

Hydrodynamic Properties of Dilute Solutions of Ring Polymers. (II). Twisted Ring Polymers

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Sedimentation constants of twisted ring macromolecules are calculated by using the spring-bead model of the polymeric chain. In the limit of large hydrodynamic interaction, the sedimentation constant is found to be a monotonously increasing function of the degree of twisting. Its ratio to the sedimentation constant of a linear chain of the same molecular weight is 1.18 for the untwisted ring, 1.32 for the singly twisted ring, 1.40 for the doubly twisted ring, ... and it approaches to the asymptotic limit, 2.00, for the case of extremely high twisting. These values are found to be practically independent of the nature of solvent.

1. INTRODUCTION

In the preceding paper of this series, Fukatsu presented a theory of the hydrodynamic properties of flexible (untwisted) ring macromolecules, and showed that the ring:linear ratio of the sedimentation constant was 1.18 in the limit of large hydrodynamic interaction¹⁾. This result is in a rough agreement with the recent experimental values, 1.14 and 1.11, which have been reported on polyoma virus DNA²⁾ and human papilloma virus DNA,³⁾ respectively. However, according to these same experiments, the DNA molecules still occur in another form called the twisted ring in addition to the linear and untwisted ring forms, and the twisted ring:linear ratio of the sedimentation coefficient is found to be 1.44 for the polyoma virus DNA and 1.55 for the human papilloma virus DNA. Thus, this paper intends to calculate the theoretical sedimentation coefficient of a series of twisted ring molecules and to estimate the degree of twisting of these DNA molecules.

2. SEDIMENTATION COEFFICIENT OF A SINGLY TWISTED RING MOLECULE

Let us start with a linear flexible chain polymer consisting of $n+1$ segments, say 0, 1, ... n th segments, and assume the Gaussian probability density for each bond vector \mathbf{r}_k ,

$$\tau(\mathbf{r}_k) = (3/2\pi a^2)^{3/2} \exp[-(3/2a^2)\mathbf{r}_k^2]. \quad (1)$$

Let \mathbf{R}_{st} be the displacement vector between two segments s and t . Then, according to Fixman,⁴⁾ the trivariate Gaussian probability density of $\mathbf{V}_1 = \mathbf{R}_{0n}$, $\mathbf{V}_2 = \mathbf{R}_{ij}$ and $\mathbf{V}_3 = \mathbf{R}_{st}$ of the linear chain is given as

$$P_0(\mathbf{V}_1, \mathbf{V}_2, \mathbf{V}_3) = (3/2\pi a^2)^{9/2} |C|^{-3/2} \times \exp[-(3/2a^2 |C|) \sum_k \sum_l c^{kl} \mathbf{V}_k \mathbf{V}_l], \quad (2)$$

where c^{kl} is the cofactor of the element c_{kl} in the 3×3 determinant $|C|$, whose elements are

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$$\begin{aligned}
 c_{11} &= n, \quad c_{22} = c_{12} = c_{21} = j - i, \\
 c_{33} &= c_{13} = c_{31} = t - s, \\
 c_{23} = c_{32} &= \begin{cases} 0 & \text{for } 0 \leq s < t < i \text{ or } j \leq s < t < n \\ t - i & \text{for } 0 \leq s < i \leq t < j < n \\ j - i & \text{for } 0 \leq s < i < j \leq t < n \\ t - s & \text{for } 0 \leq i < s < t < j < n \\ j - s & \text{for } 0 \leq i \leq s < j < t < n. \end{cases} \quad (3)
 \end{aligned}$$

An untwisted ring molecule may be derived from the linear molecule by joining its O th segment with n th segment, or in other words by giving the zero value for \mathbf{R}_{on} . Then, by jointing another pair of segments, say i th with j th segment, we obtain a double ring molecule or the so-called singly twisted ring molecule. Thus, the probability density of \mathbf{R}_{st} of the double ring molecule can be readily derived from the above trivariate Gaussian function $P_0(\mathbf{V}_1, \mathbf{V}_2, \mathbf{V}_3)$ as

$$\begin{aligned}
 P_2(\mathbf{R}_{st}) &= NP_0(O_{on}, O_{ij}, \mathbf{R}_{st}) \\
 &= N \int \delta(\mathbf{R}_{on}) \delta(\mathbf{R}_{ij}) P_0(\mathbf{R}_{on}, \mathbf{R}_{ij}, \mathbf{R}_{st}) d\mathbf{R}_{on} d\mathbf{R}_{ij}, \quad (4)
 \end{aligned}$$

where $\delta(\mathbf{R})$ is the three dimensional Dirac delta function and the subscript 2 attached to P represents the number of rings per molecule. N is the normalization constant which is given by

$$N^{-1} = \int P_0(O_{on}, O_{ij}, \mathbf{R}_{st}) d\mathbf{R}_{st}. \quad (5)$$

