LENGTH DEPENDENCE OF RATE CONSTANTS FOR END-TO-END ASSOCIATION AND DISSOCIATION OF EQUILIBRIUM LINEAR AGGREGATES

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ABSTRACT A semi-quantitative analysis is given of the length dependence of the rate constant for association (annealing) of two long linear aggregates in solution. The equilibrium constant for this process, or its inverse (fragmentation or dissociation), is relatively easy to formulate from appropriate partition functions. From these two ingredients, the length dependence of the rate constant for spontaneous fragmentation can be deduced. Numerical examples are included.

INTRODUCTION

This paper is concerned with the length dependence of the rate constants for spontaneous breaking or end-to-end association of rodlike linear aggregates. These processes may be of importance for F-actin or microtubules. To the extent that they occur, they will have an effect on the kinetics of nucleation and aggregation, on the polymer length distribution at steady state, and on the rate of ATPase or GTPase activity.

It is generally assumed, incorrectly, that a linear aggregate in solution, at equilibrium, has a length distribution of the form $P_N \sim (c/c_e)^N$, where P_N is the probability that an aggregate contains N monomers, c is the concentration of free monomers, and c_e is the critical concentration. This distribution (1, 2) is appropriate for an immobile polymer molecule but not for one moving freely in solution, with translational and rotational degrees of freedom (3–5). In the immobile case, the canonical partition function of one N-mer has the form $C'j^N$ (1, 3), where C' is a constant and j is the partition function of a monomer in the polymer. From the partition function for an open polymer (N fluctuates), we then find (1) $P_N \sim (c/c_e)^N$.

For a freely mobile polymer, the partition function for one N-mer has the form $q_N = Cj^N N^n V$ (3, 5), where the factors $j^N V$ suffice for an infinite polymer, and CN^n includes all of the finite N effects. C is a composite of many constant factors, and N^n arises as follows. The translational partition function is proportional to mass$^{3/2}$, or $N^{3/2}$. The two-dimensional rotational partition function of a rigid rod is proportional to the moment of inertia, or to mass × length,^{2} or to N^3. The contribution from rotation about the axis of a rigid rod would depend on mass$^{1/2}$, or $N^{1/2}$. This exponent would be larger for a real rod that bends. So far these factors add up to N^5. The finite N part of the vibrational partition function of a strictly one-dimensional array or crystal of N particles is $N^{-1/2}$ (3). But the particles of a real linear crystal actually vibrate in three dimensions, not one. Furthermore, F-actin and, expecially, microtubules have structures more complicated than a single linear array of monomers. If, indeed, the finite N vibrational factor can be represented simply as an end effect (both ends), independent of the polymer length, then this factor would appear in C and not contribute to n. In summary, there is some uncertainty, but we might expect n to be between 4 and 6. This assumes, of course, that the N-mer has completely free motion in solution.

The atomic vibrations within a monomer are included in j. The missing nearest-neighbor interactions between monomers at the two ends of the polymer contribute to C; otherwise nearest-neighbor interactions are in j. Thus, for a freely mobile polymer, the above q_N, when used in the partition function for the open polymer, leads to the distribution function $P_N \sim N^n (c/c_e)^N$ (3, 4). In the immobile case, $n = 0$.

The above results have significant implications for the length dependence of the rate constants and equilibrium constant for spontaneous fragmentation (dissociation) and annealing (association) of these polymers. We consider only equilibrium polymers here. Fragmentation and annealing will not alter the length distribution of an equilibrium polymer, because this distribution is independent of kinetic mechanism. However, the length distribution of a steady-state polymer (4, 6) would be affected.

This topic is not included in the present paper. We further limit ourselves here to a consideration of the length dependence of rate and equilibrium constants; we do not attempt to calculate absolute values of these constants, though most of the ingredients for such a calculation are included.

The sequence of topics is the following. The dissociation equilibrium constant is deduced from partition functions; the association rate constant is treated approximately, as a diffusion problem; finally, the dissociation rate constant is deduced from the above two constants. Some numerical examples are included.

