whatever the initial concentration ratio ρ of both components is. In addition, GPC gives the mean complex composition for all ρ and for the different α' studied; it is found that [Lys]/[Glu] equals the fraction of dissociated carboxylic sites α_T.

Finally, CD experiments confirm the one-way process for $\alpha' = 1$, resulting in a structure certainly similar to the β-pleated sheet. X-ray diffraction work in progress will presumably yield more detailed structural information.

One remaining problem to be solved concerns the question why, for $\alpha' \neq 1$, the complexation is stopped at a certain ρ value ρ_{eq}. Polyelectrolyte behavior must be responsible for the lowering of the attractive strength when the charge density decreases; nevertheless, for ρ_{eq}, the [Glu] sites not associated to a lysine group are undissociated; this can be explained only by an important enhancement of pK_a due to a local modification of the dielectric constant as a consequence of the formation of ordered structures.

References and Notes

(1) Katchalsky, A.; Spitnik, P. *Colloq. Int. CNRS* **1954 (1955)**, *No. 57*, 103-25.
(2) Veis, A.; Bodor, E. "Structure and Function of Connective and Skeletal Tissues"; Butterworths: London, 1965; pp 228-35.
(3) Veis, A. "Biological Polyelectrolytes"; Marcel Dekker: New York, 1970; pp 211-71.
(4) Michaels, A. S.; Miekka, R. G. *J. Phys. Chem.* **1961**, *65*, 1765-73.
(5) Michaels, A. S.; Mir, L.; Schnieder, N. S. *J. Phys. Chem.* **1965**, *69*, 1447-55.
(6) Michaels, A. S.; Falkenstein, G. L.; Schnieder, N. S. *J. Phys. Chem.* **1965**, *69*, 1456-65.
(7) Tsuchida, E.; Osada, Y.; Sanada, K. *J. Polym. Sci., Polym. Chem. Ed.* **1972**, *10*, 3397-3404.
(8) Tsuchida, E.; Osada, Y.; Abe, K. *Makromol. Chem.* **1974**, *175*, 583-92, 593-601. Tsuchida, E. *Ibid.* **1974**, *175*, 603-11.
(9) Tsuchida, E.; Osada, Y.; Abe, K. *J. Polym. Sci., Polym. Chem. Ed.* **1976**, *14*, 767-71.
(10) Abe, K.; Koide, M.; Tsuchida, E. *Polym. J.* **1977**, *9*, 73-8. Abe, K.; Tsuchida, E. *Ibid.* **1977**, *9*, 79-86.
(11) Zezin, A. B.; Lutsenko, V. V.; Rogacheva, V. B.; Aleksina, O. A.; Kalyuzhanaya, R. I.; Kabanov, V. A.; Kargin, V. A. *Polym. Sci. USSR (Engl. Transl.)* **1974**, *14*, 857-65.
(12) Zezin, A. B.; Lutsenko, V. V.; Izumrudov, V. A.; Kabanov, V. A. *Polym. Sci. USSR (Engl. Transl.)* **1974**, *16*, 694-8.
(13) Gulyayeva, Z. G.; Zezin, A. B.; Bazvodovskii, E. F.; Berestetskaya, T. Z. *Polym. Sci. USSR (Engl. Transl.)* **1974**, *16*, 2145-52.
(14) Izumrudov, V. A.; Zezin, A. B. *Vysokomol. Soedin., Ser. A* **1976**, *18*, 2488-94.
(15) Rinaudo, M.; Domard, A. *Biopolymers* **1975**, *14*, 2035-48.
(16) Domard, A.; Rinaudo, M.; Rochas, C. *J. Polym. Sci., Polym. Phys. Ed.* **1979**, *17*, 673-81.
(17) Rochas, C.; Domard, A.; Rinaudo, M., *Eur. Polym. J.* **1980**, *16*, 135-40.
(18) Rinaudo, M.; Domard, A. *J. Am. Chem. Soc.* **1976**, *98*, 6360-4.

Averaged Principal Moments of the Inertia Tensor for Unperturbed Poly(hydroxybutyl-L-glutamine) As It Passes through the Helix-Coil Transition

Wayne L. Mattice

Department of Chemistry, Louisiana State University, Baton Rouge, Louisiana 70803.

Received December 6, 1979

ABSTRACT: Averaged principal moments, $\langle L_1^2 \rangle \geq \langle L_2^2 \rangle \geq \langle L_3^2 \rangle$, of the inertia tensor have been obtained for polypeptides undergoing a helix-coil transition. Polypeptide chains containing 101, 201, 401, and 801 amino acid residues were studied. Conformational energy surfaces and Zimm-Bragg statistical weights used are those appropriate for aqueous poly(hydroxybutyl-L-glutamine) when it is unperturbed by long-range interactions. At all degrees of polymerization studied, $\langle L_1^2 \rangle$ and the mean square radius of gyration pass through a minimum during the transition from random coil to α helix. In contrast, the qualitative behavior of the remaining two principal moments is strongly dependent on the degree of polymerization. These two principal moments experience monotonic changes at a low degree of polymerization, but at a high degree of polymerization they pass through both a minimum and a maximum. Principal moment ratios $\langle L_2^2 \rangle / \langle L_1^2 \rangle$ and $\langle L_3^2 \rangle / \langle L_1^2 \rangle$ experience monotonic changes even when the changes in all three $\langle L_i^2 \rangle$ are not monotonic. Behavior of the principal moments is related to the presence of short helical segments at low average helicity and long helical segments at high helicity.

