onto a long prolate spheroid of length $2\,l$ and diameter b which is the radius of encounter between DNA and repressor. A standard von Smoluchowski picture led to $k_{\mathrm{ass}} = 4\pi D_3 l/\ln(2l/b)$, or a slightly more involved formula if electrostatic forces are taken into account; D_3 is the bulk

---

[*] Present address: Dept. of Chemistry, Mass. Inst. of Technology, Cambridge, Mass. 02139, USA.

diffusion constant. Satisfactory agreement with the experimental findings could be established which also qualitatively fitted in with the ionic strength effects. The prediction was made that upon shortening the total length $2L$ of DNA, the association rate would decrease as soon as $L \lesssim l$.

No precise formula was given, however, for the expected L-dependence of k_{ass}. Furthermore, as pointed out by Berg and Blomberg [7], the mathematical procedure of [3] was not entirely rigorous because diffusion along the strand was not explicitly coupled to the motion in solution. Finally, no investigation of the time dependence had been carried out, the tacit assumption being that the transient time for the steady state to build up is short compared to the experimental times involved in the determination of the rate constants (which takes seconds or minutes).

The present paper is devoted to an extensive analysis of these questions and will essentially confirm the earlier statements. The treatment will be entirely in the classical spirit of von Smoluchowski [8], Debye [9], and Eigen [10], and yet take full account of the coupling between three- and one-dimensional diffusion. The following section outlines the method and formulates the basic equations, including a digression on electrostatic effects. In sections 3 and 4, various steady state situations are analyzed that differ in their geometry and boundary conditions. The dependence of the specific association rate on the total length of DNA will be given for three different cases, showing that the qualitative behaviour is quite independent of the details in the set-up. In sect. 5 we solve the time dependent equations for two situations, with a twofold aim: one, the steady state assumption will be justified for the experiments that have been carried out so far, and two, comparison with the work of Berg and Blomberg [7] will be made showing that their approach to k_{ass} involves unnecessary difficulties. Finally, in sect. 6 a summary is given and the results are discussed in the light of experiments.

2. Coupling of three- and one-dimensional diffusion

2.1. The physical picture

Let us first describe in words what is to be put into precise mathematical terms in the following section. A particle diffuses in three-dimensional space (diffusion constant D_3) until it comes into contact with a one-dimensional chain. At each such encounter the particle gets bound to the chain, i.e. we assume the unspecific association to be diffusion controlled. While at the chain, however, the particle continues to diffuse with a reduced diffusion constant D_1. The time available for this erratic search along the chain is limited by the rate λ at which the particle returns to the pool of freely moving particles. Once dissociated, it may leave the chain behind forever or find it again and become reassociated. In that sense we also take the dissociation as diffusion controlled. At one point along the chain ($z = 0$) there is a trap. Whenever a particle gets there it will be tightly bound and spend a long time there before being released, at rate μ, to go on with one-dimensional diffusion, dissociation, reassociation etc.

In the stationary state detailed balance holds between any two of the three reservoirs:

– the bulk, number of particles per cm^3 denoted by $n(r)$;

– the unspecifically bound particles, their number per cm chain being $u(z)$;

– the specifically bound particles, s in number.

Given constant bulk concentration everywhere on the outer boundaries of the reaction vessel, $n(\text{o.b.}) = n_0$, the absence of net internal flows implies homogeneity within each compartment, $n(r) = n_0$, $u(z) = u_0$, $s = s_0$. The concentrations are related by thermodynamic equilibrium constants:

$$u_0/n_0 = K_{un}, \quad s_0/u_0 = K_{su},$$

$$s_0/n_0 = K_{su}K_{un} = K_{sn}\,. \tag{1}$$

Our interest is mainly in the specific binding, i.e. we want to interpret K_{sn} as the ratio of a specific association and a corresponding dissociation rate:

$$K_{sn} = k_{ass}/k_{diss}\,. \tag{2}$$

For that purpose we follow the reasoning of v. Smoluchowski, Debye, and Eigen [8–10] and regard the stationary situation as a superposition of two flows, one describing association, the other dissociation. For the association it is fictitiously assumed that at the outer boundaries we have n_0 as before but at the binding site $z = 0$ every particle is taken away as soon as it arrives. The specific association rate k_{ass} is then identified as the number of particles which enter this sink per unit time and concentration:

$$n_{\text{ass}}(\text{o.b.}) = n_0, \quad s = 0,$$

$$k_{\text{ass}} = \frac{2}{n_0} D_1 \left.\frac{\partial u_{\text{ass}}}{\partial z}\right|_{z=0} . \tag{3}$$

The factor 2 accounts for the fact that association is from two sides of $z = 0$. Conversely, dissociation is envisaged as the stationary flow of particles in case s is held at its equilibrium concentration s_0, and every particle crossing the outer surface is removed:

$$n_{\text{diss}}(\text{o.b.}) = 0, \quad s = s_0,$$

$$k_{\text{diss}} = -\frac{1}{s_0} \oint_{\substack{\text{outer}\\ \text{boundary}}} D_3 \nabla n_{\text{diss}} \cdot \mathrm{d}^2 r . \tag{4}$$

The similarity of the two problems (3) and (4) is apparent and we shall mainly restrict ourselves to the case of association. The question now is: how do we properly take into account the unspecific binding to the chain?

2.2. *The basic equations*

To be explicit we shall be using cylindrical coordinates here. Translation to spheroidal or other geometries is straightforward and will be done when required (sect. 4). We place the chain between $-L$ and $+L$ along the z-axis, its total length being $2L$, the radius of encounter with the particle b. The idealization of taking a straight chain will be discussed at the end (sect. 6).

We now analyze the problem of association and derive a complete set of equations and boundary conditions for n_{ass} and u_{ass}. The equation for the bulk concentration is obviously

$$\partial n_{\text{ass}}/\partial t = D_3 \nabla^2 n_{\text{ass}} . \tag{5}$$

For the unspecifically bound particles consider fig. 1 and add up the four flux contributions to $\partial u_{\text{ass}}/\partial t$, the particles which are associated to the chain, the dissociating particles, the particles entering at the top minus those leaving at the bottom:

$$\frac{\partial u_{\text{ass}}}{\partial t} = 2\pi b D_3 \left.\frac{\partial n_{\text{ass}}^{\text{in}}}{\partial r}\right|_b - \lambda u_{\text{ass}} + D_1 \frac{\partial^2 u_{\text{ass}}}{\partial z^2} . \tag{6}$$

The difficulty rests in the connection of the two equations (5) and (6) at $r = b$. However, it is familiar from electrodynamics or quantum mechanics that whenever a flow crosses a boundary, the total solution may be decomposed into an ingoing, a reflected, and a transmitted part. There is no question of identifying u_{ass} as the transmitted part. But how do we distinguish an ingoing part $n_{\text{ass}}^{\text{in}}$ from a reflected one, $n_{\text{ass}}^{\text{refl}}$, in the bulk? The answer can again be given in the framework of a von Smoluchowski–Debye–Eigen picture. Assuming that the unspecific association is diffusion controlled, the incoming part should be totally consumed by the chain. Consequently, the reflected part originates from the particles that leave the chain, and is taken away at the outer boundary:

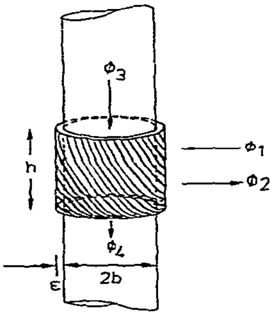

Fig. 1. The rate of change of total particle number $h \cdot u$ within the dashed box is made up of four contributions: $\phi_1 = 2\pi(b+\epsilon)h D_3\, \partial n^{\text{in}}/\partial r|_{r=b+\epsilon}$, $\phi_2 = -\lambda h u(z)$, $\phi_3 = D_1\, \partial u/\partial z|_{z+h}$, $\phi_4 = -D_1\, \partial u/\partial z|_z$. The change of particle density is therefore $\partial u/\partial t = \lim_{\epsilon,h\to 0} \Sigma_i \phi_i/h$.

$$n_{\text{ass}} = n_{\text{ass}}^{\text{in}} + n_{\text{ass}}^{\text{refl}} , \tag{7}$$

$$n_{\text{ass}}^{\text{in}}|_{r=b} = 0, \quad n_{\text{ass}}^{\text{in}}(\text{o.b.}) = n_0 , \tag{8}$$

$$2\pi b D_3 \left.\frac{\partial n_{\text{ass}}^{\text{refl}}}{\partial r}\right|_{r=b} = -\lambda u_{\text{ass}}, \quad n_{\text{ass}}^{\text{refl}}(\text{o.b.}) = 0 . \tag{9}$$

the reason for our writing $n_{\text{ass}}^{\text{in}}$ in eq. (6) should now be clear. The boundary conditions for u_{ass} are

$$u_{\text{ass}}(z=0) = 0, \quad \left.\frac{\partial u_{\text{ass}}}{\partial z}\right|_{z=\pm L} = 0 . \tag{10}$$

To sum up, the problem of calculating the specific association rate may be solved in the following sequence. Since we are interested in steady state solutions of eqs. (5) and (6), first determine $n^{\mathrm{in}}_{\mathrm{ass}}$ from Laplace's equation and boundary conditions (8). Next compute the flow $2\pi b D_3 \partial n^{\mathrm{in}}_{\mathrm{ass}}/\partial r|_b$ onto the chain and solve eq. (6) for u_{ass}, using boundary conditions (10). k_{ass} is then obtained from (3). The total bulk distribution of particles may subsequently be calculated by solving for $u^{\mathrm{refl}}_{\mathrm{ass}}$, using eq. (5) and boundary conditions (9).