DISSOCIATION EQUILIBRIUM CONSTANT

We consider the concentration dissociation equilibrium constant for the process $N \rightleftharpoons N_1 + N_2$, where $N_1 + N_2 = N$. That is, an N-mer dissociates or fragments into two pieces, an N_1-mer and an N_2-mer. The equilibrium constant is (Eq. 10–6, reference 7)

$$K_{N_1N_2} = c_{N_1}c_{N_2}/c_N = (q_{N_1}/V)(q_{N_2}/V)/(q_N/V). \qquad (1)$$

As already mentioned, we have for an N-mer

$$q_N/V = Cj^N N^n. \qquad (2)$$

If a detailed model is adopted, C, j, and n can be given explicitly from a consideration of the inter-subunit interactions and the translational, rotational, and vibrational partition functions (vibration includes atoms within a subunit and subunits relative to each other). On substituting Eq. 2 into Eq. 1, we have

$$K_{N_1N_2} = C(N_1N_2/N)^n. \qquad (3)$$

This same result is found from $P_{N_1}P_{N_2}/P_N$, which quotient (see Eq. 1) is equal to $K_{N_1N_2}$ divided by the total polymer concentration. Eq. 3 predicts that the polymer is most likely to fragment in the middle, as illustrated in Fig. 1. In this example, $N = 1{,}000$ and $n = 5$. The dissociation constant $K_{N_1N_2}$ (normalized to unity at its maximum) is plotted against N_2, one of the fragment sizes (N constant). The curve has a Gaussian shape, symmetrical around the maximum at $N_2 = 500$. Note that if $n = 0$, $K_{N_1N_2}$ would be a constant.

Eq. 3 also predicts that a longer polymer is more likely to fragment. Fig. 2 shows how $K_{N_1N_2}$ varies with $N(= 2N_1)$ for breakage in the middle ($N_1 = N_2$), normalized to unity at $N = 1{,}000$. From Eq. 3, $K_{N_1N_1} \sim N^n$. If $n = 0$, $K_{N_1N_1}$ would be a constant.

ASSOCIATION RATE CONSTANT

In the equilibrium process under consideration, namely, $N \rightleftharpoons N_1 + N_2$, the second-order association rate constant is denoted $\beta_{N_1N_2}$ and the first-order dissociation rate constant is denoted $\alpha_{N_1N_2}$, so that

$$K_{N_1N_2} = \alpha_{N_1N_2}/\beta_{N_1N_2}. \qquad (4)$$

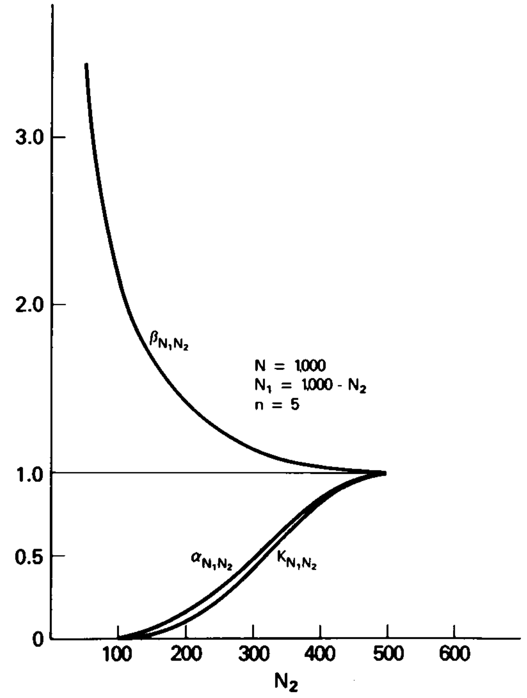

FIGURE 1 Plots of $\alpha_{N_1N_2}$ (dissociation), $\beta_{N_1N_2}$ (association), and $K_{N_1N_2}$ (dissociation equilibrium constant) as functions of N_2 in a numerical example with $n = 5$, $N = 1{,}000$, and all functions normalized to unity at the mid-point $N_2 = 500$, about which there is symmetry.