Measurement of the molecular weight dependence of the mean square radius of gyration or intrinsic viscosity is a classic method for establishing whether a polypeptide in a given solvent is in the random coil state or exists instead as an α helix.[1] These same parameters are also used to follow a helix-coil transition induced in a certain polypeptide by alteration of solvent composition or temperature. However, the experimentally measured parameter is not necessarily found to be a monotonic function of the perturbing influence. In the case of the pH-induced helix-coil transition of aqueous poly(L-glutamic acid), for example, the intrinsic viscosity at the midpoint of the transition is substantially lower than that of either the helix or the completely disordered form.[2] This result implies the mean square radius of gyration passes through a minimum as the polypeptide undergoes the helix-coil transition.

Using matrix methods,[3] Miller and Flory[4] obtained theoretical verification that the characteristic ratio passes through a minimum during the helix-coil transition. The characteristic ratio is defined here as $\langle r^2 \rangle_0 / n_p l_p^2$, where $\langle r^2 \rangle_0$ is the mean square unperturbed end-to-end distance for a polypeptide containing $n_p + 1$ amino acid residues and l_p is the distance (3.80 Å) between neighboring C^α atoms. Classification of amino acid residues as helical or nonhelical was achieved by Zimm-Bragg treatment.[5] This procedure assigns a statistical weight of unity to each nonhelical amino acid residue, while the statistical weights of helical amino acid residues are σS or S, depending on whether the amino acid residue initiates or propagates,

0024-9297/80/2213-0904$01.00/0

respectively, a helical segment. The conformational energy surface used for the nonhelical amino acid residues was that appropriate for those bearing a CH_2R side chain in the L configuration.[6] If σ was held constant at 0.003 while S was varied, the characteristic ratio for a polypeptide of degree of polymerization 10^3 went through a minimum when about one-third of the amino acid residues were in the helical state. The minimum moved to higher helical content for short chains.

With the behavior of the characteristic ratio established, attention now shifts to the asymmetry of the spatial distribution of amino acid residues in the polypeptide. For a rigid chain

$$s^2 = L_1^2 + L_2^2 + L_3^2 \quad (1)$$

where s^2 is the squared radius of gyration and L_i^2 are the principal moments of the inertia tensor. These moments are indexed so that $L_1^2 \geq L_2^2 \geq L_3^2$. Excellent approximations for the helical form of a polypeptide of high degree of polymerization are $L_1^2 = r^2/12$ and $L_2^2 = L_3^2 = 0$. A flexible molecule, such as the completely disordered polypeptide, can be viewed as an ensemble of configurations to each of which eq 1 applies. The mean square radius of gyration, $\langle s^2 \rangle$, is then

$$\langle s^2 \rangle = \langle L_1^2 \rangle + \langle L_2^2 \rangle + \langle L_3^2 \rangle \quad (2)$$

where $\langle L_i^2 \rangle$ denotes the statistical mechanical average of all principal moments with the indicated subscript. If the completely disordered polypeptide is unperturbed and of sufficiently high degree of polymerization, $\langle L_1^2 \rangle_0 : \langle L_2^2 \rangle_0 : \langle L_3^2 \rangle_0 = 0.765:0.172:0.0627$.[7-9]

At a high degree of polymerization s^2 and L_1^2 for the helix will be larger than $\langle s^2 \rangle$ and $\langle L_1^2 \rangle$ for the completely disordered polypeptide. The observed minimum in $\langle s^2 \rangle$ would occur if $\langle L_1^2 \rangle$ goes through a minimum during the helix-coil transition and does so when it completely dominates the remaining two principal moments. However, this behavior of $\langle L_1^2 \rangle$ is not mandatory if there is to be a minimum in $\langle s^2 \rangle$. The behavior of the remaining two principal moments, which are larger in the random coil than in the α helix, might contribute to the observation of a minimum in $\langle s^2 \rangle$. The minimum would arise with a monotonic change in $\langle L_1^2 \rangle$, provided this monotonic change took place primarily at high helicity while the change in the remaining two principal moments was concentrated at low helicity.

The purpose here is to characterize the relationship between helical content and $\langle L_i^2 \rangle$ for polypeptides of varying degree of polymerization. Asymmetry of the spatial distribution of amino acid residues is assessed through evaluation of the ratios $\langle L_2^2 \rangle / \langle L_1^2 \rangle$ and $\langle L_3^2 \rangle / \langle L_1^2 \rangle$. Poly(hydroxybutyl-L-glutamine) is chosen as the model polypeptide because appropriate conformational energy surfaces[6,10] and Zimm-Bragg statistical weights[11,12] are known. Results should have wider applicability because σ for poly(hydroxybutyl-L-glutamine) is of the same magnitude as that found with many other polypeptides. All calculations refer to the polypeptide in a state unperturbed by long-range interactions.