Thus, using Eqs. (2) and (3), we obtain

$$\begin{aligned}
 P_2(\mathbf{R}_{st}) &= (3/2\pi a^2)^{3/2} (c^{33}/|C|)^{3/2} \\
 &\quad \times \exp[-(3/2a^2)(c^{33}/|C|)\mathbf{R}_{st}^2]. \quad (6)
 \end{aligned}$$

According to Kirkwood and Riseman,⁵⁾ the sedimentation coefficient s_0 of a polymer chain is generally given as

$$s_0 = M(1 - v_2 \rho_0) N_A f \quad (7)$$

$$f^{-1} = (1/n\zeta) [1 + (\zeta/6\pi\eta_0 n) \Sigma_s \Sigma_t \langle \mathbf{R}_{st}^{-1} \rangle], \quad (8)$$

where M , v_2 and f are the molecular weight, the partial specific volume and the translational friction constant of the polymer, ρ_0 and η_0 are the density and the viscosity of solvent, N_A is the Avogadro number and ζ is the friction constant of a segment, respectively. Using the hydrodynamic interaction parameter h , we may rewrite Eq. (8) in the form,

$$f^{-1} = (1/n\zeta) [1 + h(2\pi a^2/3)^{1/2} \eta^{-3/2} \Sigma_s \Sigma_t \langle \mathbf{R}_{st}^{-1} \rangle], \quad (9)$$

with

$$h = \zeta n^{1/2} / (12\pi^3)^{1/2} a \eta_0. \quad (10)$$

Thus, our task is simply to evaluate the statistical average of the inverse distance \mathbf{R}_{st}^{-1} between two segments.

Using the probability density $P_2(\mathbf{R}_{st})$ given by Eq. (6), we readily obtain

$$\begin{aligned}
 \langle \mathbf{R}_{st}^{-1} \rangle &= \int \mathbf{R}_{st}^{-1} P_2(\mathbf{R}_{st}) d\mathbf{R}_{st} \\
 &= (6/\pi a^2)^{1/2} (c^{33}/|C|)^{1/2}. \quad (11)
 \end{aligned}$$

Then, substituting Eq. (11) with Eq. (3) into Eq. (9), and replacing the summation with respect to s and t by the integration, we obtain

$$f^{-1} = (1/n\zeta) (1 + \sqrt{2} h K_2), \quad (12)$$

$$K_2 = \hat{p}_1^{3/2} \pi + \hat{p}_2^{3/2} \pi + 2(\hat{p}_1 \hat{p}_2^{1/2} + \hat{p}_1^{1/2} \hat{p}_2 - \hat{p}_1^{1/2} \hat{p}_2^{1/2}) \pi, \quad (13)$$

$$\hat{p}_1 = [i + (n - j)]/n, \quad (14)$$

$$p_2 = 1 - p_1 = (j - i) / n. \quad (15)$$

The first and the second terms in Eq. (13) represent the contribution from ring 1 and 2, respectively, while the remaining terms in parentheses represent the contributions from cross configurations in which the segments s and t belong to different rings from each other. When $p_2 = 0$, the molecule becomes identical with the untwisted ring molecule, and we have

$$K_2(p_2 = 0) = \pi. \quad (16)$$

which has already been obtained in Part I of this series.¹

It is now widely accepted that the hydrodynamic properties of any type of flexible macromolecules are well approximated by the so-called non-free-draining limit of the hydrodynamic interaction, *i. e.* $h \gg 1$. In this case, the first term in Eq. (12) is negligible, and the ratio of the sedimentation coefficients between two different forms of molecule with the same molecular weight is simply represented by the ratio of K values. For the linear flexible chains, K is given as $8/3$.⁵ Accordingly, the twisted ring: linear ratio of the sedimentation coefficient is given by $3K_2/8$. Table 1 shows the values of $3K_2/8$ calculated by Eq. (13). As is seen from

Table 1. Effect of Ring Formation on the Sedimentation Constant s_0 .

No. of rings m	Size distribution			$\frac{s_0(\text{ring})}{s_0(\text{linear})}$ $= K_m/K_0$
	p_1	p_2	p_m	
1	1	—	—	1.178
2	0.95	0.05	—	1.206
	0.90	0.10	—	1.230
	0.80	0.20	—	1.270
	0.70	0.30	—	1.299
	0.60	0.40	—	1.315
	0.50	0.50	—	1.321
3	uniform size		1/3	1.400
4	"		1/4	1.458
5	"		1/5	1.500
10	"		1/10	1.617
∞	"		~ 0	2.000

this table, the ratio is rather insensitive to the partition of the size between two rings unless $p_1/p_2 > 5$.

