Polymer Journal Vol. 1, No. 3, pp. 341–347 (1970)

Solution Properties of Synthetic Polypeptides.
VIII. Further Study of the Light-Scattering Function

Takashi NORISUYE and Akio TERAMOTO

Department of Polymer Science, Osaka University, Toyonaka, Osaka, Japan.

(Received January 16, 1970)

ABSTRACT: The Rayleigh scattering of light from solutions of polypeptide molecules which are in the helix—coil transition region is discussed in terms of the particle scattering function $P(\theta)$ expanded in powers of $k^2 = (16\pi^2/\lambda'^2)\sin^2(\theta/2)$, where λ' is the wavelength of the light in the scattering medium. Approximate expressions are derived, under the conditions that $N \gg 1$, $\sqrt{\sigma} \ll 1$, and $N\sqrt{\sigma} > 3$, for the coefficients in the series up to the third one and for the fourth moment $\langle R^4 \rangle$ of the end—to—end distance of the chain. Here N refers to the degree of polymerization and σ to the helix-initiation parameter. It can be shown that a polypeptide chain tends to obey Gaussian statistics at the limit of infinite N unless the helical content is unity. Examination of numerical results indicates that the behavior of a finite chain significantly differs from the Gaussian limit even at an N as large as 4000. Features of $P(\theta)^{-1/2}$ as a function of $\sin^2(\theta/2)$ are examined in relation to the determination of the mean-square radii of gyration of polypeptide samples from light-scattering measurements.

KEY WORDS Light-Scattering / Molecular Dimensions / Polypeptide / Helix-coil Transition / Statistical Theory /

The particle scattering function $P(\theta)$ of a homopolymer (degree of polymerization is N) in a single solvent may be expanded in ascending powers of $k^2 = [(16\pi^2/\lambda'^2)\sin^2(\theta/2)]$ in the form:

$$P(\theta) = 1 - (k^2/3N^2)\sum_{i<j}\langle r_{ij}^2\rangle + (k^4/60N^2)\sum_{i<j}\langle r_{ij}^4\rangle - (k^6/2520N^2)\sum_{i<j}\langle r_{ij}^6\rangle + \cdots \quad (1)$$

where λ' is the wavelength of the light in the scattering medium, θ is the scattering angle, r_{ij} is the distance between residues i and j ($i, j = 1, 2, \ldots, N$), and $\langle\cdots\rangle$ denotes the average taken over all conformations of the polymer molecule. Strictly speaking, Eq. 1 is not applicable to a polypeptide molecule at intermediate stages of a helix—coil transition. This is because the helical and randomly coiled sections of such a molecule may have different refractive index increments in a given solvent. In Part VII[1] of this series we have shown, however, that this copolymer nature of a polypeptide molecule has little effect on the apparent mean-square radius of gyration $\langle S^2\rangle_{app}$ as determined from the initial slope of $P(\theta)^{-1}$ *vs.* $\sin^2(\theta/2)$. In other words, the experimentally measured $\langle S^2\rangle_{app}$ may be taken to an excellent approximation as equal to the true mean-square radius of gyration $\langle S^2\rangle$ of the molecule. This result implies that even if a polymer assumes a conformation of an interrupted helix, Eq. 1 is applied quite correctly at least up to the second term of the series.

In the present paper, we anticipate that the above-mentioned copolymer nature of a polypeptide molecule has a negligible effect also on the higher terms in the k^2-expansion of $P(\theta)$ and we investigate how its $P(\theta)$ varies with k^2 by evaluating the third and fourth terms in Eq. 1 subject to the same conditions as have been imposed in Part VII, *i.e.*, $N \gg 1$, $\sqrt{\sigma} \ll 1$, and $N\sqrt{\sigma} > 3$. Here σ is the helix-initiation parameter and is known to have values of the order of 10^{-4} for typical polypeptides.[2,3] The essential problem here is to obtain expressions for $\sum_{i<j}\langle r_{ij}^4\rangle$, $\sum_{i<j}\langle r_{ij}^6\rangle$, etc., under the imposed conditions of N and σ. In carrying out the calculations, we assume that no excluded-volume effect is present between any pair of monomeric residues and we follow Nagai's[4] formalism for the statistical mechanics of homopolypeptides valid under this restriction.