Calculations

Random Coil. The conformational energy surface used for a nonhelical amino acid residue is that obtained by Brant et al.[6] for unperturbed polypeptides bearing a CH_2R side chain in the L configuration. A variety of experimentally measured configuration-dependent physical properties of such polypeptides can be reproduced by calculations using this conformational energy surface. Such properties include the unperturbed dimensions of several homopolypeptides bearing CH_2R side chains,[10,13] dipole moments of 14 small peptides made up of L- and D-alanyl residues,[14] and proton magnetic resonance coupling constants between NH and $C^\alpha H$ of several homopolypeptides.[15] One of the homopolypeptides whose characteristic ratio was determined experimentally is poly(hydroxyethyl-L-glutamine),[10] an analogue of poly(hydroxybutyl-L-glutamine). The characteristic ratio deduced from experimental measurements in water is in excellent agreement with the conformational energy surface obtained by Brant et al.[6] for poly(L-alanine). A recent theoretical analysis[16] verifies poly(L-alanine) and poly(hydroxyethyl-L-glutamine) should have experimentally indistinguishable characteristic ratios. Further evidence for the validity of this conformational energy surface comes from experimental studies of configuration-dependent properties of copolypeptides containing amino acid residues bearing CH_2R side chains in the L configuration.[17-22]

Generation of a representative sample of nonhelical polypeptide chains by Monte Carlo methods was carried out as follows. Bond lengths and angles between bonds were those used by Brant et al.[6] Conformational states were defined at 10° intervals for φ and ψ, yielding 36^2 states for each amino acid residue. The number of states was reduced to 343 by elimination of those having a conformational energy more than 5 kcal mol^{-1} above that in the minimum-energy state. Each state retained was assigned a probability of $\exp[-E(\varphi,\psi)/RT]/\sum\exp[-E(\varphi,\psi)/RT]$, the summation extending over the 343 states. A representative sample of chains, grown using these probabilities and a random number generator, was found to have $\langle r^2 \rangle_0$ and $\langle s^2 \rangle_0$, in harmony with those computed with the exact generator matrix formulated with the averaged transformation matrix.[6]

Helix. The α helix was constructed by using $\varphi = 122.2°$ and $\psi = 133.0°$, dihedral angles being reported using the convention in which φ and ψ are each 0° in the completely extended chain.[23]

Partially Helical Polypeptides. Representative samples of partially helical polypeptide chains were generated by using a priori and conditional probabilities obtained from the matrix expression for the configuration partition function, Z.[3] The configuration partition function for a homopolypeptide can be written as

$$Z = \text{row } (1, 0)\ \mathbf{U}^{n_p}\ \text{col } (1, 1) \quad (3)$$

$$\mathbf{U} = \begin{bmatrix} 1 & \sigma S \\ 1 & S \end{bmatrix} \quad (4)$$

where $\mathbf{U}$ is the statistical weight matrix for an amino acid residue. Columns in $\mathbf{U}$ index the state of that amino acid residue, rows index the state of the preceding amino acid residue, and the order of indexing is c, h (coil, helix).

Let $p_{\eta;i}$ denote the a priori probability that amino acid residue i is in state η.[3] Then

$$p_{\eta;i} = Z^{-1}\ \text{row } (1, 0)\ \mathbf{U}^{i-1}\mathbf{U}_\eta'\mathbf{U}^{n_p-i}\ \text{col } (1, 1) \quad (5)$$

where $\mathbf{U}_\eta'$ is obtained from $\mathbf{U}$ by replacing with zero all elements except those in the column whose index is η. The average helical content, $\langle f \rangle$, is obtained as

$$\langle f \rangle = n_p^{-1} \sum p_{h;i} \quad (6)$$

The a priori probability that amino acid residue i ($i \neq 1$) is in state η, with amino acid residue $i - 1$ simultaneously in state ξ, is

$$p_{\xi\eta;i} = Z^{-1}\ \text{row } (1, 0)\ \mathbf{U}^{i-1}\mathbf{U}_{\xi\eta}'\mathbf{U}^{n_p-i}\ \text{col } (1, 1) \quad (7)$$

Here $\mathbf{U}_{\xi\eta}'$ denotes the matrix obtained from $\mathbf{U}$ when all

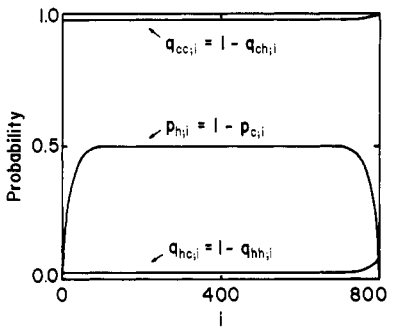

Figure 1. A priori and conditional probabilities for $n_p = 800$, $\sigma = 0.00068$, and $S = 1$.

elements except $u_{\xi\eta}$ are replaced by zero. Conditional probabilities can be calculated from the above a priori probabilities. The conditional probability that amino acid residue i ($i \neq 1$) is in state η, given that amino acid residue $i - 1$ is in state ξ, is

$$q_{\xi\eta;i} = p_{\xi\eta;i}/p_{\xi;i-1} \tag{8}$$

Representative chains were generated by using one or two random numbers for each amino acid residue in a growing chain. The amino acid residue was assigned as coil or helix by using the first random number and either $p_{\eta;1}$ or $q_{\xi\eta;i}$ ($i \neq 1$). If the amino acid residue was assigned as coil, a second random number was used to specify its φ and ψ (see above under Random Coil).

An illustrative set of probabilities is depicted in Figure 1 for the case where $n_p = 800$, $\sigma = 0.00068$, and $S = 1$. As expected for the case where S is unity, $p_{h;i}$ is found to be $^1/_2$ for those amino acid residues sufficiently remote from either chain end. The well-known end effect, producing a decline in helicity at the chain ends, is readily apparent. The end effect extends about 100 amino acid residues into the polypeptide chain for the case depicted in Figure 1. Representative chains, generated with probabilities calculated from eq 5 and 8, properly incorporate the end effects.