A straightforward generalization may account for cases where the unspecific association is not purely diffusion controlled. The standard way to describe a reaction barrier, for the incoming particles, is in terms of a parameter k:

$$n^{\mathrm{in}}_{\mathrm{ass}}|_{r=b} = \frac{1}{k} \left.\frac{\partial n^{\mathrm{in}}_{\mathrm{ass}}}{\partial r}\right|_{r=b} . \tag{11}$$

In the limit $k \to \infty$ we recover condition (8) whereas for $k = 0$ no association takes place at all. The remaining boundary conditions would not be affected.

It is now easy to analogously formulate the problem for dissociation from the trap. Some care must be taken, however, to properly state the boundary condition for u_{diss} at $z = 0$. It may be helpful, for that matter, to consult the appendix where we present a discrete model which illustrates the kinetic nature of our system. The basic assumption throughout this work is, of course, that on a molecular scale diffusion along the chain is a faster process than dissociation,

$$D_1/a^2 \gg \lambda . \tag{12}$$

Here a is the distance between neighbouring binding sites on the chain, and diffusion is visualized as a random walk over these sites. Let μ be the kinetic parameter for release from the trap at $z = 0$ to a neighbouring unspecific binding site which we also assume a distance a apart. Then if (12) is fulfilled, the boundary condition at $z = 0$ reads

$$u_{\mathrm{diss}}|_{z=0} = \frac{\mu a}{D_1} s_0 = u_0 . \tag{13}$$

This means that most of the particles that once leave the specific binding site do not immediately dissociate from the chain but first diffuse in z-direction and eventually return to the trap. In the near neighbourhood of $z = 0$ the equilibrium between s_0 and u_0 is not disturbed. In a certain sense, therefore, the boundary condition (13) automatically accounts for a "reflected" part in u_{diss} which is the solution of

$$\frac{\partial u_{\mathrm{diss}}}{\partial t} = D_1 \frac{\partial^2 u_{\mathrm{diss}}}{\partial z^2} - \lambda u_{\mathrm{diss}}, \qquad \left.\frac{\partial u_{\mathrm{diss}}}{\partial z}\right|_{\pm L} = 0 . \tag{14}$$

The bulk concentration n_{diss} finally derives from Laplace's equation and the boundary conditions

$$2\pi b D_3 \left.\frac{\partial n_{\mathrm{diss}}}{\partial r}\right|_{r=b} = -\lambda u_{\mathrm{diss}}, \qquad n_{\mathrm{diss}}(\mathrm{o.b.}) = 0 . \tag{15}$$

The dissociation rate k_{diss} is given by (4) or, because of conservation of matter, by

$$k_{\mathrm{diss}} = -\frac{2}{s_0} D_1 \left.\frac{\partial u_{\mathrm{diss}}}{\partial z}\right|_{z=0} . \tag{16}$$

2.3. Electrostatic effects

Taking into account electrostatic forces, the particle fluxes are no longer driven by the concentration gradient alone but rather by the inhomogeneities of the chemical potential μ:

$$\nabla n \to n \nabla \frac{\mu}{kT} = \nabla n + n \nabla \frac{U}{kT} , \tag{17}$$

where U is the electrostatic potential. In the simplest possible approximation we neglect screening effects, or free charges in the bulk. Then the potential U obeys Laplace's equation,

$$\nabla^2 U = 0 , \tag{18}$$

and instead of eq. (5) we have

$$\frac{\partial n}{\partial t} = D_3 \nabla^2 n + D_3 \nabla n \cdot \nabla \frac{U}{kT} , \tag{19}$$

both for association and dissociation. The chain may reasonably be taken as an equipotential surface, $U(r = b) = \text{const.}$, and the outer boundary is assumed to be grounded, $U(\mathrm{o.b.}) = 0$. Diffusion along the chain is not affected by the electric forces because it proceeds orthogonal to the field. The boundary conditions of the previous section also need no modification.

As a first and simple illustration let us discuss the case of unspecific binding to the chain, i.e. we disregard the sink at $z = 0$. For simplicity we take an infinitely long chain, $L \to \infty$, so that the problem depends on coordinate r only. The outer boundary be located at $r = R$, and we have $n(R) = n_0$, $U(R) = 0$. From eq. (18) we obtain the familiar potential

$$U(r) = U(b)\,\frac{\ln r/R}{\ln b/R}\,. \tag{20}$$

The ingoing part of the bulk concentration then follows from (19) and (8)

$$n^{\mathrm{in}}(r) = \frac{e^{-U(r)/kT} - e^{-U(b)/kT}}{1 - e^{-U(b)/kT}}\, n_0\,. \tag{21}$$

The flux of incoming particles per cm chain and concentration is nothing else than the phenomenological unspecific association rate

$$k_{\mathrm{in}} = \frac{1}{n_0}\, 2\pi b D_3\, \frac{\partial n^{\mathrm{in}}}{\partial r}\bigg|_{r=b} = \frac{2\pi D_3}{\ln R/b}\, \frac{-U(b)/kT}{1 - e^{U(b)/kT}}\,. \tag{22}$$

The dissociation from the chain is similarly described by an outgoing part $n^{\mathrm{out}}(r)$ which fulfils the boundary conditions (9) with $u(z) \equiv u_0$ given by detailed balance

$$\lambda u_0 = k_{\mathrm{in}} n_0\,. \tag{23}$$

We find

$$n^{\mathrm{in}}(r) + n^{\mathrm{out}}(r) = n_0\,, \tag{24}$$

and the flux of outgoing particles at $r = R$ gives the observed dissociation rate:

$$k_{\mathrm{out}} = -\frac{1}{u_0}\, 2\pi R D_3\, \frac{\partial n^{\mathrm{out}}}{\partial r}\bigg|_R$$

$$= \frac{n_0}{u_0}\, k_{\mathrm{in}}\, e^{U(b)/kT} = \lambda e^{U(b)/kT}\,. \tag{25}$$

The expressions (22) and (25) allow for an interpretation of the unspecific binding in terms of electrostatics alone. Let us assume an attractive interaction between the chain and the particle, $U(b) < 0$. Without any intrinsic affinity to the chain the particle would dissociate at a rate $\lambda_0 \approx D_3/d^2$ where d is the mean free path for the tumbling motion in solution; reasonable assumptions for D_3 ($\approx 10^{-7}$ cm^2/s) and d ($\approx 1 - 10$ Å) lead to $\lambda_0 \approx 10^7 - 10^9$ s^{-1}. An observed λ of 10/s could then be ascribed to a potential $U(b)$ in the order of 8–11 kcal/mole which seems quite reasonable for a molecule with the size of the repressor. For the association rate k_{in} the dependence on electrostatic forces is much weaker and can be interpreted as an effective increase of the radius of encounter; screening effects would reduce the rate enhancement due to attraction. Thus if we adjust b to contain the influence of the electrostatic factor in (22), and if λ is taken to contain the factor $e^{U(b)/kT}$, we may include electrostatic effects without explicitly mentioning them.

3. Cylindrical geometry

The rod shape of DNA makes the choice of cylindrical coordinates most obvious. We consider chains of radius b and length $2L$ extending along the z-axis. Assuming that they may be regarded isolated each DNA chain may be embedded in a larger cylinder of the same length[‡] but radius $R > b$. These cylinders are assumed to fill the reaction volume and thus approximately $2\pi L R^2 = s_0^{-1}$ where s_0 is the DNA concentration (the results do not depend critically on R which is confirmed also by the results of sect. 4). According to (8) we assume $n = n_0$ on the outer cylinder, for the case of association. This will be done in sect. 3.2. Another kind of boundary condition has been treated by Berg and Blomberg [7] in their "closed cell approach": The condition of vanishing flux across the boundary. In this case there is no stationary state except the trivial one $n(r) = 0$, and the only interesting feature is the time it takes for the sink at $z = 0$ to consume all the particles initially present. Since this time can be related to the association rate we shall discuss it in connection with transient behaviour in sect. 5.2. The first section, however, will be devoted to the case of "mixed boundary conditions" which is mathematically the simplest one, yet exhibits all the features of the more realistic cases.