CALCULATION OF $\langle r_{ij}^4 \rangle$, $\langle R^4 \rangle$, AND $\sum_{i<j} \langle r_{ij}^4 \rangle$

Statistical Model Used and Expression for $\langle r_{ij}^4 \rangle$

As before,[1] we take the following simplified model of Nagai[4] for a real polypeptide chain. Here a helical section consisting of n_1 monomeric residues is replaced by a straight rod of $n_1 b_1$ in length; a randomly coiled section consisting of n_0 residues is approximated by a random flight chain of n_0 steps each of which has a length b_0; and these two sections are connected alternately at their ends by a universal joint. It is assumed that there is no excluded-volume effect between any pair of monomeric residues. Let N residues be numbered 1, 2, ..., N, from one end of the chain to the other and $\boldsymbol{r}_i$ ($i = 1, 2, \ldots, N$) be the displacement vector of residue i. We first consider the average fourth moment of r_{ij}, the distance between residues i and j, in some detail. It can be shown that

$$\begin{aligned} r_{ij}^4 = {} & (\sum_{i<m\le j} r_m^2)(\sum_{i<n\le j} r_n^2) \\ & + 4(\sum_{i<m\le j} r_m^2)(\sum_{i<s<t\le j} \boldsymbol{r}_s \cdot \boldsymbol{r}_t) \\ & + 4(\sum_{i<m<n\le j} \boldsymbol{r}_m \cdot \boldsymbol{r}_n)(\sum_{i<s<t\le j} \boldsymbol{r}_s \cdot \boldsymbol{r}_t) \end{aligned} \qquad (2)$$

where $r = |\boldsymbol{r}|$. The first two sums on the right-hand side of this equation can be rewritten

$$(\sum_{i<m\le j} r_m^2)(\sum_{i<n\le j} r_n^2) = \sum_{i<m\le j} r_m^4 + 2\Big[\sum_{i<m<n\le j} r_m^2 r_n^2\Big] \qquad (3)$$

and

$$\begin{aligned} & (\sum_{i<m\le j} r_m^2)(\sum_{i<s<t\le j} \boldsymbol{r}_s \cdot \boldsymbol{r}_t) \\ & = 2\Big[\sum_{i<m<n\le j} \overline{r_m^3 r_n}\Big] + 2\Big[\sum_{i<m<s<t\le j} r_m^2 \overline{r_s r_t}\Big] \\ & \quad + \sum_{i<s<m<t\le j} \overline{r_s r_m^2 r_t} \end{aligned} \qquad (4)$$

where a bar attached to the product of r_i's indicates that the residues specified by it belong to the same helical section. The last sum in Eq. 2 can conveniently be divided into the following three groups of sum according to the relative magnitudes of the running indices m, n, s, and t. Thus for $m = s$ and $n = t$ the sum can be recast in the form:

$$\sum_{i<m<n\le j} (r_m^2)^*(r_n^2) \cos^2\varphi + \sum_{i<m<n\le j} \overline{r_m^2 r_n^2} \qquad (5)$$

where the symbol * implies that the residues or sections appearing on both sides of it (residues m and n in the present case) do not belong to the same helical section and where φ is the angle between the corresponding displacement vectors. When two of the four indices are equal and the rest are different, the sum in question can be put in the form:

$$\begin{aligned} & 6\Big[\sum_{i<m<s<n\le j} \overline{r_m^2 r_s r_n}\Big] \\ & + 4\Big[\sum_{i<m<s<n\le j} (r_m^2)^*(\overline{r_s r_t}) \cos^2\varphi\Big] \end{aligned} \qquad (6)$$

Finally when all the four indices are different from each other, the sum is expressed by

$$\begin{aligned} & 6\Big[\sum_{i<m<s<t<n\le j} \overline{r_m r_s r_t r_n}\Big] \\ & + 2\Big[\sum_{i<m<s<t<n\le j} (\overline{r_m r_s})^*(\overline{r_t r_n})(1 + 2\cos^2\varphi)\Big] \end{aligned} \qquad (7)$$