Configuration-Dependent Properties. Four sets, containing 1000–2000 chains each, were generated for each S and n_p. Nine different values of S were used for each n_p. All results depicted here use $\sigma = 0.00068$, which is the result obtained experimentally for poly(hydroxybutyl-L-glutamine) in water.[11] The value of S for this polypeptide is about 1.044 at 0 °C, and it falls to near 0.973 at 70 °C. Calculations reported here cover a broader range of S so that the entire helix–coil transition can be observed.

Standard deviations were calculated from the averages of each configuration-dependent property for the four sets. Bars on the data points denote ±1 standard deviation. In many cases the standard deviation is so small that the points are not distinguishable from the lines drawn through them.

Properties evaluated for each chain were f, r^2, s^2, L_1^2, L_2^2, and L_3^2. Only the C^α atoms were considered in the evaluation of the last five properties. The last four properties were obtained by first computing the inertia tensor, $\mathbf{S}_{x2}$, as[24]

$$\mathbf{S}_{x2} = (n_p + 1)^{-1}\sum \mathbf{r}_{0i}^{x2} - (n_p + 1)^{-2}(\sum \mathbf{r}_{0i})^{x2} \tag{9}$$

Here $\mathbf{r}_{0i}$ denotes the vector from the zeroth to the ith C^α atom, $x2$ as a superscript denotes the self-direct product, and $\mathbf{S}_{x2}$ is generated as a column of nine elements. The principal moments are obtained via an appropriate similarity transform, and s^2 is their sum. Validity of the sampling technique was demonstrated by successful reproduction of the dependence reported by Miller and Flory[4] for $\langle r^2 \rangle$ on $\langle f \rangle$ when σ is 0.003.

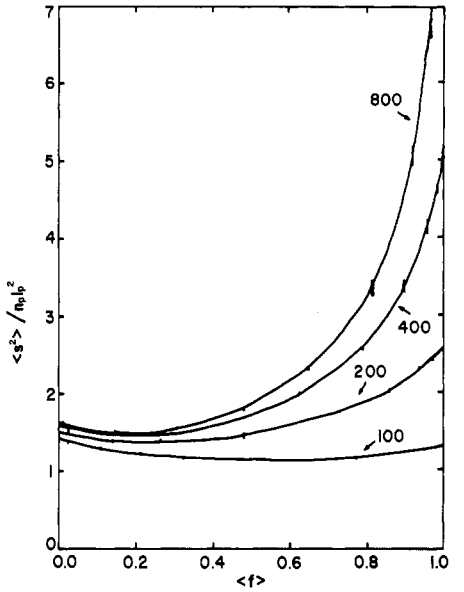

Figure 2. Relationship between $\langle s^2 \rangle / n_p l_p^2$ and helical content at the indicated n_p when σ is 0.00068.

Results and Discussion

Mean Square Radius of Gyration. Figure 2 depicts the dependence of $\langle s^2 \rangle / n_p l_p^2$ on $\langle f \rangle$ for four different n_p. This dimensionless ratio is found to be directly proportional to n_p when $\langle f \rangle$ is unity, as expected for a rigid helix. In contrast, only a small dependence on n_p is found when $\langle f \rangle$ is zero. The dimensionless ratio is then about 1.6 when n_p is 400 or 800 and becomes slightly lower at the two smaller n_p. This behavior is in harmony with that obtained with generator matrices which achieve an exact averaging over the conformational energy surface.[25]

In each case investigated the mean square unperturbed radius of gyration goes through a minimum as $\langle f \rangle$ increases from zero to unity. The minimum occurs at an $\langle f \rangle$ near 0.6 when n_p is 100. It moves to lower $\langle f \rangle$ as n_p increases, being observed at about $\langle f \rangle = 0.2$ for the longest polypeptide chain studied. The minimum occurs because of the small translation per residue along the axis of an α helix, causing short helices to have smaller dimensions than short segments of random coil.[4,26] Thus unperturbed dimensions for the completely disordered chain are slightly larger than those of the helix when n_p is 100. This situation is reversed at the higher degrees of polymerization.

Unperturbed dimensions for the longer polypeptide chains are especially sensitive to helical content when $\langle f \rangle$ is high. In the case where $n_p = 800$, for example, half the total change in $\langle s^2 \rangle$ is achieved when $\langle f \rangle$ is 0.95. This polypeptide has about 300 amino acid residues in an average helical segment at this helical content. The average number of amino acid residues in a helical segment has fallen substantially below the total number of amino acid residues in the polypeptide chain, causing a pronounced reduction in $\langle s^2 \rangle$.

Ratio of $\langle r^2 \rangle$ to $\langle s^2 \rangle$. Figure 3 depicts the behavior of $\langle r^2 \rangle / \langle s^2 \rangle$ for polypeptide chains as they undergo a helix–coil transition. An infinitely long polypeptide would have values for this ratio of 12 and 6, respectively, in the completely helical and completely disordered states. The behavior at the extremes of helical content in Figure 3 is in accord with this prediction.