3.1. Mixed boundary conditions

We assume fixed concentration at the outer cylinder $r = R$ and no flux passing through its top and bottom:

$$n(r = R) = n_0\,, \quad \partial n/\partial z\,|_{z=\pm L} = 0\,. \tag{26}$$

Let us first analyze the case of association. The incoming part fulfils $n^{\mathrm{in}}_{\mathrm{ass}}(r = b) = 0$. Thus it is easily verified that

$$n^{\mathrm{in}}_{\mathrm{ass}}(r) = n_0\, \frac{\ln r/b}{\ln R/b} \tag{27}$$

[‡] It would be mathematically quite difficult though more realistic to have the outer cylinder larger than $2L$. The spheroidal coordinates, sect. 4, help to simulate such a situation.

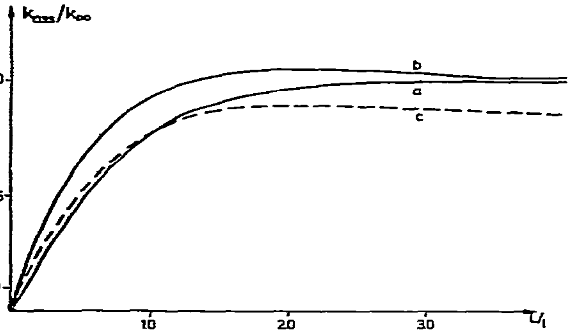

Fig. 2. L-dependence of the association rate k_{ass} given in reduced units for a: mixed boundary conditions (sect. 3.1), b: $n = n_0$ everywhere on the outer cylinder (sect. 3.2), c: spheroidal arrangement (sect. 4). For comparison, the cylinder radius R has been chosen equal to $2l$, $b = 10^{-3} l$, $k_\infty = 4\pi D_3 l/\ln(R/b)$.

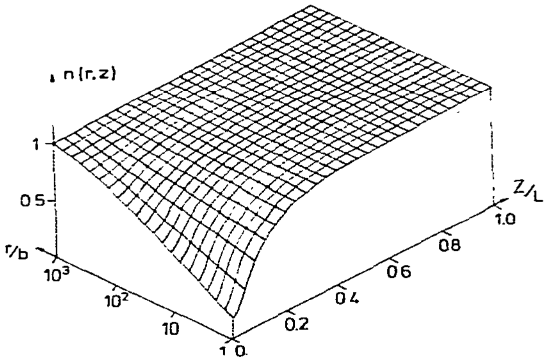

Fig. 3. Steady state distribution of particles in the bulk, for the case of association ($l^{-2} = 5, R = 1, L = 5$). The dependence on r is shown on a logarithmic scale as r/b ranges from 1 to 10^3, $n_0 = 1$.

independent of z. The flux onto the chain is therefore

$$2\pi b D_3 \left.\frac{\partial n_{ass}^{in}}{\partial r}\right|_b = \frac{2\pi D_3}{\ln R/b} n_0 \equiv k_{in} n_0 , \qquad (28)$$

cf. (22). Next solve eq. (6) for the stationary state $\partial u_{ass}/\partial t = 0$, using (28). The result is

$$u_{ass}(z) = n_0 \frac{k_{in}}{\lambda}\left(1 - \frac{\cosh(z-L)/l}{\cosh L/l}\right), \qquad (29)$$

where l is the length defined by

$$l = \sqrt{D_1/\lambda} . \qquad (30)$$

For large L, $L \gg l$, the profile (29) increases from 0 at $z = 0$ to $n_0 k_{in}/\lambda$ at $z \gtrsim 1$. The slope at $z = 0$ gives the specific association rate, according to (3):

$$k_{ass} = \frac{4\pi D_3}{\ln R/b} l \tanh L/l = 2 k_{in} l_{eff} . \qquad (31)$$

The interpretation is straightforward: k_{in} is the rate of unspecific association to the chain, per unit length, l_{eff} therefore represents the effective range of the central sink. For $L \gg l$ it coincides with the length l a particle diffuses along the chain during time λ^{-1}; if L is smaller than l the total length L becomes the limiting factor for the association rate. Fig. 2 shows the L-dependence of k_{ass}. For small L the present boundary conditions underestimate the rate because no particles are allowed to come in through the top.

The reflected contribution to $n_{ass}(r, z)$ may be calculated from Laplace's equation and boundary condition (9) using the result (29). Numerical evaluation leads to the picture of fig. 3 where the range of the operator sink is shown both in r- and z-direction.

The modification induced by taking the boundary condition (11) instead of the pure case of diffusion control is obvious. The r-dependence of n_{ass}^{in} is smoother than in (27),

$$n_{ass}^{in}(r) = n_0 \frac{1 + kb \ln r/b}{1 + kb \ln R/b} \qquad (32)$$

and the unspecific association rate is reduced accordingly:

$$k_{in} = \frac{2\pi D_3}{\ln R/b}\left(1 + \frac{1}{kb \ln R/b}\right)^{-1} . \qquad (33)$$

The specific association rate is again $2k_{in} l_{eff}$. Assuming that the unspecific equilibrium $u_0/n_0 = k_{in}/\lambda$ is not affected by the reaction barrier, λ changes in proportion to k_{in}, and $l \sim k_{in}^{-1/2}$ is increased. At large L, therefore, the reduction in k_{ass} is weaker than in k_{in},

$$k_{ass} \sim \left(1 + \frac{1}{kb \ln R/b}\right)^{-1/2} \quad (L \gg l) . \qquad (34)$$

For small chain lengths, on the other hand, $l_{eff} \approx L$ and the reaction barrier is fully apparent in k_{ass}:

$$k_{ass} \sim \left(1 + \frac{1}{kb \ln R/b}\right)^{-1} \quad (L \ll l) . \qquad (35)$$

We now turn to the calculation of the specific dissociation rate. The diffusion equation (14) with boundary condition (13) is solved by

$$u_{\text{diss}}(z) = \frac{\mu a}{D_1} s_0 \frac{\cosh(z-L)/l}{\cosh L/l} = u_0 - u_{\text{ass}}(z) \,. \tag{36}$$

and k_{diss} is derived by means of (16):

$$k_{\text{diss}} = 2\frac{\mu a}{l^2} l_{\text{eff}} = \begin{cases} 2\mu a/l & (L \gg l) \\ 2\mu a L/l^2 & (L \ll l) \end{cases} \,. \tag{37}$$

At first sight it might be surprising that the dissociation rate is reduced to the same extent as the association rate is enhanced, $k_{\text{ass}} \sim l$, $k_{\text{diss}} \sim l^{-1}$ $(L \gg l)$ so that the equilibrium constant

$$s_0/n_0 = k_{\text{ass}}/k_{\text{diss}} \sim l^2 \tag{38}$$

seems to depend on a geometrical factor. This is not in contradiction, however, to s_0/n_0 being the product of two equilibrium constants,

$$s_0/n_0 = \frac{k_{\text{in}}}{\mu a} l^2 = \frac{k_{\text{in}}}{\lambda} \cdot \frac{D_1}{\mu a} = \frac{u_0}{n_0} \cdot \frac{s_0}{u_0} \tag{39}$$

The point is that a modification of these equilibria by means of λ or D_1 may be expressed in geometrical terms because $\sqrt{D_1/\lambda}$ is a length with a well defined physical meaning.

3.2. *Concentration fixed at $r = R$ and $z = \pm L$*

For small chain lengths $2L$ it is certainly not realistic to have so markedly different boundary conditions at $r = R$ and $z = \pm L$. We therefore proceed to calculating k_{ass} for the more interesting case $n = n_0$ everywhere on the outer boundary. From now on we shall only consider association because the dissociation rate can be inferred from k_{ass} and the equilibrium constant. The index "ass" will henceforth be omitted.