The required r_{ij}^4 is the sum of all these terms. Substituting Eqs. 3 through 7 into Eq. 2 and averaging over all conformations of the molecule gives

$$\begin{aligned} \langle r_{ij}^4 \rangle = {} & \sum_{i<m\le j} \langle r_m^4 \rangle + (32/3)\Big[\sum_{i<m<n\le j} \langle \overline{r_m^2 r_n^2} \rangle\Big] \\ & + (68/3)\Big[\sum_{i<m<s<t\le j} \langle \overline{r_m^2 r_s r_t} \rangle\Big] \\ & + (10/3)\Big[\sum_{i<m<n\le j} \langle r_m^2 r_n^2 \rangle\Big] \\ & + (40/3)\Big[\sum_{i<m<s<t\le j} \langle \overline{r_m^2 r_s r_t} \rangle\Big] \\ & + (40/3)\Big[\sum_{i<m<s<t<n\le j} \langle \overline{r_m r_s}\ \overline{r_t r_n} \rangle\Big] \\ & + (32/3)\Big[\sum_{i<m<s<t<n\le j} \langle \overline{r_m r_s r_t r_n} \rangle\Big] \end{aligned} \qquad (8)$$

In deriving this equation, the symbol * has been eliminated by utilizing such a relation as

$$\langle (r_m^2)^*(\overline{r_s r_n}) \rangle = \langle r_m^2 \overline{r_s r_n} \rangle - \langle \overline{r_m^2 r_s r_n} \rangle$$

together with

$$\langle \cos^2\varphi \rangle = 1/3 \quad \text{and} \quad \langle \overline{r_m^3 r_n} \rangle = \langle \overline{r_m^2 r_n^2} \rangle$$

Go, Saito, and Ochiai[6] attempted deriving another form of $\langle r_{ij}^4 \rangle$ on the basis of the Lifson-Roig theory,[7] but their equation as well as numerical results deduced therefrom were erroneous as pointed out recently by Saito himself[8]

and by Birshtein.[9] These latter authors have given the correct form of $\langle r_{ij}^4 \rangle$, which proves to be recast in the form of Eq. 8.

Expression for $\langle R^4 \rangle$

Substitution of $i = 1$ and $j = N$ into Eq. 2 gives an expression for the fourth moment $\langle R^4 \rangle$ of the end-to-end distance of a polypeptide chain. Its explicit form as a function of the molecular parameters considered can be derived by utilizing the theory of Nagai quoted above.[4] The resulting equation, however, becomes too complex to be amenable to a straightforward numerical computation. In order to obtain a relevant approximate expression for $\langle R^4 \rangle$, we again follow the approximation used previously[1,5] for calculating $\langle S^2 \rangle$, $\langle R^2 \rangle$, and other quantities. This treatment has recourse to series expansions of the eigenvalues λ_i ($i = 1$, 2, 3, and 4) for the statistical weight matrix[4] in powers of $\sqrt{\sigma}$. It has been shown[1,5] that averages of various quantities can be expressed to a good approximation by the sums of terms involving $\sqrt{\sigma}$, N^{-1}, $(N\sqrt{\sigma})^{-1} = \beta^{-1}$, $\exp(-\beta\sqrt{(1-f)/f})$, etc., and their higher powers, where f refers to the helical content for infinite N. Since we are interested in a relatively large N, we confine ourselves to the case where $N \gg 1$, $\sqrt{\sigma} \ll 1$, and $N\sqrt{\sigma} > 3$. Thus in the development which follows, terms of the order of N^{-1} and of $\sqrt{\sigma}$ are neglected as compared with unity, but terms multiplied by $(N\sqrt{\sigma})^{-1}$ and by its higher powers are retained. Then it is shown that the first three terms in Eq. 8 with $i = 1$ and $j = N$ can be dropped out of the equation, giving an expression of the form:

$$\langle R^4 \rangle = (10/3)\left(J_1 + 4J_2 + 4J_3 + \frac{16}{5} J_4 \right) \quad (9)$$

where

$$J_1 = \sum_{0<i<j\leq N} \langle r_i^2 r_j^2 \rangle \quad (10)$$

$$J_2 = \sum_{0<i<j\leq s<N} \langle r_i^2 \overline{r_j r_s} \rangle \quad (11)$$

$$J_3 = \sum_{1<i<j\leq s<t<N} \langle \overline{r_i r_j}\, \overline{r_s r_t} \rangle \quad (12)$$

and

$$J_4 = \sum_{1<i<j\leq s<t<N} \langle \overline{r_i r_j r_s r_t} \rangle \quad (13)$$