3. SEDIMENTATION COEFFICIENT OF MULTIPLY TWISTED RINGS

The extension of the above calculation to a more general case is straightforward though tedious. Here we show only the results of the calculations. If a molecule consists of m rings linearly connected by universal joints, the quantity K_m corresponding to K_2 in Eq. (12) is obtained as

$$K_m = \sum_{k=1}^m p_k^{3/2} \pi + \sum_{k=1}^{m-1} \sum_{l=1}^{m-k} J_{k, k+l}, \quad (17)$$

$$\begin{aligned}
 J_{k,k+l} = & 4 \{ p_k p_{k+l}^{1/2} \sin^{-1} (p_{k+l}/p - p_k)^{1/2} \\
 & + p_k^{1/2} p_{k+l} \sin^{-1} (p_k/p - p_{k+l})^{1/2} \\
 & - (p_k p_{k+l} p)^{1/2} \sin^{-1} p_k p_{k+l}/p (p - p_k - p_{k+l}) \\
 & + p_k p_{k+l} \}^{1/2}, \quad (18)
 \end{aligned}$$

$$p = p_k + p_{k+l} + 4 \sum_{h=k+1}^{k+l-1} p_h q_h (1 - q_h). \quad (19)$$

Here p_k is the fractional size of the k th ring of the molecule, and q_k and $1 - q_k$ are the fractional lengths of two paths connecting two junctions of the k th ring with $k-1$ th and $k+1$ th rings. If the molecule consists of m symmetric rings of equal size, we can simply put

$$p_k = 1/m, \quad q_k = 1/2 \text{ for all } k. \quad (20)$$

Accordingly, we obtain

$$\begin{aligned}
 K_m = & m^{-1/2} \pi + 4m^{-3/2} \sum_{k=1}^{m-1} \sum_{l=1}^{m-k} 2 \sin^{-1}(l^{-1/2}) \\
 & - (l+1)^{1/2} \sin^{-1}(l^{-1}). \quad (21)
 \end{aligned}$$

The values of $3K_m/8$ calculated from Eq. (21) are given in Table 1. This ratio has the asymptotic limit, two, for large m .

4. DISCUSSION

Comparing the theoretical values with the experimental values for the twisted ring: linear ratio of the sedimentation coefficient, we can obtain $m=4$ in the case of the polyoma virus DNA, and $m=6$ in the case of the human papilloma virus DNA. However, these values of the degree of twisting may be somewhat underestimated. That is, the DNA chains are not completely flexible because of their double stranded structure. According to Stockmayer and Hearst,⁶⁾ the s_0 versus M relationship of the native DNA chains can be expressed in the form,

$$s_0 = b + K_s M^{0.5}. \quad (22)$$

The constant b is about 2.2~2.7 and K_s is 7.60×10^{-3} in Svedberg unit. Very recently, new experimental data on the DNA's with extremely high molecular weight (T2 and T7 phage DNA) becomes available due to Crothers and Zimm,⁷⁾ and the inclusion of these new data slightly modifies Eq. (22): *i.e.*

$$s_0 = 2.7 + 1.52 \times 10^{-2} M^{0.445}. \quad (23)$$

The first terms in Eqs. (22) and (23) represent the effect of the stiffness of the DNA chains, while the second terms represent the part of s_0 which is subjected to the flexible chain treatment. The effect of chain stiffness on the sedimentation constant of ring molecules is still unknown.

The sedimentation constants of the linear, ring and twisted ring forms of polyoma virus DNA are 14, 16 and 20 in Svedbergs, respectively, as reported by Weil and Vinograd.²⁾ These values for human papilloma virus DNA are 18, 20 and 28 in Svedbergs, respectively, as reported by Crawford. The effect of the chain stiffness on s_0 is still unknown for the ring molecules. But, if we tentatively assume for the ring forms of DNA a correction of the same order as that for the linear DNA's, say 3 Svedbergs, the ring: linear ratio of these molecule can be estimated as $(16-3)/(14-3) = 1.18$ for polyoma DNA and $(20-2)/(18-3) = 1.13$ for human papill-

oma DNA, respectively. These values are in better agreement with the theoretical estimate, 1.18, than are the uncorrected values, $16/14=1.14$ and $20/18=1.11$. Similarly, the corrected ratio of the sedimentation coefficient between the twisted ring and linear forms is evaluated as $(20-3)/(14-3)=1.54$ and $(28-3)/(18-3)=1.67$ for the two species of DNA. From these values, the number of rings m per a molecule is estimated as about 6 to 10. In order to obtain a more reliable estimate on the degree of twisting, it is necessary to develop a theory of the sedimentation coefficient of semiflexible ring macromolecules and also to know the behavior of the intrinsic viscosity both experimentally and theoretically.

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Note added in proof: A theory essentially similar to the present has been recently published by V. A. Bloomfield, *Proc. Natl. Acad. Sci. U. S.* **55**, 717 (1966).