We have to solve Laplace's equation

$$\left(\frac{\partial^2}{\partial z^2} + \frac{1}{r}\frac{\partial}{\partial r} r \frac{\partial}{\partial r}\right) n^{\text{in}}(r,z) = 0 \,, \tag{40}$$

with boundary conditions

$$n^{\text{in}}(r, \pm L) = n_0, \quad \partial n^{\text{in}}/\partial z\,|_{z=0} = 0,$$

$$n^{\text{in}}(R, z) = n_0, \quad n^{\text{in}}(b, z) = 0 \,. \tag{41}$$

The solution may be expanded in the following way:

$$n^{\text{in}}(r,z) = n_0 + \sum_{n=0}^{\infty} A_n\, y(k_n r) \cos k_n z \,, \tag{42}$$

where

$$k_n = (n + \tfrac{1}{2})\pi/L \tag{43}$$

in order to satisfy the first two boundary conditions, and

$$y(k_n r) = I_0(k_n r)\, K_0(k_n R) - I_0(k_n R)\, K_0(k_n r) \tag{44}$$

is a suitable combination of Bessel functions for the third boundary condition to be fulfilled ($y(k_n R) = 0$). Imposing $n^{\text{in}}(b, z) = 0$ upon (42) and using the orthogonality relation

$$\int_0^L \cos k_n z \cos k_m z \,\mathrm{d}z = L\delta_{nm}/2$$

finally determines the A_n. The result is

$$n^{\text{in}}(r,z) = n_0 \left(1 - \frac{2}{L} \sum_{n=0}^{\infty} \frac{(-1)^n}{k_n} \frac{y(k_n r)}{y(k_n b)} \cos k_n z\right), \tag{45}$$

which immediately gives the flux onto the chain

$$2\pi b D_3 \left.\frac{\partial n}{\partial r}\right|_b$$

$$= 2\pi D_3 n_0 \frac{2}{L} \sum_{n=0}^{\infty} \frac{(-1)^n}{k_n} \frac{-k_n b\, y'(k_n b)}{y(k_n b)} \cos k_n z \,. \tag{46}$$

The equation for u must be solved with (46) as an inhomogeneity. The ansatz

$$u(z) = A \cosh \frac{z}{L} + B \sinh \frac{z}{L} + \sum_{n=0}^{\infty} C_n \cos k_n z \tag{47}$$

leads to

$$u(z) = -\frac{2\pi D_3}{\lambda} n_0 \frac{2}{L} \sum_{n=0}^{\infty} \frac{-k_n b\, y'(k_n b)}{y(k_n b)} \frac{1}{1 + (k_n l)^2} \times \left\{ l \frac{\sinh z/l}{\cosh L/l} - \frac{(-1)^n}{k_n} \frac{\cosh(L-z)/l}{\cosh L/l} + \frac{(-1)^n}{k_n} \cos k_n z \right\} . \tag{48}$$

The association rate is obtained from $\partial u/\partial z\,|_0$, according to (3)

$$k_{\text{ass}} = 4\pi D_3\, l \tanh\frac{L}{l}\cdot\frac{2}{L}$$
$$\times \sum_{n=0}^{\infty} \frac{(-1)^n}{k_n} \frac{1}{1+(k_n l)^2} \frac{-k_n b\, y'(k_n b)}{y(k_n b)} \left(1 + \frac{(-1)^n k_n l}{\sinh L/l}\right). \tag{49}$$

This somewhat cumbersome expression has been evaluated numerically. The L-dependence is displayed in fig. 2 where comparison with the earlier result (31) is made. At small chain lengths, $L \lesssim l$, the rate (49) is higher due to contributions from the cylinder's top and bottom. For $L \gg l$, on the other hand, the two formulae both lead to $k_{\text{ass}} = 4\pi D_3\, l/\ln(R/b)$. In order to derive this from (49) note that $-k_n b\, y'(k_n b)/y(k_n b)$ tends to $1/\ln(R/b)$ for $L \to \infty$, and

$$\lim_{L\to\infty} \frac{2}{L} \sum_{n=0}^{\infty} \frac{(-1)^n}{k_n} \frac{1}{1+(k_n l)^2} = 1\,. \tag{50}$$

Although (49) may appear somewhat more realistic than (31) it is nevertheless not entirely satisfactory for small chain lengths $L \lesssim l$. The reason is that near the ends, $z = \pm L$, the external concentration is artificially held at the high value n_0. Thus (49) is expected to overestimate k_{ass} and the true value will be somewhere in between (31) and (49). The outer boundary is nowhere too close to the chain if spheroidal coordinates are used to solve the problem.

4. Spheroidal geometry

The main advantage of spheroidal coordinates is to allow for a smooth transition from spherical to cylindrical problems (prolate spheroids) or from spherical to discoidal situations (oblate spheroids). We are interested here in the prolate case, and represent the chain of length $2L$ and radius b by an ellipsoid which has rotational symmetry with respect to the z-axis. Its foci are located at $z = \pm(L^2 - b^2)^{1/2} =: \pm a/2$, and we shall consider the set of all spheroids that have the same foci. These are labelled by the coordinate ξ such that their long half axis is $a\xi/2$ and the small one $a(\xi^2 - 1)^{1/2}/2$. In particular, since our chain has small half axis b, it is characterized by

$$\xi_b = L/(L^2 - b^2)^{1/2} \tag{51}$$

which is close to 1 for DNA-like molecules. The outer surface may be any spheroid $\xi_0 > \xi_b$. The case $\xi_0 \to \infty$ presents no such difficulty as $R \to \infty$ would in the cylindrical arrangement.

The two remaining spheroidal coordinates are η and φ, each η denoting one sheet of a hyperboloid of revolution, φ the azimuthal angle. The complete relations between cartesian and spheroidal coordinates are

$$x = \tfrac{1}{2}a((\xi^2 - 1)(1 - \eta^2))^{1/2} \cos\varphi\,, \tag{52a}$$

$$y = \tfrac{1}{2}a((\xi^2 - 1)(1 - \eta^2))^{1/2} \sin\varphi\,, \tag{52b}$$

$$z = \tfrac{1}{2}a\,\xi\eta \tag{52c}$$

The Laplace equation for the incoming part now reads

$$\frac{4}{a^2(\xi^2 - \eta^2)} \frac{\partial}{\partial\xi}(\xi^2 - 1)\frac{\partial}{\partial\xi} n^{\text{in}}(\xi) = 0\,. \tag{53}$$

Its solution satisfying $n^{\text{in}}(\xi_b) = 0$ and $n^{\text{in}}(\infty) = n_0$ is

$$n^{\text{in}}(\xi) = n_0 \ln\left(\frac{\xi - 1}{\xi_b - 1}\cdot\frac{\xi_b + 1}{\xi + 1}\right)\Big/\ln\left(\frac{\xi_b + 1}{\xi_b - 1}\right). \tag{54}$$

The flux onto the ring between η and $\eta + \mathrm{d}\eta$, of the spheroid ξ_b, is

$$D_3 \int_0^{2\pi} |\nabla n^{\text{in}}(\xi)| \tfrac{a}{2}((\xi^2 - 1)(1 - \eta^2))^{1/2}\, \mathrm{d}\varphi$$
$$= \frac{4\pi D_3 n_0}{\ln(\xi_b + 1)/(\xi_b - 1)} \left(\frac{1 - \eta^2}{\xi_b^2 - \eta^2}\right)^{1/2}. \tag{55}$$

The diffusion equation for the unspecifically bound particles therefore reads as follows:

$$\left\{\frac{4D_1}{a^2}\left(\frac{1 - \eta^2}{\xi_b - \eta^2}\right)^{1/2} \frac{\mathrm{d}}{\mathrm{d}\eta}\left(\frac{1 - \eta^2}{\xi_b^2 - \eta^2}\right)^{1/2} \frac{\mathrm{d}}{\mathrm{d}\eta} - \lambda\right\} u(\eta)$$
$$= -\frac{4\pi D_3 n_0}{\ln(\xi_b + 1)/(\xi_b - 1)} \left(\frac{1 - \eta^2}{\xi_b - \eta^2}\right)^{1/2}. \tag{56}$$

No approximation has so far been made. It appears, however, that solving (56) for arbitrary ξ_b is a difficult task. We thus confine ourselves to the limit of long rods, $\xi_b \approx 1$ or $b \ll L$, which implies $a/2 \approx L$, $\xi_b - 1 \approx b^2/2L^2$. With $l \equiv \sqrt{D_1/\lambda}$ as before, the equation for u reduce to

$$\left\{\frac{\mathrm{d}^2}{\mathrm{d}\eta^2} - \left(\frac{L}{l}\right)^2\right\} u = -\frac{2\pi D_3 n_0 L^2}{D_1 \ln 2L/b}\,. \tag{57}$$

The solution that vanishes at $\eta = 0$ and is regular at

$\eta = 1$, $\partial u/\partial\eta|_{\eta=1} = 0$, is very similar to (29):

$$u(\eta) = \frac{2\pi D_3 n_0}{\lambda \ln 2L/b}\left(1 - \frac{\cosh L(1-\eta)/l}{\cosh L/l}\right). \tag{58}$$

Finally, the association rate is given by $(2/n_0)D_1 \nabla u|_{\eta=0}$ and reads

$$k_{\text{ass}} = \frac{4\pi D_3}{\ln 2L/b}\, l \tanh L/l\,. \tag{59}$$

This result coincides with (31) if the somewhat arbitrary distance R is chosen to be the chain's total length $2L$. The L-dependence of (59) is slightly different from that in (31) or (49): the logarithm takes into account to what extent a rod is not as good a target, for a diffusing particle, as a disc or a sphere. Fig. 2 compares the three formulae: For $2L \lesssim R$, the spheroidal result (59) interpolates between the two expressions (31) and (49), as expected. For longer chains (59) predicts a decrease in k_{ass} which becomes noticeable for $L \gg l$ because $\ln(2L/b) = \ln(2l/b) + \ln L/l$. As the chain grows in length it steadily extends its range to farther distances, thereby decreasing the average flow onto a piece of given length. This did not occur in the cylindrical arrangements because the total particle number was increased and R was held constant as L became larger.