Let μ_i be the probability that residue i is in the helical state. We follow Nagai[4] who assumed that $\mu_1 = \mu_N = 0$. Then Eq. 10 can be put in the form:

$$J_1 = \sum_{0<i<j\leq N} \{\langle \mu_i\mu_j \rangle b_1^4 + [\langle \mu_i \rangle + \langle \mu_j \rangle - 2\langle \mu_i\mu_j \rangle] b_1^2 b_0^2 + [1 - \langle \mu_i \rangle - \langle \mu_j \rangle + \langle \mu_i\mu_j \rangle] b_0^4\} \quad (14)$$

J_2 may be divided into two groups depending on whether $j = s + 1$ or $j > s + 1$, leading to

$$J_2 = \sum_{0<i<j<N-1} \langle r_i^2 \overline{r_j r_{j+1}} \rangle + \sum_{0<i<s<j-1<N-1} \langle r_i^2 \overline{r_s r_j} \rangle \quad (15)$$

An approximate calculation shows that the first sum in Eq. 15 is of the order of $\sqrt{\sigma}$ in comparison with the second one and hence may be neglected to our present approximation. Thus we have for J_2

$$J_2 = \sum_{0<i<s<j<N} [\langle \mu_i\mu_s\mu_{s+1} \cdots \mu_j \rangle b_1^4 + (\langle \mu_s\mu_{s+1} \cdots \mu_j \rangle - \langle \mu_i\mu_s\mu_{s+1} \cdots \mu_j \rangle) b_0^2 b_1^2] \quad (16)$$

Similarly we obtain for J_3 and J_4

$$J_3 = b_1^4 \sum_{1<i<s<t<j<N} \langle \mu_i\mu_{i+1} \cdots \mu_s\mu_t\mu_{t+1} \cdots \mu_j \rangle \quad (17)$$

and

$$J_4 = b_1^4 \sum_{1<i<s<t<j<N} \langle \mu_i\mu_{i+1} \cdots \mu_j \rangle \quad (18)$$

Expressions for the averages of the products of μ_i's appearing in these equations can be derived from the theory of Nagai.[4] In fact, all of them but one that appears in Eq. 17 have been calculated by Nagai[4] and also by Teramoto *et al.*[1] Thus the only average to be evaluated here is the following:

$$\langle \mu_i\mu_{i+1} \cdots \mu_s\mu_t\mu_{t+1} \cdots \mu_j \rangle = Z_N^{-1}(\sigma u)^{-1} u^{j-i-t+s-4} \sum_{p,q,w} c(\lambda_p)c(\lambda_q)c(\lambda_w)\lambda_p^{i+1}\lambda_q^{t-s+2} \times \lambda_w^{N-j+2}(\lambda_p - u)(\lambda_w - u) \quad (19)$$

where Z_N is the partion function of the molecule and the summation extends over the four eigenvalues λ_i ($i = 1$, 2, 3, and 4). The expressions for Z_N and $c(\lambda)$ are given in the article by Nagai.[4]

Substitution of λ_i's, $c(\lambda)$, Z_N, etc., expressed in terms of σ, f, and N into such expressions as Eq. 19 and replacement of the summations over i, j, s, and t by integrals, subject to the conditions that $N \gg 1$, $\sqrt{\sigma} \ll 1$, $N\sqrt{\sigma} > 3$, and $0 \leq f < 1$, eventually leads to

$$\langle R^4\rangle = (5/3)(Nb_0^2)^2(1-f)^2 \times \{K_1 + (4/\sqrt{\sigma})(b_1/b_0)^2[f/(1-f)]^{3/2}K_2 + (4/\sigma)(b_1/b_0)^4[f/(1-f)]^3K_3\} \quad (20)$$

where

$$K_1 = 1 + (6/\beta)f^{3/2}(1-f)^{-1/2} + (6/\beta^2)f^2(2f-1)/(1-f) \quad (21)$$

$$K_2 = 1 - (6/\beta)f^{1/2}(1-f)^{1/2} + (12/\beta^2)f(1-f) \quad (22)$$

and

$$K_3 = 1 + (6/\beta)f^{-1/2}(1-f)^{-1/2}[(1-f)^2 - 2/5] + (2/\beta^2)[(1-f)(6f-5) + 4(f-2/5) + (15f-12)/5(1-f)] + P(\beta,f)\exp\left(-\beta\sqrt{\frac{1-f}{f}}\right) \quad (23)$$

with $P(\beta, f)$ defined by

$$P(\beta,f) = (4/5f^3) + (2/\beta)f^{-1/2}(1-f)^{-1/2} \times [f-2+(1+f)(1-f)^3/f^3 + 8(2f-1)/5f^3] + (2/\beta^2)[(f-4)/(1-f) + 4(2+f)/5f(1-f) + 3(1-f)/f^2 + 8(1-2f)(1-f)/5f^3 - (15f-7)(1-f)^3/5f^4] \quad (24)$$