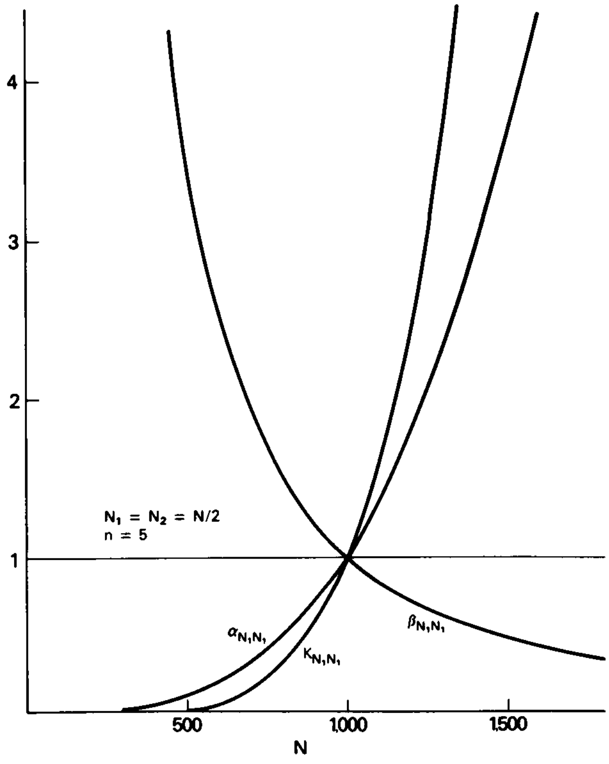

FIGURE 2 Plots of $\alpha_{N_1N_1}$ (dissociation), $\beta_{N_1N_1}$ (association), and $K_{N_1N_1}$ (dissociation equilibrium constant) as functions of N in a numerical example with $n = 5$, $N_1 = N_2 = N/2$ (i.e., symmetrical dissociation and association), and all functions normalized to unity at $N = 1{,}000$.

The association rate constant is easier to handle theoretically. With it available, the dissociation rate constant can then be found from Eqs. 3 and 4.

It is well-known (8–10) that the rate constant for a diffusion-controlled bimolecular association is $4\pi(D_1 + D_2)R$, where D_1 and D_2 are the diffusion coefficients of the two molecules, and R is the approach distance between molecular centers needed for association to occur. In the present problem (Fig. 3), when the inter-center distance, r, between an N_1-mer and an N_2-mer is R_{12}, we let p_{12} be the probability that the mutual orientation of the two linear polymers is suitable for joining. Then, as in earlier work (9), we approximate $\beta_{N_1N_2}$ by

$$\beta_{N_1N_2} = 4\pi(D_1 + D_2)R_{12}p_{12}. \qquad (5)$$

A more elaborate treatment would include explicit rotational as well as translational diffusion, and also the mutual orientational interference of the two long rods when $r < R_{12}$. R_{12} (Fig. 3) is equal to $(N_1 + N_2)/2$ multiplied by the axial length per subunit of the polymer. Thus, $R_{12} \sim N$. We turn now to a consideration of D_1, D_2, and p_{12}.

For a linear string of touching spheres of diameter d, the formula of Riseman and Kirkwood (11) reduces to

$$\begin{aligned} D_i &= (kT/N_i\zeta)\ln N_i \qquad (i = 1, 2) \\ \zeta &= 6\pi\eta(d/2), \end{aligned} \qquad (6)$$

where ζ is the friction constant for the monomer in the solvent and η is the viscosity of the solvent. Also, the formula of Perrin (12) reduces, for a very long ellipsoid of revolution (length N_id, diameter at the center d), to virtually the same result, except for small differences in numerical factors. Hence, we take $D_i \sim (\ln N_i)/N_i$.

To estimate p_{12}, we assume that in order for association to occur at R_{12} (Fig. 3), two conditions must be satisfied. The angle θ (Fig. 4 *a*) between the two long rods must be less than some small maximum value θ_m; and the ends of the two rods must be within some small distance δ of each other. Actually, it is simpler to modify these two conditions at R_{12} slightly, as indicated in Fig. 4 *b*; either end (I or II) of the N_1-mer must be within the circle of radius a (a is determined by θ_m); and the opposite end (II or I) of the N_2-mer must be within a distance δ of the end of the N_1-mer. Or, because of symmetry, the designations of the two N_i-mers may be reversed. Because θ_m is small, we have (Fig. 4 *a*)

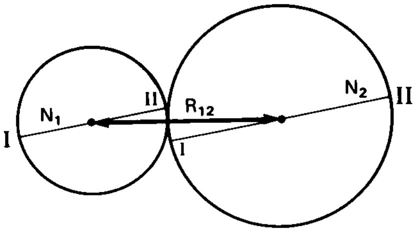

FIGURE 3 An N_1-mer and an N_2-mer with centers at the proper distance $r = R_{12}$ apart for association. The two ends of a polymer are different (I, II); association occurs with opposite ends only.

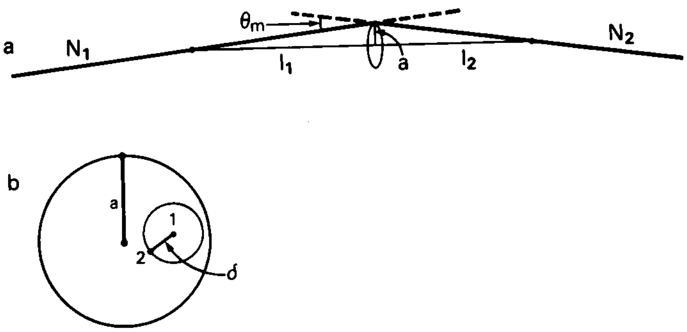

FIGURE 4 (*a*) Orientational notation for an N_1-mer and an N_2-mer with $r \simeq R_{12} \simeq l_1 + l_2$ (the angle θ_m is small). (*b*) The circle of radius a in *a* is seen here from along the line l_1, l_2, and enlarged. Points 1 and 2 are possible positions of the ends of the N_1-mer and N_2-mer. See text.