5. Transient behaviour

So far we have only investigated various stationary states of the diffusion equation. The question now is how fast will these steady states of flow build up from given initial conditions. During this transient process the rate constants must be considered as time dependent [11], and it is important to know if this will affect the experimental observations.

In order to analyze the problem we solve eqs. (5) and (6) for the general time dependent case, the initial condition being a homogeneous distribution of particles outside the chain, and no particles bound:

$$n(r, t=0) = n_0, \quad u(z, t=0) = 0 \tag{60}$$

There is of course some arbitrariness in this choice but the ensuing time constants are expected to be of the same order of magnitude for all reasonable initial conditions.

The equations will be solved by means of a Laplace transformation. In the following we shall denote the Laplace transform of a quantity $f(t)$ by $\widetilde{f}(s)$:

$$\widetilde{f}(s) = \int_0^\infty e^{-st} f(t)\,dt\,. \tag{61}$$

Two cases will be treated. First and for simplicity we shall analyze the case of mixed boundary conditions, as in sect. 3.1. In sect. 5.2. we then consider a reflecting boundary, in order to allow for comparison with the work of Berg and Blomberg [7]. The stationary state in this case is trivial, $n(r) \equiv 0$, so that the transient behaviour is really its only interesting aspect.

5.1. Mixed boundary conditions

The incoming part $n^{\text{in}}(r, t)$ obeys the following equations:

$$\left(D_3 \frac{1}{r}\frac{d}{dr} r \frac{d}{dr} - s\right)\widetilde{n}^{\text{in}}(r, s) + n_0 = 0\,, \tag{62a}$$

$$\widetilde{n}^{\text{in}}(r=b) = 0, \quad \widetilde{n}^{\text{in}}(r=R) = n_0/s\,. \tag{62b}$$

It is easily verified that the solution is

$$\widetilde{n}^{\text{in}}(r, s) = \frac{1}{s} n_0 \left(1 - \frac{y(qr)}{y(qb)}\right), \tag{63}$$

where y is defined in (44) and q is a wave number defined by

$$q^2 = s/D_3\,. \tag{64}$$

The flux onto the chain is readily determined from (63), and the equation for the unspecifically bound particles turns out to be

$$D_1 \frac{d^2\widetilde{u}}{dz^2} - (\lambda + s)\widetilde{u} - \frac{2\pi n_0}{q^2}\frac{qb\, y'(qb)}{y(qb)} = 0\,. \tag{65}$$

The solution showing the usual behaviour at $z = 0$ and $z = L$ is

$$\widetilde{u}(z, s) = \frac{2\pi D_3 n_0}{s(\lambda + s)}\frac{-qb\, y'(qb)}{y(qb)}\left(1 - \frac{\cosh(L-z)/l_s}{\cosh L/l_s}\right) \tag{66}$$

where

$$l_s \equiv \left(\frac{D_1}{\lambda + s}\right)^{1/2}. \tag{67}$$

The Laplace transformed rate constant finally is

$$\tilde{k}_{\text{ass}}(s) = \frac{1}{s} 4\pi D_3 \frac{-qb\, y'(qb)}{y(qb)} l_s \tanh L/l_s \,. \tag{68}$$

This looks very similar to expression (31), and indeed for long times $t \to \infty$ we expect $k_{\text{ass}}(t)$ to approach the steady state result k_{ass} (31). For the Laplace transform this means that $\lim_{s\to 0} \tilde{k}_{\text{ass}}(s) = k_{\text{ass}}/s$ should hold. Noting that $l_s \to l$ and $-qb\, y'(qb)/y(qb) \to 1/\ln(R/b)$ we confirm this expectation.

The formula (68) contains the whole time dependence of $k_{\text{ass}}(t)$ in disguised form. Since the explicit representation requires a difficult inverse transformation we shall content ourselves with a typical time τ which is the mean time for relaxation to the steady state and is defined as follows:

$$k_{\text{ass}} \cdot \tau = \int_0^\infty (k_{\text{ass}}(t) - k_{\text{ass}})\,\mathrm{d}t \,, \tag{69}$$

i.e. we assume that the deviation $k_{\text{ass}}(t) - k_{\text{ass}}$ is typically of the order of the stationary rate k_{ass} itself. In terms of Laplace transforms this reads

$$k_{\text{ass}} \cdot \tau = \lim_{s\to 0} (\tilde{k}_{\text{ass}}(s) - k_{\text{ass}}/s) \tag{70}$$

and expanding (68) to the appropriate order of s we find

$$\tau = \frac{R^2 - b^2}{4D_3 \ln R/b} - \frac{b^2(1 + \ln R/b)}{2D_3} - \frac{1}{2\lambda}\left(1 - \frac{L/l}{\sinh L/l \cosh L/l}\right), \tag{71}$$

which for $R \gg b$ takes on the simpler form

$$\tau \approx \frac{(R/2)^2}{D_3 \ln R/b} - \frac{1}{2\lambda}\left(1 - \frac{2L/l}{\sinh 2L/l}\right) \equiv \tau_1 - \tau_2 \,. \tag{72}$$

We see that there are two times involved. The first, τ_1, is essentially the time it takes for an average particle, located at a distance $R/2$, to find the chain by diffusion. The second time τ_2 is the mean residence time of the unspecifically bound particles; for long chains $L \gg l$ it reduces to $1/2\lambda$ whereas for $l \gg L$ it reads $L^2/3D_1$ which is the time the particle needs to find the trap and disappear. The minus sign in (72) means that when τ_2 is the dominant time the mean excess current $n_0 \int_0^\infty (k_{\text{ass}}(t) - k_{\text{ass}})\mathrm{d}t$ is directed outward; it is then easier for the particles to reach the outer cylinder wall at R than to find the sink at $z = 0$.

The order of magnitude of τ_1 and τ_2 depends on the experimental conditions. For $R \sim 10^4$ Å, $b \sim 10$ Å, $D_3 \sim 10^{-7}$ cm^2/s which might apply to the repressor-operator association, we get $\tau_1 \sim 10^{-2}$ s. From the unspecific binding constant [6] it may be estimated that τ_2 is of a similar order of magnitude, although it should depend on the salt concentration. The experimental determination of k_{ass} [12], on the other hand, involves seconds or minutes which shows that the steady state assumption usually is well justified.

5.2. Reflecting boundary conditions

In their "closed cell approach", Berg and Blomberg [7] have considered the case of impenetrable outer cylinder walls. This implies that every particle initially present will sooner or later disappear in the sink at $z = 0$. The steady state $n(r) \equiv 0$ does not reveal anything about the association kinetics. It is therefore inevitable to consider the time dependence to get any nontrivial result. This is obviously not a straightforward way to derive the rate constants but it does present a possibility.

The main difference to the preceding calculations is that the boundary condition $\partial n/\partial r|_{r=R} = 0$ requires both n^{in} and n^{refl} to be determined as

$$\partial n^{\text{in}}/\partial r|_R = -\partial n^{\text{refl}}/\partial r|_R \,. \tag{73}$$

This makes the computation very cumbersome. In fact, it is convenient to first solve the equation for n^{refl} which vanishes at $t = 0$ so that according to (9), and after Laplace transformation, we have

$$(D_3\nabla^2 - s)\tilde{n}^{\text{refl}} = 0, \quad \tilde{n}^{\text{refl}}|_R = \partial\tilde{n}^{\text{refl}}/\partial z|_L = 0 \,,$$
$$2\pi b D_3\, \partial\tilde{n}^{\text{refl}}/\partial r|_b = -\lambda\tilde{u} \,. \tag{74}$$