For the compactness of the results we have expressed all equations in terms of f by using the relation:[1,5]

$$(\ln u)/\sqrt{\sigma} = (2f-1)[f(1-f)]^{-1/2} + 0(\sqrt{\sigma})$$

At the limit of infinite N, K_1, K_2, and K_3 tend to unity, and Eq. 20 reduces to

$$\langle R^4\rangle = (5/3)(Nb_0^2)^2(1-f)^2 \times \{1 + (2/\sqrt{\sigma})(b_1/b_0)^2[f/(1-f)]^{3/2}\}^2 \quad (25)$$

Since to the same approximation the mean-square end-to-end distance $\langle R^2\rangle$ for infinite N is given by[1]

$$\langle R^2\rangle = (Nb_0^2)(1-f) \times \{1 + (2/\sqrt{\sigma})(b_1/b_0)^2[f/(1-f)]^{3/2}\} \quad (26)$$

unless $f = 1$, it follows that

$$\langle R^4\rangle = (5/3)\langle R^2\rangle^2 \quad (27)$$

This relation is known to hold for a Gaussian coil. Although we have omitted the first sum in Eq. 15 to derive Eq. 25, we can show that Eq. 27 is strictly valid unless $f = 1$. Birshtein[9] has reached the same conclusion from a different approach. The value of $\langle R^4\rangle$ relative to $(5/3)\langle R^2\rangle^2$ varies from unity for a Gaussian coil to 3/5 for a rigid rod.

Expression for $\sum_{i<j}\langle r_{ij}^4\rangle$

Summation of Eq. 8 over i and j along with the substitution of explicit expressions for the various averages involved should give an expression for $\sum_{i<j}\langle r_{ij}^4\rangle$. Although this operation can be carried through in principle under the same approximations as above, the resulting equation involves too many terms to be of practical interest. Therefore we are content here with obtaining the desired expression by imposing an additional condition that $\beta\sqrt{(1-f)/f} > 5$. This allows us to ignore terms associated with the factor $\exp(-\beta\sqrt{(1-f)/f})$ in comparison with unity. The result reads

$$(1/5N^2)\sum_{i<j}\langle r_{ij}^4\rangle = (Nb_0^2/6)^2(1-f)^2 \times \{K_1^* + (4/\sqrt{\sigma})(b_1/b_0)^2[f/(1-f)]^{3/2}K_2^* + (4/\sigma)(b_1/b_0)^4[f/(1-f)]^3K_3^*\} \quad (28)$$

where

$$K_1^* = 1 + (4/\beta)f^{3/2}(1-f)^{-1/2} + 12(f/\beta)^2 - (24/\beta^3)f^{5/2}(3-5f)(1-f)^{-1/2} + (120/\beta^4)f^3(1-2f) \quad (29)$$

$$K_2^* = 1 - (4/\beta)(2-f)[f/(1-f)]^{1/2} + (12/\beta^2)f(2-f^2)/(1-f) - (24/\beta^3) \times [f/(1-f)]^{3/2}[2-f^2+(1-f)^2(5f-2)] + (24/\beta^4)[f/(1-f)]^2 \times [2-f^2+3f(1-f)^2-(1-f)^3(7-10f)] \quad (30)$$

and

$$K_3^* = 1 + (4/\beta)[f(1-f)]^{-1/2}[(1-f)^2 - 2f + 4/5] + (12/\beta^2)[f^2+2f-6+3/5(1-f)] + (24/\beta^3)f^{1/2}(1-f)^{-3/2}[(1-f)^3(5f-1) + (1-f)^2(2f+17/5) + 4(6/5-f)] - (24/\beta^4)\{10f^2(1-f)(2-f) + f(5f-8) \times [(1-f)^3 - 1]/(1-f)^2 + 26f^2/5\} \quad (31)$$

For infinitely large N, K_1^*, K_2^*, and K_3^* become unity, and hence Eq. 28 is simplified to give

$$(1/5N^2)\sum_{i<j}\langle r_{ij}^4\rangle = (Nb_0^2/6)^2(1-f)^2 \times \{1+(2/\sqrt{\sigma})(b_1/b_0)^2[f/(1-f)]^{3/2}\}^2 \quad (32)$$

The right-hand side of this equation stands in agreement with the square of our previously derived expression[1] for $\langle S^2\rangle$. Thus unless $f=1$, $\sum_{i<j}\langle r_{ij}^4\rangle$ of the polypeptide chain with infinite N is found to obey the relation expected for a Gaussian chain.