While $\langle s^2 \rangle$ (Figure 2) and $\langle r^2 \rangle$ (not shown) pass through a minimum during the helix–coil transition, the ratio $\langle r^2 \rangle / \langle s^2 \rangle$ is found to be a monotonic function of helical

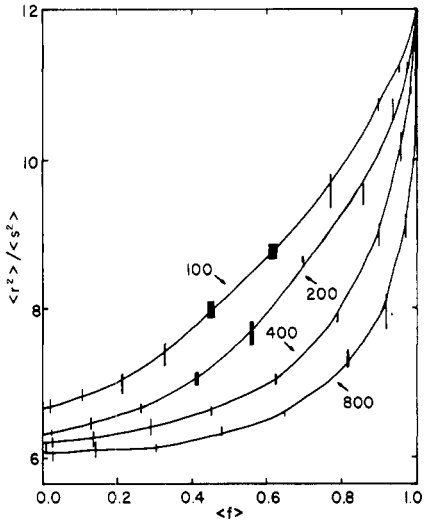

Figure 3. Dependence of $\langle r^2\rangle/\langle s^2\rangle$ on helical content at the indicated n_p when σ is 0.000 68.

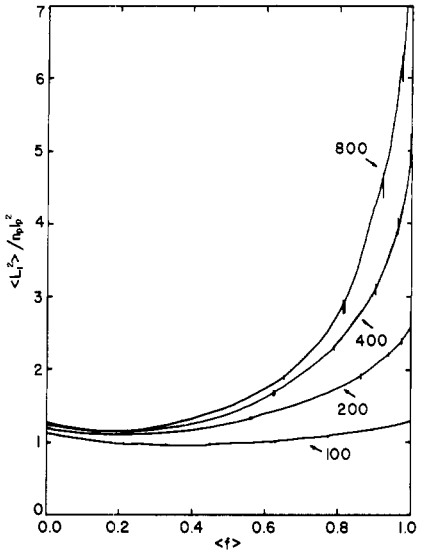

Figure 4. Dependence of $\langle L_1^2\rangle/n_p l_p^2$ on helical content at the indicated n_p when σ is 0.000 68.

content. It is nearly a linear function of $\langle f\rangle$ at a sufficiently low degree of polymerization. At a high degree of polymerization, on the other hand, the change in $\langle r^2\rangle/\langle s^2\rangle$ occurs only at high helical content. For example, $\langle r^2\rangle/\langle s^2\rangle$ has undergone half its total change when $\langle f\rangle$ is 0.97 if n_p is 800. Long polypeptide chains at low to moderate $\langle f\rangle$ may contain several helical segments connected by sequences of disordered residues. Such chains can retain $\langle r^2\rangle/\langle s^2\rangle$ characteristic of random coils even though a substantial number of their amino acid residues are helical. The increase in $\langle r^2\rangle/\langle s^2\rangle$ does not become pronounced until the average number of amino acid residues in a helical segment approaches n_p.

Averaged Principal Moments of the Inertia Tensor. Behavior of $\langle L_1^2\rangle/n_p l_p^2$ is depicted in Figure 4. Curves in this figure bear a strong similarity to those depicted in Figure 2. Corresponding curves in these two figures become identical as $\langle f\rangle$ approaches unity because L_1^2 is essentially equal to s^2 for a helix of small cross section. When $\langle f\rangle$ is zero, on the other hand, $\langle L_1^2\rangle$ is only about four-fifths as large as $\langle s^2\rangle$, as expected.[7-9]

Each $\langle L_1^2\rangle$ in Figure 4 goes through a minimum as the helix–coil transition takes place. This minimum occurs near $\langle f\rangle = 0.2$ for the polypeptide chain of highest degree of polymerization. The minimum $\langle s^2\rangle$ for these chains was

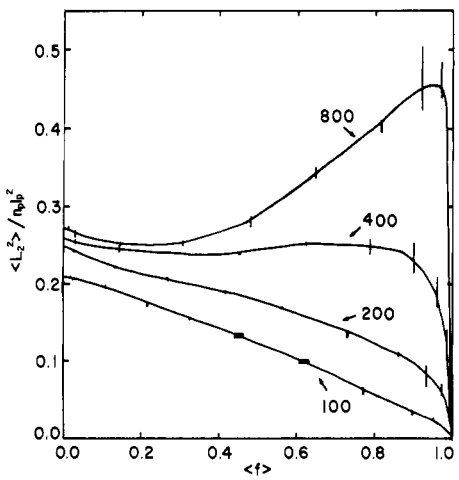

Figure 5. Dependence of $\langle L_2^2\rangle/n_p l_p^2$ on helical content at the indicated n_p when σ is 0.000 68.

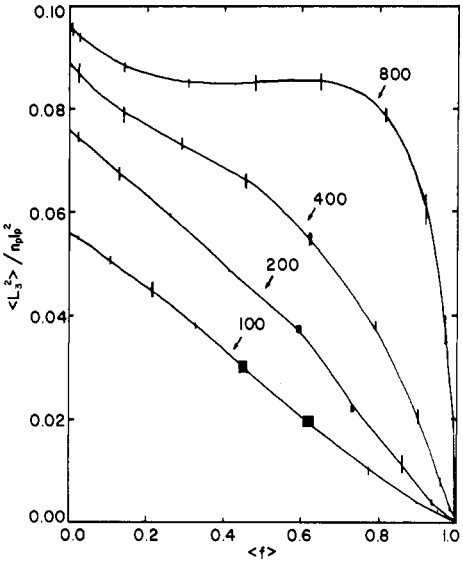

Figure 6. Dependence of $\langle L_3^2\rangle/n_p l_p^2$ on helical content at the indicated n_p when σ is 0.000 68.

observed at about the same helical content (Figure 2). Short chains, however, experience their minimum $\langle L_1^2\rangle$ at helical contents substantially lower than those giving the minimum $\langle s^2\rangle$.