The formal solution is

$$\tilde{n}^{\text{refl}} = \frac{\lambda}{2\pi D_3 L} \sum_{n=-\infty}^{\infty} \frac{y(K_n r)}{-K_n b\, y'(K_n b)} \tilde{u}_n \cos k_n z \,, \tag{75}$$

where

$$k_n = n\pi/L, \quad K_n^2 = k_n^2 + s/D_3 = k_n^2 + q^2 \tag{76}$$

and

$$\tilde{u}_n \equiv \int_0^L \tilde{u}(z, s) \cos k_n z\, \mathrm{d}z. \tag{77}$$

has yet to be determined from the equation for u. Next, however, we calculate n^{in} which fulfils

$$(D_3\nabla^2 - s)\tilde{n}^{\text{in}} + n_0 = 0, \quad \tilde{n}^{\text{in}}|_b = \left.\frac{\partial \tilde{n}^{\text{in}}}{\partial z}\right|_L = 0 \quad (78)$$

and the boundary condition (73). Using (75) we find

$$\tilde{n}^{\text{in}}(r, s) = \frac{n_0}{s}\left(1 - \frac{K_0(qr)}{K_0(qb)}\right) - \frac{n_0}{s}\frac{K_1(qR)}{x'(qR)}\frac{x(qr)}{K_0(qb)}$$
$$+ \frac{\lambda}{2\pi D_3 L}\sum_{n=-\infty}^{\infty} \varphi_n(s)\, x(K_n r)\tilde{u}_n \cos k_n z\,, \quad (79)$$

where $x(K_n r)$ is a combination of Bessel functions that vanishes at $r = b$ (cf. (44) for y),

$$x(K_n r) = I_0(K_n r)K_0(K_n b) - I_0(K_n b)K_0(K_n r)\,, \quad (80)$$

and $\varphi_n(s)$ is an abbreviation for

$$\varphi_n(s) = \frac{1}{K_n b\, y'(K_n b)}\,\frac{1}{K_n R\, x'(K_n R)}$$
$$= \frac{x'(K_n b)\, y'(K_n R)}{y'(K_n b)\, x'(K_n R)}\,. \quad (81)$$

From (79) we derive the flux onto the chain

$$2\pi b D_3 \left.\frac{\partial \tilde{n}^{\text{in}}}{\partial r}\right|_b = n_0\psi(s) + \frac{\lambda}{L}\sum_{n=-\infty}^{\infty} \varphi_n(s)\tilde{u}_n \cos k_n z \quad (82)$$

with $\psi(s)$ standing for

$$\psi(s) = \frac{2\pi D_3}{s K_0(qb)}\left(qb\, K_1(qb) - \frac{K_1(qR)}{x'(qR)}\right). \quad (83)$$

Inserting (82) into the equation for u,

$$D_1\frac{\mathrm{d}^2\tilde{u}}{\mathrm{d}z^2} - (\lambda + s)\tilde{u} = -2\pi b D_3 \left.\frac{\partial \tilde{n}^{\text{in}}}{\partial r}\right|_b\,, \quad (84)$$

multiplying by $\cos k_n z$ and integrating we find

$$(\lambda + s)\tilde{u}_n = \frac{n_0\psi(s)L\delta_{n,0} - D_1\,\mathrm{d}\tilde{u}/\mathrm{d}z\,|_0}{1 + (k_n l_\mathrm{s})^2 - (l_\mathrm{s}/l)^2\varphi_n(s)} \quad (85)$$

where l_s has been defined in (67). The complete integration of (84) gives

$$\tilde{u}(z, s) = \frac{n_0\psi(s)}{\lambda + s}\left(1 - \frac{\cosh(L - z)/l_\mathrm{s}}{\cosh L/l_\mathrm{s}}\right) + \frac{\lambda}{L(\lambda + s)}$$
$$\times \sum_{-\infty}^{\infty} \frac{\varphi_n(s)}{1 + (k_n l_\mathrm{s})^2}\tilde{u}_n\left(\cos k_n z - \frac{\cosh(L - z)/l_\mathrm{s}}{\cosh L/l_\mathrm{s}}\right). \quad (86)$$

The flux into the sink at $z = 0$ is therefore

$$D_1 \left.\frac{\mathrm{d}\tilde{u}}{\mathrm{d}z}\right|_0$$
$$= \left(n_0\psi(s) + \frac{\lambda}{L}\sum_{-\infty}^{\infty}\frac{\varphi_n(s)}{1 + (k_n l_\mathrm{s})^2}\tilde{u}_n\right) l_\mathrm{s} \tanh L/l_\mathrm{s}\,. \quad (87)$$

Finally we substitute (85) for $\tilde{u}_n$ and after rearrangement obtain the time dependent association rate as

$$\tilde{k}_{\text{ass}}(s) = 2L\psi(s)\Bigg[1 + 2(1 - (l_\mathrm{s}/l)^2\varphi_0(s))$$
$$\times \sum_{n=1}^{\infty}\frac{1}{1 + (k_n l_\mathrm{s})^2 - (l_\mathrm{s}/l)^2\varphi_n(s)}\Bigg]^{-1}. \quad (88)$$

This resembles very much the formula obtained by Berg and Blomberg [7] except that here we do not use their artificial length a. As in the previous section, we now investigate the behaviour at small s:

$$\tilde{k}_{\text{ass}}(s) = \frac{\phi}{n_0}(1 - \tau \cdot s + \ldots)\,. \quad (89)$$

No contribution $\sim s^{-1}$ appears because of the trivial steady state. ϕ is the integrated flux into the sink, $\phi = 2\int_0^\infty D_1\,\mathrm{d}u/\mathrm{d}z\,|_0\,\mathrm{d}t$, and indeed we derive from (88)

$$\phi = 2L\psi(0)n_0 = 2L\pi(R^2 - b^2)n_0\,, \quad (90)$$

which is the total number of particles initially present in the cylinder. The time constant τ requires somewhat more computation. For $\lambda = 0$ the unspecific association is rate limiting and we get $\tau = \tau_0 + L^2/3D_1$

$$\tau_0 = \frac{R^4 \ln R/b}{2D_3(R^2 - b^2)} - \frac{3R^2 - b^2}{8D_3}$$
$$\approx \frac{R^2 \ln R/b}{2D_3} \quad (R \gg b)\,. \quad (91)$$

τ_0 is the time it takes for a typical particle to find the chain. Berg and Blomberg argue that the phenomenological association rate should be identified as $1/n_0\tau_0$ with $2L\pi R^2 = 1/n_0$, i.e. R is determined such that the cylinder comprises one particle. Inserting this into (91) we find $k_{\text{ass}} = 4\pi D_3 L/\ln(R/b)$ which is indeed the unspecific association rate (28) taken for the total length of the chain.

In the general case $\lambda \neq 0$ the typical time is

$$\tau = \tau_0 + \left(\frac{1}{\lambda} + \frac{R^2 - b^2}{2D_3} \ln \frac{R}{b}\right)\left(\frac{L}{l \tanh L/l} - 1 + M\right)$$
$$\approx (L/l_{\text{eff}} + M)\tau_0 + (L/l_{\text{eff}} + M - 1)/\lambda \quad (R \gg b)\,, \tag{92}$$

where l_{eff} is the same as in (31) and

$$M = 2 \sum_{n=1}^{\infty} \frac{1}{1 + (k_n l)^2} \frac{\varphi_n(0)}{1 + (k_n l)^2 - \varphi_n(0)} \tag{93}$$

is a number which depends on R, b, L, and l. The interpretation of (92) is obvious: before getting to the sink at $z = 0$ the particles has to make $N = L/l_{\text{eff}} + M$ trials on the average, and $N - 1$ times it gets released from the chain. Since l_{eff} is the distance that the particle covers each time it is unspecifically bound, L/l_{eff} is indeed the minimum number of trials to be expected. Assuming $M \lesssim 1$ and $L \gg l$, as Berg and Blomberg do, their argument leads to the association rate (31). This assumption, however, requires $R \gtrsim L$; for long rod shape cylinders, $b \ll R \ll L$, we have $\varphi_n(0) \approx 1$ and $M \approx 1/3(L/l)^2$. In this case the particle's fate may be described as a random walk along the cylinder, in steps of length l, each step taking the time $\tau_0 + 1/\lambda$. A connection between the total time τ and the phenomenological association rate k_{ass} is then no more obvious. Considering this and the fact that the results of these transient calculations do not provide more information than the simple models dealt with in sect. 3 and 4 we conclude that the use of time dependent situations to derive rate constants is not only quite awkward but also not generally applicable. A real need for considering the transient behaviour only arises when the steady state assumption is to be justified (as e.g. in [11]).

6. Summary and discussion

A detailed investigation has been presented of a particular kind of catalysis, namely rate enhancement due to guided diffusion. The target of a diffusing particle is assumed to be part of a string for which that particle has a general affinity, the unspecific attachment being loose enough, however, to allow for motion along the string. In such a case the particle can use the efficiency of one-dimensional diffusion to more easily find its target. This mechanism becomes the more pronounced the higher the diffusion constant D_1 and the lower the unspecific dissociation rate λ; it may be conveniently characterized in terms of a length $l \equiv \sqrt{D_1/\lambda}$ which is for large strings the effective range of the target and determines the cross section for an incoming particle.