It does not seem tractable to derive simple analytical expressions for the averages of higher even moments $\langle r_{ij}^{2p}\rangle (p>2)$ when N is finite. However, the asymptotic expression for $\langle r_{jj}^6\rangle$ valid at the limit of infinite N can be obtained without difficulty. It reads

$$\langle r_{ij}^6\rangle = (70/3)\Bigg\{\sum_{i<m<s<n\le j}\langle r_m^2 r_s^2 r_n^2\rangle + 6\Bigg[\sum_{i<m<s<t<n\le j}\langle r_m^2\overline{r_s r_t} r_n^2\rangle\Bigg] + 12\Bigg[\sum_{i<m<s<t<h<n\le j}\langle r_m^2 r_s\overline{r_t r_h} r_n\rangle\Bigg] + 8\Bigg[\sum_{i<m<s<t<h<k<n\le j}\langle \overline{r_m r_s}\,\overline{r_t r_h}\,\overline{r_k r_n}\rangle\Bigg]\Bigg\} \quad (33)$$

which, upon applying a procedure similar to that used for the derivation of Eq. 28, eventually gives

$$\langle R^6\rangle = (35/9)\langle R^2\rangle^3 \quad (34)$$

and

$$\sum_{i<j}\langle r_{ij}^6\rangle = 42N^2\langle S^2\rangle^3 \quad (35)$$

provided that $f<1$. These relations are known to hold for a Gaussian coil. This and other arguments so far presented suffice to infer that a polypeptide chain with infinite N should behave as a Gaussian coil unless $f=1$. In fact, the validity of this inference can be concluded without recourse to a particular molecular model from the general consideration of Nagai[10] on the conformation of stereoregular polymer molecules. Evidently, substitution of Eqs. 32 and 35 into Eq. 1 results in $P(\theta)$ which agrees with that for a Gaussian coil up to the term of k^6. Our principal interest here is the extent to which the behavior of a polypeptide chain with finite N deviates from that of a Gaussian coil. This problem is discussed with numerical results in the following section.

NUMERICAL RESULTS AND DISCUSSION

The nature of the approximation on which the present calculations are based has been fully discussed in our previous publications,[1,5] and will not be repeated here. In order to demonstrate the degree of our approximation, we have calculated values of $\langle R^2\rangle$ from the approximate expression which was derived previously[1] under the same conditions as used for the present derivation of $\langle R^4\rangle$. In Figure 1, the results for

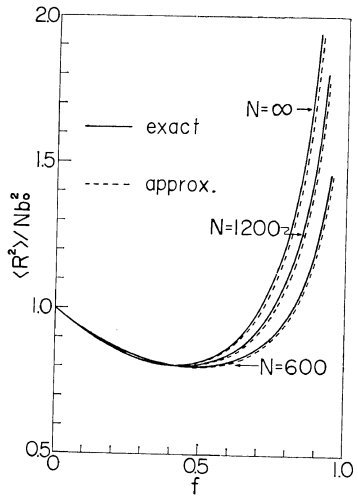

Figure 1. Comparison of exact and approximate values of the mean-square end-to-end distance $\langle R^2\rangle$ for the parameters: σ, 2×10^{-4}; b_0, 22.4 A; b_1, 1.5 A. Exact values (solid lines) taken from the paper by Nagai;[4] approximate values (dotted lines) calculated according to Eq. 20 of ref. 1.