$$\begin{aligned} \theta_m &= (a/l_1) + (a/l_2) \\ a &= l_1l_2\theta_m/(l_1 + l_2). \end{aligned} \qquad (7)$$

Then the probability that either end of the N_1-mer is inside the circle of radius a is

$$p_1 = 2(\pi a^2)/4\pi l_1^2. \qquad (8)$$

The probability that the opposite end of the N_2-mer is inside the circle of radius δ is

$$p_2 = \pi\delta^2/4\pi l_2^2. \qquad (9)$$

Finally, from Eqs. 7–9,

$$p_{12} = p_1p_2 = \delta^2\theta_m^2/8(l_1 + l_2)^2, \qquad (10)$$

where δ and θ_m are constants that do not depend on the polymer lengths. Thus, $p_{12} \sim 1/N^2$. The treatment in Eqs. 5–10 is certainly not rigorous, but the essential features are introduced into the problem in a semi-quantitatively reliable way.

Using the above results, Eq. 5 becomes

$$\beta_{N_1N_2} \sim (N_2 \ln N_1 + N_1 \ln N_2)/N_1N_2N. \qquad (11)$$

The example in Fig. 1 shows that the association rate constant, $\beta_{N_1N_2}$, has a minimum at $N_1 = N_2$ ($\beta_{N_1N_2}$ is symmetrical about $N_1 = N_2$, and is normalized to unity at $N_2 = 500$ in Fig. 1). Note that n does not appear in Eq. 11 (but the translation and rotation of rigid rods are implicit in the derivation of Eq. 11). From Eq. 11, we see that the dependence of $\beta_{N_1N_1}$ on N at $N_1 = N_2$ (the minimum in Fig. 1) is

$$\beta_{N_1N_1} \sim N^{-2}\ln(N/2). \qquad (12)$$

This is illustrated in Fig. 2, with $\beta_{N_1N_1}$ normalized to unity at $N = 1{,}000$. The association rate constant decreases with increasing N.

DISSOCIATION RATE CONSTANT

From Eqs. 3, 4, and 11 we can now deduce

$$\alpha_{N_1N_2} \sim (N_1N_2)^{n-1}\,(N_2 \ln N_1 + N_1 \ln N_2)/N^{n+1}. \qquad (13)$$

The dependence of the dissociation rate constant, $\alpha_{N_1N_2}$, on N_2, at constant N is illustrated in Fig. 1; $\alpha_{N_1N_2}$ is approximately Gaussian, like $K_{N_1N_2}$, with a maximum value for a symmetrical break in the N-mer. $K_{N_1N_2}$ and $\alpha_{N_1N_2}$ are so similar in Fig. 1 because the N_2 dependence of $\beta_{N_1N_2}$ is really quite modest. If $n = 0$, $\alpha_{N_1N_2}$ has the same N_2 dependence as $\beta_{N_1N_2}$ in Fig. 1. (Incidentally, $\alpha_{N_1N_2}$ is practically independent of N_2 if $n = 0.75$.)

Fig. 2 includes the dependence of $\alpha_{N_1N_1}$ on N; $\alpha_{N_1N_1} \sim N^{n-2} \ln(N/2)$. If $n = 0$, the N dependence is the same as for $\beta_{N_1N_1}$ (Eq. 12). (If $n = 1.825$, $\alpha_{N_1N_1}$ is practically independent of N.)

DISCUSSION

It seems reasonable to expect that a steady-state polymer such as actin would fragment spontaneously like an equilibrium polymer, that is, following Eq. 13 with $n = 4$ to 6. The assumption that $\alpha_{N_1N_2}$ is a constant for actin (13) is therefore probably an oversimplification. The dependence on polymer length of rate constants for adding or losing one monomer at a time from the end of a polymer is very weak (3, 4). However, as shown here, this is not the case for annealing and fragmentation; both the total length of the polymer and the relative lengths of the two fragments make a significant difference to the rate constants. This is not to say, however, that the rate constants, $\alpha_{N_1N_2}$ and $\beta_{N_1N_2}$, are large enough in magnitude to be important; this question can be settled reliably at the present time only by experiments (13–15).

I am indebted to Drs. E. D. Korn and S. L. Brenner for a helpful discussion.

Received for publication 22 April 1983 and in final form 21 July 1983.

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