By that means nature may overcome the upper limit set to the association rate by diffusion in three-dimensional space [17]. However, the effective range l may not be pushed arbitrarily high by simply reducing λ and enlargening D_1. As for other catalytic mechanisms, e.g. in enzymic reactions [17], there has to be a balance between the affinity (i.e. λ) and the mobility (i.e. D_1) of the particle on the string, giving an upper limit to l. The following schematic picture illustrates this situation from an energetic viewpoint (fig. 4). Let $-g_{\text{u}}$ and $-g_{\text{s}}$ be the free energies per particle upon unspecific and specific association. The equilibrium is then characterized by the constant

$$K = k_{\text{ass}}/k_{\text{diss}} = e^{g_{\text{s}}/kT} \tag{94}$$

For the various rates we have to consider the corresponding energy barriers. Since we assume diffusion controlled reactions throughout, these barriers are directly related to the diffusion constants, $D_3 \sim \exp(-\Delta_3/kT)$, $D_1 \sim \exp(-\Delta_1/kT)$. The reverse rates are accordingly $\lambda \sim \exp(-(g_{\text{u}} + \Delta_3)/kT)$, $\mu \sim \exp(-(g_{\text{s}} - g_{\text{u}} + \Delta_1)/kT)$. The main result of our investigations was $k_{\text{ass}} \sim D_3\sqrt{0_1/\lambda}$ or

$$k_{\text{ass}} \sim \exp((g_{\text{u}} - \Delta_1 - \Delta_3)/2kT)\,. \tag{95}$$

Let us put $g_{\text{u}} = xg_{\text{s}}$, and vary x between 0 and 1. Assume $\Delta_3 \ll \Delta_1$ so that Δ_3 may be neglected. How does the diffusion barrier Δ_1 depend on x? For $x = 0$ there is obviously $\Delta_1 \to \Delta_3 \approx 0$; for $x = 1$ the unspecific binding would be as tight as the specific, thus any jump along the chain should be as difficult as total dissociation, $\Delta_1 \approx g_{\text{s}}$. Let us therefore assume $\Delta_1 = x^{\nu} g_{\text{s}}$. This gives $k_{\text{ass}} \sim l \sim \exp((x - x^{\nu})g_{\text{s}}/2kT)$ which has a maximum at $x^{\nu-1} = 1/\nu$. The simplest conceivable case $\nu = 2$ yields $x = 1/2$ and $l_{\max} \sim \exp(g_{\text{s}}/8kT)$ for the maximum possible effective range. This would imply

$$\lambda \sim K^{-1/2}, \quad D_1 \sim K^{-1/4}, \quad l_{\max} \sim K^{1/8}\,. \tag{96}$$

With $K \approx 10^{12}$ the optimal unspecific binding constant D_3/λ should be 10^6, D_1 would be three orders of magnitude smaller than D_3, and the rate enhancement would be by a factor of 30 (assuming that the

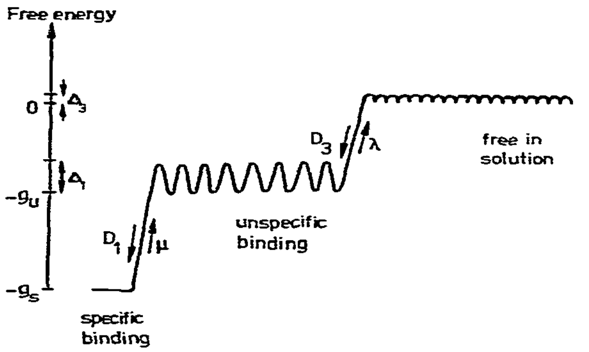

Fig. 4. Free energy scheme of diffusing particle. The equilibrium distribution between the three possible states is given by the free energies 0, g_u, g_s. The kinetic parameters depend on the height of the energy barriers Δ_1 and Δ_3, see text. Δ_1 must be expected to increase as $-g_u$ is lowered.

typical step size of the random walk along the chain equals the extent of the specific binding site). These figures do not appear unreasonable for the *lac* repressor-operator system indicating that evolution has indeed operated to maximize the association rate while at the same time retain specificity.

We have calculated k_{ass} for three different arrangements, two cylindrical ones and a spheroidal one. The results are given in (31), (49), and (59) respectively; they are graphically displayed in fig. 2. Comparison shows that there are no pronounced effects to be expected from changing the boundary conditions at the outer surfaces, the largest differences occuring for short chains, $L \lesssim l$. The most realistic formula has been derived using spheroidal coordinates:

$$k_{ass} = \frac{4\pi D_3}{\ln 2L/b}\, l \tanh L/l \,. \qquad (59)$$

It applies as long as $L \gg b$ and the chain is a straight line. For very short chains $L \approx b (\ll l)$, however, the discrepancies to the result for spherical shape, $4\pi D_3 L$, are hardly noticeable so that (59) has indeed a wide range of validity. An advantage with respect to the cylinder formulae (31) and (49) is that the somewhat arbitrary radius R is not needed here.

Two points must be taken into account before comparison with experiments can be made.

(i) For long chains the assumption of a linear shape will break down since DNA has a tendency to form random coils. This becomes effective when L exceeds the so called persistence length p which for DNA is of the order of 600 Å [13]. How is k_{ass} then modified? We need not worry about $l_{eff} \approx l$ because this is not appreciably greater than p in the cases of interest [3]. The logarithmic term, however, should be affected: according to the concluding remarks in sect. 4 and comparison with (31), the logarithm takes into account how far the total chain extends its influence into the bulk. This should generally be determined by the molecule's largest diameter D which replaces L in (59). For a random coil, if $2L = np$, we expect this diameter to be $2D \approx n^{0.6} p = (2L)^{0.6} p^{0.4}$ [14]. This yields

$$k_{ass} \approx \frac{4\pi D_3 l}{0.6 \ln 2L/b + 0.4 \ln p/b} \quad (L \gg p, l) \,, \qquad (97)$$

which has a slightly weaker L-dependence than (59).

(ii) For small DNA sizes it becomes necessary to account for an L-dependence of the diffusion constant D_3 [15]. The reason is that D_3 must be understood as the sum of the diffusion constants for the two reacting partners. As long as L is very large, the diffusion of the chain may be neglected but short DNA pieces may diffuse faster than the enzymes operating on them.

In addition to calculating k_{ass} for various steady state flow situations, following the von Smoluchowski–Debye–Eigen procedure, we have analyzed the time dependent diffusion equations in order to establish that the steady states are well attained within the times of typical experiments [12,16]. For cylindrical geometry it turned out that the two characteristic times are R^2/D_3 and $1/\lambda$. For spheroids we would again have to replace R by the maximum length $2L$, and for randomly coiled DNA the diameter $n^{0.6}p$ should be taken. The DNA used by Riggs et al. [12] had molecular weight 3×10^7 corresponding to $n \approx 300$ and gives relaxation times in the order of 10^{-3}–10^{-2} s which is quite small on the scale of minutes that is relevant in the filter binding experiments. Nonetheless it should be remarked that compared to the transient times associated with small molecules, $\lesssim 10^{-9}$ s [11], those related to macromolecules are considerably larger.

Electrostatic forces have only been mentioned in connection with unspecific binding, in sect. 2.3. The reason is that we consider their influence to be quite unspecific in reactions like the repressor-operator association. In fact, they are probably the main cause for the unspecific attraction of repressors to the chain, as suggested by the strong ionic strength dependence of the unspecific equilibrium, and we have shown how λ may be interpreted in electrostatic terms. Of course, the electrostatic contribution is also part of the specific binding energy but the difference between specific and unspecific attraction is certainly due to short range chemical forces.

The most obvious test for our formulae would consist in a series of experiments with operator-containing DNA segments of varying length $2L$. So far only the limiting cases $L \gg l$ [12] and $L \ll l$ [16] are available, and both agree well with our picture. The work of Riggs et al. [12] has already been discussed in ref. [3]. Let us now briefly comment on the recent work on synthetic *lac* operators [16], 21 resp. 26 base pairs long, with wild-type (SQ) and tight-binding (QX 86) *lac* repressors. At low ionic strength ($I = 0.05$ M) the association rates are $k_{\mathrm{ass}} = 2 \times 10^9\ \mathrm{M}^{-1}\ \mathrm{s}^{-1}$ for SQ and $3.3 \times 10^8\ \mathrm{M}^{-1}\ \mathrm{s}^{-1}$ for QX 86. The larger rate is well within the limits of what diffusion can achieve without electrostatic support. Taking a length $2L \approx 80$ Å, $b \approx 10$ Å, and $D_3 \approx 10^{-6}\ \mathrm{cm}^2/\mathrm{s}$ (which may even underestimate the actual diffusion constant of the small DNA segments) we get

$$k_{\mathrm{ass}} = \frac{2\pi \cdot 2D_3 \cdot 2L}{\ln 2L/b} \approx 3 \times 10^9\ \mathrm{M}^{-1}\ \mathrm{s}^{-1}$$

Therefore SQ association appears to be nearly diffusion controlled whereas QX 86 has to overcome a reaction barrier before association takes place. Electrostatic forces could do little to enhance the rates as determined by diffusion because shielding would definitely not allow their influence to extend further than some 10 Å. What they could do, however, is modify the reaction barrier for QX 86. This might indeed explain the different sensitivity of the two repressors with respect to salt concentration. At $I = 0.20$ M, the rate for SQ has only moderately decreased to $1 \times 10^9\ \mathrm{M}^{-1}\ \mathrm{s}^{-1}$ whereas $k_{\mathrm{ass}} = 4 \times 10^7\ \mathrm{M}^{-1}\ \mathrm{s}^{-1}$ for QX 86 indicates that with shielded electrostatic attraction the reaction barrier is quite high for the tight-binding repressor. With long operator-containing DNA these differences between SQ and QX 86 largely disappear which strongly points to the availability of a new more effective pathway, using unspecific binding as an intermediate state. Part of the explanation, however, may also be found in eqs. (34) and (35) which show that k_{ass} is much less sensitive to reaction barriers in the case of long chains than it is for short segments.