The behavior of $\langle L_2^2\rangle$ and $\langle L_3^2\rangle$ is depicted in Figures 5 and 6. The smaller two principal moments are essentially zero when the helical content is unity, and they are positive when the helical content is zero, as expected. All three $\langle L_i^2\rangle/n_p l_p^2$ for the completely disordered polypeptide chain converge to an asymptotic limit as n_p increases. Based on $(\langle s^2\rangle/n_p l_p^2)_\infty$[6] and the limiting ratios of the principal moments,[7-9] the $(\langle L_i^2\rangle/n_p l_p^2)_\infty$ are 1.18, 0.27, and 0.097. Convergence to the limiting values is not equally rapid for all principal moments. Convergence is obtained most rapidly with $\langle L_1^2\rangle$ and most slowly with $\langle L_3^2\rangle$.

The $\langle L_i^2\rangle$, $i = 2, 3$, are found to be a monotonic, nearly linear, function of $\langle f\rangle$ at a sufficiently low degree of polymerization. Markedly different behavior is seen at a high degree of polymerization. These principal moments then pass through a minimum and a maximum as the coil–helix transition occurs. The minimum and maximum for $\langle L_2^2\rangle$ occur at $\langle f\rangle$ near 0.2 and 0.95, respectively, when n_p is 800. A less dramatic maximum and minimum are seen with $\langle L_3^2\rangle$, and they are located closer to $\langle f\rangle = 0.5$.

A rationalization can be devised for the behavior of the $\langle L_i^2\rangle$ at high $\langle f\rangle$ when n_p is 800. The maximum for $\langle L_2^2\rangle$

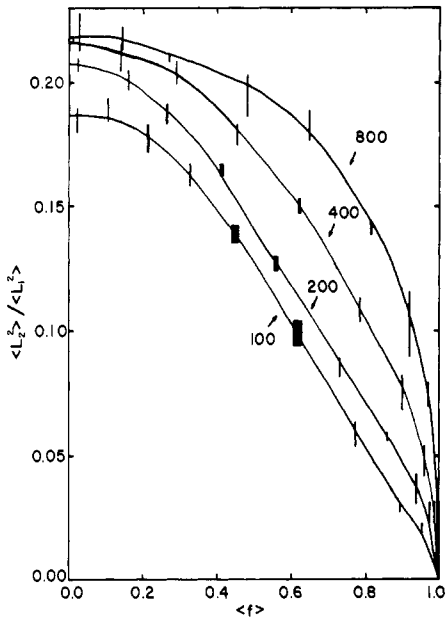

Figure 7. Dependence of $\langle L_2^2\rangle/\langle L_1^2\rangle$ on helical content at the indicated n_p when σ is 0.000 68.

is especially dramatic with this polypeptide. It occurs near the helical content at which the average number of amino acid residues in a helical segment is $n_p/2$. An illuminating, even though oversimplified, model of the polypeptide chain under these conditions is two rodlike segments connected by a short random-coil segment. The rodlike segments will not, in general, be coaxial, and there will frequently be a large angle between their axes. Consequently $\langle L_1^2\rangle$ will be much smaller than it was when $\langle f\rangle$ was unity. However, $\langle L_2^2\rangle$ will be considerably larger than the value of essentially zero found in the completely rigid helix. A further decline in the average number of amino acid residues in a helical segment, achieved when $\langle f\rangle$ falls below about 0.95, produces a decline in both $\langle L_1^2\rangle$ and $\langle L_2^2\rangle$. If thin rods are connected by a universal joint, $\langle L_3^2\rangle$ is zero when the number of rods is two, but $\langle L_3^2\rangle > 0$ if there are three or more rods. Therefore the maximum for $\langle L_3^2\rangle$ is observed at smaller $\langle f\rangle$ than the maximum for $\langle L_2^2\rangle$.

At sufficiently low helical content, $d\langle L_i^2\rangle/d\langle f\rangle$ is invariably found to be negative. The average helical segment is short at low $\langle f\rangle$, and introduction of short helical segments into a disordered polypeptide is known to cause a reduction in $\langle s^2\rangle$.[4,26] Contraction of the distribution of C^α atoms along all three principal axes contributes to the decline in $\langle s^2\rangle$.

If n_p is 800, both $\langle L_1^2\rangle$ and $\langle L_2^2\rangle$ go through a minimum when $\langle f\rangle$ is about 0.2, and $\langle L_3^2\rangle$ goes through a minimum near $\langle f\rangle = 0.4$. Hence both $\langle L_1^2\rangle$ and $\langle L_2^2\rangle$ (and $\langle L_3^2\rangle$ to a lesser extent) contribute to the observation of a minimum $\langle s^2\rangle$ near $\langle f\rangle = 0.2$. A different situation arises at a low degree of polymerization. A minimum still occurs with $\langle L_1^2\rangle$, but the changes in $\langle L_2^2\rangle$ and $\langle L_3^2\rangle$ are now monotonic. The largest principal moment is sufficiently dominant to still produce a minimum in $\langle s^2\rangle$, but the monotonic change of the smaller two principal moments causes $\langle s^2\rangle$ to pass through its minimum at higher $\langle f\rangle$ than the minimum for $\langle L_1^2\rangle$.