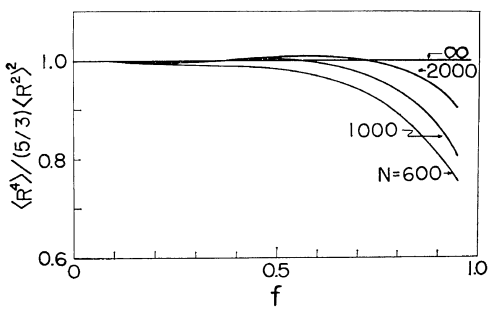

Figure 2. Fourth moments of the end-to-end distance $\langle R^4\rangle$ relative to $(5/3)\langle R^2\rangle^2$ for various N. The parameters chosen are: σ, 10^{-4}; b_0, 15A; b_1, 1.5 A. The value of the moment at $f=1$ (rigid rod) is 3/5.

different values of N are plotted against f and compared with the exact values taken from the paper by Nagai;[4] the parameters chosen here are $\sigma = 2 \times 10^{-4}$, $b_0 = 22.4$ A, and $b_1 = 1.5$ A. It is seen that the agreement between the exact and approximate values is satisfactory over the range of f examined. Similar agreement was demonstrated also for $\langle S^2 \rangle$.[1]

Figure 2 illustrates how the ratio $\langle R^4 \rangle / (5/3) \cdot \langle R^2 \rangle^2$ for a given N varies with f for the case where $\sigma = 10^{-4}$, $b_0 = 15$ A, and $b_1 = 1.5$ A. As has been noted in the preceding section, this ratio should vary from unity for a Gaussian coil ($f = 0$) to 3/5 for a rigid rod ($f = 1$). Figure 2 shows that the ratio stays almost constant near unity for $f < 1/2$ and decreases gradually toward 3/5 as f approaches unity. The deviation from Gaussian behavior becomes less pronounced as N increases. The curve for $N = 2000$ appears to display a slight maximum at a point near $f = 0.6$. Whether this trend is real or simply due to an error arising from the present approximate treatment remains unanswered until exact calculations become available. It must be remarked that detailed features of the curve also depend on the ratio b_1/b_0. At any rate, the curves depicted in Figure 2 show distinctly different behavior from those reported by Go *et al.*[6]

Values of $\sum_{i<j} \langle r_{ij}^4 \rangle$ for fixed values of σ, N, b_1, and b_0 calculated from Eq. 28, together with those of $\langle S^2 \rangle$ obtained for the same parameters from our previous expression,[1] have been used to compute a quantity G defined by

$$G = \sum_{i<j} \langle r_{ji}^4 \rangle / 5N^2 \langle S^2 \rangle^2 \tag{36}$$

This quantity becomes unity for a Gaussian coil and 24/25 for a rigid rod, and appears to be very insensitive to the geometry of the molecule. In Figure 3 are presented the curves of G against f for various N and $\sigma = 10^{-4}$, $b_0 = 15$ A, and $b_1 = 1.5$ A. We have restricted the calculations of G to f smaller than 0.9 in order to ensure the condition that $\beta\sqrt{(1-f)/f} > 5$, on which Eq. 28 for $\sum_{i<j} \langle r_{ij}^4 \rangle$ was derived. It is of interest that G increases toward a broad maximum before decreasing to the value for a rod and that the Gaussian behavior characteristic of infinite chains is not approached even

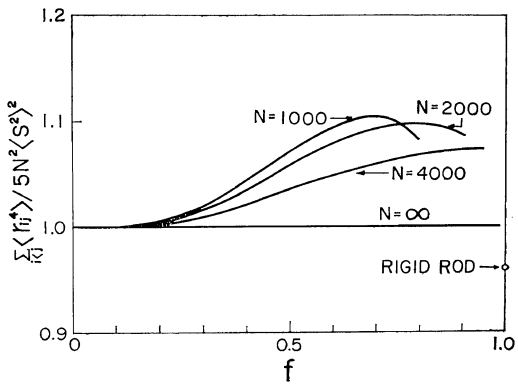

Figure 3. Plots of G *vs.* f as a function of N. The same parameter as those used in Figure 2 is chosen. The value of G at $f = 1$ (rigid rod) indicated by a circle is 24/25.

at an N as large as 4000. This latter feature is associated with the fact, evident from Eqs. 20 and 28, that it is the parameter $\beta = N\sqrt{\sigma}$, not N itself, that primarily governs the extent to which the behavior for finite N deviates from the Gaussian limit.