Appendix

A discrete model chain

Let us analyze the kinetics of the reaction system shown in fig. 5 where the chain is simulated as a sequence of N unspecific binding sites. Assuming that the concentrations $u_1, u_2, ..., u_N$ of unspecifically bound particles are all in detailed balance, we want to discuss how the specific association and dissociation rates k_{ass}, k_{diss},

$$n \underset{k_{\mathrm{diss}}}{\overset{k_{\mathrm{ass}}}{\rightleftharpoons}} s\,, \tag{A.1}$$

depend on N and the kinetic parameters k, λ, d, μ:

$$\begin{aligned} \frac{\partial s}{\partial t} &= -\frac{\partial n}{\partial t} = -\mu s + d u_1 \\ 0 &= \mu s - 2du_1 + du_2 \qquad - \lambda u_1 + kn \\ 0 &= du_{i-1} - 2du_i + du_{i+1} - \lambda u_i + kn \quad (i = 2, ..., N-1) \\ 0 &= du_{N-1} - du_N \qquad - \lambda u_N + kn \end{aligned} \tag{A.2}$$

A little algebra yields the following relations for the u_i:

$$\begin{aligned} du_1 &= \left(1 - \frac{\lambda}{d} z_N\right) \mu s + z_N kn \\ du_{i+1} &= \left(1 - \frac{\lambda}{d} z_{N-i}\right) du_i + z_{N-i} kn \quad (i = 1, ..., N-1) \end{aligned} \tag{A.3}$$

where the z_i are recursively given by

$$z_i = \frac{1}{\lambda/d + 1/(1 + z_{i-1})}, \quad z_0 = 0\,. \tag{A.4}$$

Inserting du_1 into the rate equation for s we get

$$\frac{\partial s}{\partial t} = -z_N \left(\frac{\lambda\mu}{d} s - kn\right). \tag{A.5}$$

As a first consequence we see that the equilibrium between n and s is independent of N as it should be:

$$s_0/n_0 = kd/\lambda\mu\,. \tag{A.6}$$

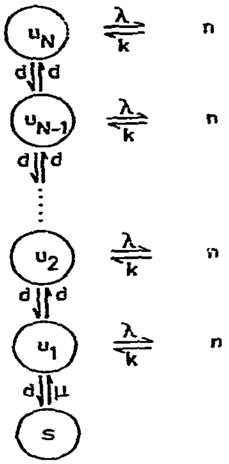

Fig. 5. Discrete reaction model for catalysis via unspecific binding. Free particles are denoted by n, unspecific binding places are $u_1, ..., u_N$, the specific target is s. The rates k and λ characterize the unspecific equilibrium, d the diffusion along the chain and μ the specific dissociation rate.

Given this equilibrium situation, the u_i are all equal to

$$u_0 = s_0/d = kn_0/\lambda\ . \tag{A.7}$$

The rates, on the other hand, are both modified by the N-dependent factor z_N:

$$k_{\mathrm{ass}} = z_N k, \quad k_{\mathrm{diss}} = z_N \lambda\mu/d \tag{A.8}$$

This factor is easily analyzed using relation (A.4). There are three ranges to be discussed:

(i) $N\lambda \ll d$. In this limit, (A.4) reduces to $z_i \approx 1 + z_{i-1}$ and therefore

$$z_N \approx N\ . \tag{A.9}$$

The rates are enhanced in proportion to the total chain length.

(ii) $d/N \ll \lambda \ll d$. In this range as well as in the third, the sequence $z_1, z_2, ...$ has converged for indices of the order of N which means that $z_N = z_{N-1}$. Inserting this into (A.4) we find

$$z_N = \tfrac{1}{2}(\sqrt{1 + 4d/\lambda} - 1)\ . \tag{A.10}$$

In the limit $\lambda \ll d$ this can be further simplified to yield

$$z_N \approx \sqrt{d/\lambda} \tag{A.11}$$

which is very reminiscent of the results for the continuous models: the effective range of the specific binding site is given by the distance the particle can diffuse during the time λ^{-1} it is unspecifically bound. Inserting (A.11) into (A.8) we find

$$k_{\mathrm{ass}} = \sqrt{d/2}\,k, \qquad k_{\mathrm{diss}} = \sqrt{\lambda/d}\,\mu\ . \tag{A.12}$$

(iii) $\lambda \gg d$. If dissociation from the chain is faster than diffusion between the sites then expansion of (A.10) shows

$$z_N \approx d/\lambda \tag{A.13}$$

which means that no rate enhancement due to unspecific binding occurs. This is of course an uninteresting case from our point of view.

This model is useful if we are looking for the boundary conditions at $z = 0$, in the continuous versions. For the transition to a continuous chain we have to introduce the molecular length a which is the distance between neighbouring binding sites:

$$L = Na, \quad D_1 = da^2, \quad u(z) = u_i/a \tag{A.14}$$

and take the limit $a \to 0, N \to \infty$.

Let us now consider the behaviour of u_1 and u_2, for the case $d \gg \lambda$. Using (A.3) we see that for association ($s \equiv 0$) the u_i increase linearly, $u_2 = 2u_1$, and that the flux into the trap is

$$\partial s/\partial t = du_1 = da(u(2a) - u(a)) \to D_1\, \partial u/\partial z\,|_0, \tag{A.15}$$

in accordance with eq. (3). For dissociation, on the other hand, $n \equiv 0$ and $u_2 \approx u_1 = \mu s/d$. Going over to the continuous limit we find

$$u(0) = (\mu/D_1)as\ , \tag{A.16}$$

which is the boundary condition (13).

The situation would be quite different in the limit $d \ll \lambda$. For $s \equiv 0$ (association) we would have $u_2 \approx u_1 \approx kn/\lambda$ whereas for $n \equiv 0$ (dissociation) the chain is completely devoid of particles, $u_i \equiv 0$.

Acknowledgements

We are grateful to Drs. M. Eigen, T.M. Jovin and H. Strehlow for stimulating discussions and to Mr. D. Hellwig for assistance in numerical calculations.

References

[1] G. Adam and M. Delbrück, in: Structural chemistry and molecular biology, eds. A. Rich and N. Davidson (Freeman, San Francisco, 1968).
[2] W. Goad, Biophys. Soc. Abstr. 12 (1972) 248a.
[3] P.H. Richter and M. Eigen, Biophys. Chem. 2 (1974) 255.
[4] H. Roberts and B. Hess, Biochim. Biophys. Acta 462 (1977) 215.
[5] S. Bourgeois and M. Pfahl, in: Adv. in protein chemistry 30, eds. C.B. Anfinsen, J.T. Edsall and F.M. Richards (Academic Press, New York, 1976).
[6] S. Lin and A.D. Riggs, J. Mol. Biol. 72 (1972) 671.
[7] O.G. Berg and C. Blomberg, Biophys. Chem. 4 (1976) 367.
[8] M. von Smoluchowski, Physik. Z. 17 (1916) 557, 585; Z. Physik. Chem. 92 (1917) 129.
[9] P. Debye, Trans. Electrochem. Soc. 82 (1942) 265.
[10] M. Eigen, Z. Phys. Chem. N.F. 1 (1954) 176.
[11] A. Weller, Z. Phys. Chem. N.F. 13 (1957) 335.
[12] A.D. Riggs, S. Bourgeois and M. Cohn, J. Mol. Biol. 53 (1970) 401.
[13] H. Eisenberg, Biopolymers 8 (1968) 545.
[14] P.G. de Gennes, Phys. Lett. 38A (1972) 339.
[15] T.M. Jovin, Ann. Rev. Biochem. 45 (1976) 889.
[16] D.V. Goeddel, D.G. Yansura and M.H. Caruthers, Proc. Natl. Acad. Sci. USA 74 (1977) 3292.
[17] M. Eigen, in: Quantum statistical mechanics in the natural sciences, eds. B. Kursunoglu, S.L. Mintz and S.M. Widmayer (Plenum Press, New York, 1974).