Ratios of the Averaged Principal Moments. The ratios $\langle L_2^2\rangle/\langle L_1^2\rangle$ and $\langle L_3^2\rangle/\langle L_1^2\rangle$ are depicted in Figures 7 and 8. They are found to be monotonic functions of $\langle f\rangle$. This behavior is in contrast to that depicted for the individual $\langle L_i^2\rangle$ in Figures 4–6. A correlation is observed between the behavior of $\langle r^2\rangle/\langle s^2\rangle$ (Figure 3) and $\langle L_2^2\rangle/\langle L_1^2\rangle$ (Figure 7). The $\langle f\rangle$ required to produce half the total

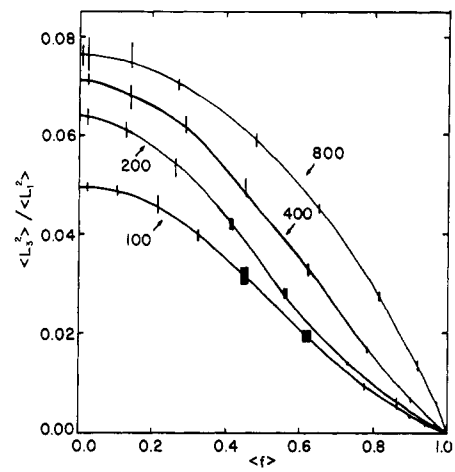

Figure 8. Dependence of $\langle L_3^2\rangle/\langle L_1^2\rangle$ on helical content at the indicated n_p when σ is 0.000 68.

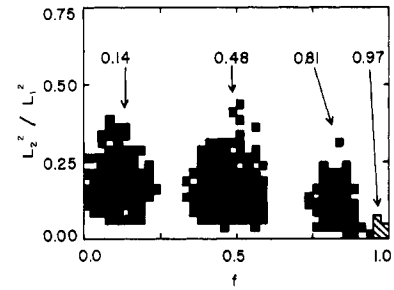

Figure 9. Relationship between fluctuations in L_2^2/L_1^2 and f when $\langle f\rangle$ is 0.14, 0.48, 0.81, or 0.97. Half of the chains generated fall within the areas indicated. In all cases n_p is 800 and σ is 0.000 68.

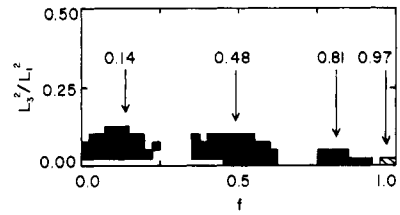

Figure 10. Relationship between fluctuations in L_3^2/L_1^2 and f when $\langle f\rangle$ is 0.14, 0.48, 0.81, or 0.97. Half of the chains generated fall within the areas indicated. In all cases n_p is 800 and σ is 0.000 68.

change in $\langle r^2\rangle/\langle s^2\rangle$ is larger by only 0.09 ± 0.04 than the $\langle f\rangle$ required to produce half the change in $\langle L_2^2\rangle/\langle L_1^2\rangle$.

As n_p approaches infinity, $\langle L_2^2\rangle/\langle L_1^2\rangle$ and $\langle L_3^2\rangle/\langle L_1^2\rangle$ for the completely disordered polypeptide should approach asymptotic limits of 0.225 and 0.082, respectively.[9] Dependence of these ratios on n_p in Figures 7 and 8 is in reasonable accord with this prediction. Disordered poly(hydroxybutyl-L-glutamine) chains with n_p = 100–400 have lower $\langle L_2^2\rangle/\langle L_1^2\rangle$ and $\langle L_3^2\rangle/\langle L_1^2\rangle$ (are more asymmetric) than a completely disordered chain of infinitely high molecular weight. A study of simple chains reveals short chains are more asymmetric than long chains if the bond angle exceeds 100°.[9] The angle between the virtual bond from C^α_i to C^α_{i+1} and the virtual bond from C^α_{i+1} to C^α_{i+2} is variable but usually greater than 100°. Therefore the observation that disordered poly(hydroxybutyl-L-glutamine) of low molecular weight is more asymmetric than a polypeptide of high molecular weight is in accord with the behavior of simpler polymer chains.

Fluctuations in Asymmetry. A 40 × 40 × 40 grid, with axes representing f, L_2^2/L_1^2, and L_3^2/L_1^2, was established, and an entry was made in this grid for each chain generated at n_p = 800. Figures 9 and 10 demonstrate that

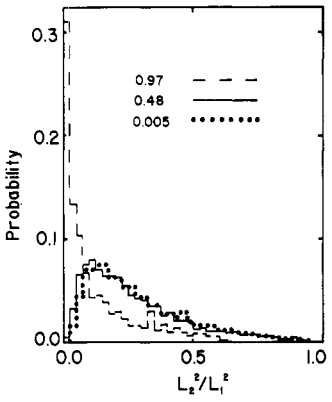

Figure 11. Probability of observation of a particular L_2^2/L_1^2 (at intervals of 0.025) for n_p = 800, σ = 0.000 68, and the indicated $\langle f \rangle$.