In terms of Eq. 36, we can recast Eq. 1 in the form:

$$P(\theta)^{-1/2} = 1 + (1/6)k^2\langle S^2 \rangle + (1/24)(1 - G)K^4\langle S^2 \rangle^2 \tag{37}$$

with the omission of terms higher than k^6. The argument[11] that a plot of $P(\theta)^{-1/2}$, rather than of $P(\theta)^{-1}$, *vs.* $k^2\langle S^2 \rangle$ for a Gaussian coil follows a straight line over a relatively wide range of $k^2\langle S^2 \rangle$ is attributed to the third term on the right-hand side of Eq. 37 which vanishes for this type of chain. The coefficient for this term is as small as 1/600 for a rigid rod. Indeed, numerical calculations indicate that Eq. 37 gives a good approximation to the exact $P(\theta)^{-1/2}$ for both coils and rods provided that $k^2\langle S^2 \rangle < 3$. We therefore discuss $P(\theta)$ for a polypeptide melecule in terms of this equation.

The solid lines in Figure 4 present values of $P(\theta)^{-1/2}$ at fixed f as a function of $\sin^2(\theta/2)$ for typical values of the parameters: $N = 2000$, $\sigma = 10^{-4}$, $b_0 = 15$ A, $b_1 = 1.5$ A and $\lambda' = 3113$A; the lines at $f = 0$ and 1 have been calculated from the exact light scattering functions relevant for each.[12] The dotted lines in the figure represent the initial tangents to the solid lines. It is seen that $P(\theta)^{-1/2}$ *versus* $\sin^2(\theta/2)$ follows

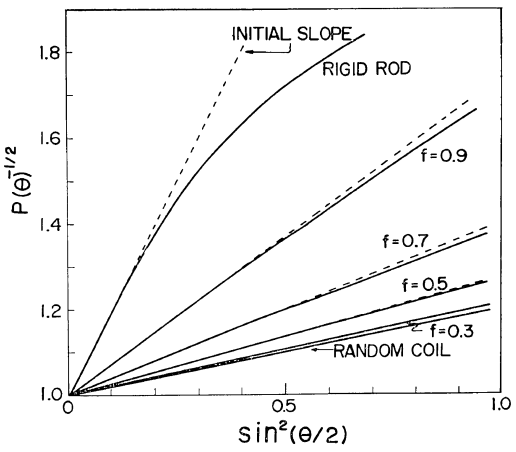

Figure 4. $P(\theta)^{-1/2}$ at fixed f as functions of $\sin^2(\theta/2)$ for the parameters: N, 2000; σ, 10^{-4}; b_0, 15 A; b_1, 1.5 A; $\lambda'(=\lambda_0/n)$, 3113 A. The solid lines for intermediate f represent the values calculated according to Eq. 37, and the dotted lines indicate the initial tangents to the solid lines. The solid lines at $f=0$ and 1 denote the values calculated from the exact light scattering functions.

the initial tangent over an extended range provided that f is not too close to unity, implying that this type of plot allows $\langle S^2 \rangle$ of a polypeptide molecule to be determined from light scattering data even when the molecule is in the state of interrupted helix.

Acknowledgment. We wish to thank Prof. H. Fujita for his interest in this work and his helpful criticism of the manuscript. Thanks are due to Dr. K. Nagai, who drew our attention to the Gaussian behavior of an infinite chain and provided us with the numerical results for $\langle R^2 \rangle$ and $\langle S^2 \rangle$.

REFERENCES

1. A. Teramoto, T. Norisuye, and H. Fujita, *Polymer J.*, **1**, 55 (1970).
2. B. H. Zimm and J. K. Bragg, *J. Chem. Phys.*, **31**, 526 (1959).
3. T. Ackermann and E. Neumann, *Biopolymers*, **5**, 649 (1967).
4. K. Nagai, *J. Chem. Phys.*, **34**, 887 (1961).
5. K. Okita, A. Teramoto, and H. Fujita, *Biopolymers*, in press.
6. M. Go, N. Saito, and M. Ochiai, *J. Phys. Soc. Japan*, **22**, 227 (1967).
7. S. Lifson and A. Roig, *J. Chem. Phys.*, **34**, 1963 (1961).
8. N. Saito, private communication.
9. T. M. Birshtein, *Biopolymers*, **7**, 433 (1969).
10. K. Nagai, *J. Chem. Phys.*, **38**, 924 (1963).
11. G. C. Berry, *J. Chem. Phys.*, **44**, 4550 (1967).
12. K. A. Stacey, "Light-Scattering in Physical Chemistry", Butterworths, London, 1956.