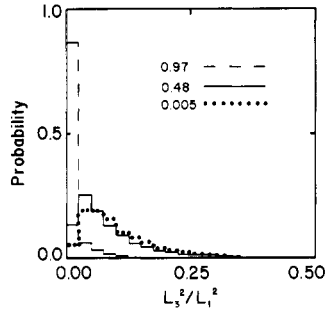

Figure 12. Probability of observation of a particular L_3^2/L_1^2 (at intervals of 0.025) for n_p = 800, σ = 0.000 68, and the indicated $\langle f \rangle$.

the greatest fluctuation in f occurs when $\langle f \rangle$ is near 0.5, as expected. The largest fluctuations in L_2^2/L_1^2 and L_3^2/L_1^2, on the other hand, occur when $\langle f \rangle$ is in the range 0.0–0.5 (Figures 9 and 10).

Distribution functions for L_2^2/L_1^2 for the polypeptide with n_p = 800 are depicted in Figure 11. Nearly identical distribution functions are found when $\langle f \rangle$ is 0.005 and 0.48, but a markedly different distribution function occurs at $\langle f \rangle$ = 0.97. This behavior is in accord with that depicted for the $\langle L_2^2 \rangle / \langle L_1^2 \rangle$ for this polypeptide in Figure 7. A predominantly disordered, long poly(hydroxybutyl-L-glutamine) chain can therefore take on substantial helical content with little alteration in either $\langle L_2^2 \rangle / \langle L_1^2 \rangle$ or the distribution for L_2^2/L_1^2.

The distribution function for L_2^2/L_1^2 displays a long tail in the random coil and in the half-helical polypeptide. A long tail persists even when the helical content has risen as high as 0.97. A once-broken rod will be a reasonable model for a significant portion of the polypeptide chains at such high helical content. Since once-broken rods can have quite high L_2^2/L_1^2, the persistence of the tail in the distribution function can be attributed to their presence.

Distribution functions for L_3^2/L_1^2 are depicted in Figure 12. Once again the polypeptide with $\langle f \rangle$ = 0.97 has a distribution function markedly different from that seen when $\langle f \rangle$ is 0.48 or 0.005. The distribution for L_3^2/L_1^2 is much sharper than that for L_2^2/L_1^2 when $\langle f \rangle$ is 0.97. Once-broken rods may have larger L_2^2/L_1^2, but they will have a small L_3^2/L_1^2.

Dimensions will be modified if there is incomplete compensation from effects of long-range and polymer-solvent interactions, as has been shown in a study where atoms are treated as hard spheres.[27]

Acknowledgment. This work was supported by National Science Foundation Research Grant PCM 78-22916.

References and Notes

(1) Doty, P.; Bradbury, J. H.; Holtzer, A. M. *J. Am. Chem. Soc.* **1956**, *78*, 947.
(2) Doty, P.; Wada, A.; Yang, J. T.; Blout, E. R. *J. Polym. Sci.* **1957**, *23*, 851.
(3) Flory, P. J. *Macromolecules* **1974**, *7*, 381.
(4) Miller, W. G.; Flory, P. J. *J. Mol. Biol.* **1966**, *15*, 298.
(5) Zimm, B. H.; Bragg, J. K. *J. Chem. Phys.* **1959**, *31*, 526.
(6) Brant, D. A.; Miller, W. G.; Flory, P. J. *J. Mol. Biol.* **1967**, *23*, 47.
(7) Solc, K.; Stockmayer, W. H. *J. Chem. Phys.* **1971**, *54*, 2756.
(8) Solc, K. *J. Chem. Phys.* **1971**, *55*, 335.
(9) Mattice, W. L. *Macromolecules*, in press.
(10) Mattice, W. L.; Lo, J. T. *Macromolecules* **1972**, *5*, 734.
(11) von Dreele, P. H.; Lotan, N.; Ananthanarayanan, V. S.; Andreatta, R. H.; Poland, D.; Scheraga, H. A. *Macromolecules* **1971**, *4*, 408.
(12) McCord, R. W.; Madison, E. L.; Mattice, W. L. *Biopolymers*, in press.
(13) Brant, D. A.; Flory, P. J. *J. Am. Chem. Soc.* **1965**, *87*, 2788.
(14) Flory, P. J.; Schimmel, P. R. *J. Am. Chem. Soc.* **1967**, *89*, 6807.
(15) Tonelli, A. E.; Bovey, F. A. *Macromolecules* **1970**, *3*, 410.
(16) Paterson, Y.; Leach, F. A. *Macromolecules* **1978**, *11*, 418.
(17) Miller, W. G.; Brant, D. A.; Flory, P. J. *J. Mol. Biol.* **1967**, *23*, 67.
(18) Miller, W. G.; Goebel, C. V. *Biochemistry* **1968**, *7*, 3925.
(19) Mattice, W. L.; Mandelkern, L. *Biochemistry* **1971**, *10*, 1934.
(20) Mattice, W. L.; Riser, J. M.; Clark, D. S. *Biochemistry* **1976**, *15*, 4264.
(21) Mattice, W. L. *Macromolecules* **1977**, *10*, 516.
(22) Mattice, W. L. *Biopolymers* **1979**, *18*, 225.
(23) Ramachandran, G. N.; Sasisekharan, V. *Adv. Protein Chem.* **1968**, *23*, 283.
(24) Flory, P. J.; Yoon, D. Y. *J. Chem. Phys.* **1974**, *61*, 5358.
(25) Brant, D. A.; Flory, P. J. *J. Am. Chem. Soc.* **1965**, *87*, 2791.
(26) Nagai, K. *J. Chem. Phys.* **1961**, *34*, 887.
(27) Neves, D. E.; Scott, R. A. *Macromolecules* **1976**, *9*, 554.