the effects of increasing temperature and potassium chloride concentration oppose each other (Figure 8). The opposite situation is encountered with poly(N^5-ω-hydroxyethyl-L-glutamine) and poly(L-glutamic acid), with the effects of increasing temperature and potassium chloride concentration being cumulative (Figure 5). This observation constitutes further evidence that the effect of salts on the circular dichroism of ionized polypeptides cannot be due to the formation of segments of α helix in the presence of the salts.

The suggestion that the isothermal addition of salts leads to the formation of α helix in charged homopolypeptides has led to conclusions which are inconsistent with other properties of polypeptides. Based on the assumption that ionized poly(L-lysine) is in an unordered conformation in the absence of added salt and becomes increasingly α helical when salt is added, curve fitting of the circular dichroism from 212 to 228 nm led to estimates of 14.7% helix in 3 *M* calcium chloride and 32.3% helix in 6 *M* calcium chloride.[7] The common experience of other investigators has been that calcium chloride destabilizes the ordered structures formed by proteins, as has been documented with ribonuclease,[31] collagen,[32,33] elastoidin fibers,[34] and glycerinated muscle fibers.[35] The positive band observed in the circular dichroism of poly(L-proline)[21,36] and poly(γ-hydroxy-L-proline)[37] in water is eliminated by the isothermal addition of calcium chloride. It is impossible, for steric reasons, that the results with poly(L-proline) and poly(γ-hydroxy-L-proline) could reflect an increase in the amount of α helix in the presence of calcium chloride.[38] Finally, Iizuka and Yang have shown that the pH at the midpoint of the helix–coil transition of poly(L-glutamic acid) is shifted to lower pH values in the presence of salts, with the exception of high concentrations of potassium fluoride.[25] This observation is not consistent with the formation of additional α helix when salt is added at constant pH.

The data presented here are rather more effective in showing what the effect of salt is not than they are in defining the mechanism of action of the salts. It can be concluded, however, that the circular dichroism spectra of poly(L-glutamic acid) and poly(N^5-ω-hydroxyethyl-L-glutamine) reported here are not due to changes in the content of α helix and do not support the proposal that the interaction of the charged side chains in ionized polypeptides leads to a locally ordered structure.

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(31) P. H. von Hippel and K. Y. Wong, *J. Biol. Chem.*, **240**, 3909 (1965).

(32) J. Bello, H. C. A. Riese, and J. R. Vinograd, *J. Phys. Chem.*, **60**, 1299 (1956).

(33) P. H. von Hippel and K. Y. Wong, *Biochemistry*, **2**, 1387 (1963).

(34) L. Mandelkern, W. T. Meyer, and A. F. Diorio, *J. Phys. Chem.*, **66**, 375 (1962).

(35) L. Mandelkern and E. A. Villarico, *Macromolecules*, **2**, 394 (1969).

(36) M. L. Tiffany and S. Krimm, *Biopolymers*, **6**, 1767 (1968).

(37) W. L. Mattice and L. Mandelkern, *Macromolecules*, **3**, 199 (1970).

(38) P. R. Schimmel and P. J. Flory, *Proc. Nat. Acad. Sci. U. S.*, **58**, 52 (1967).

Unperturbed Dimensions of Poly(N^5-ω-hydroxyethyl-L-glutamine) in Water[1]

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ABSTRACT: The characteristic ratio of poly(N^5-ω-hydroxyethyl-L-glutamine) in water at 30° is found to be 10 ± 1, in agreement with results obtained by Brant and Flory for four other polypeptides with $-CH_2R$ side chains. The circular dichroism of poly(N^5-ω-hydroxyethyl-L-glutamine) under these conditions, where the polypeptide is in a statistical conformation, exhibits a positive band at 216 nm. The circular dichroism of poly(N^5-ω-hydroxyethyl-L-glutamine) and ionized poly(L-glutamic acid) are similar but not identical under conditions where they have the same characteristic ratio. The possibility that this observation could arise from the presence of short left-handed helices in the ionized poly(L-glutamic acid) is *considered* and found to be improbable.

The Moffitt[2] parameter b_0 for poly(N^5-ω-hydroxyethyl-L-glutamine) in water is between 0 and 40 (deg cm^2)/dmol over the temperature range 0 to 70°.[3] Consequently, poly(N^5-ω-hydroxyethyl-L-glutamine) has been used as a model for a random-coil water-soluble polypeptide with a $-CH_2R$ side chain.[4,5] Poly(N^5-ω-hydroxyethyl-L-glutamine) is not a polyelectrolyte, in contrast to two other frequently used models for random-coil polypeptides, poly(L-glutamic acid) and poly(L-lysine) at neutral pH.[6-14] Recently, suggestions based upon calculations and circular dichroism measurements have indicated that ionized homopolypeptides such as poly(L-

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(1) This work was supported by a Frederick Gardner Cotrell grant in aid.

(2) W. Moffitt, *J. Chem. Phys.*, **25**, 467 (1956).

(3) N. Lotan, A. Yaron, and A. Berger, *Biopolymers*, **4**, 365 (1966).

(4) A. J, Adler, R. Hoving, J. Potter, M. Wells, and G. D. Fasman, *J. Amer. Chem. Soc.*, **90**, 4736 (1968).

(5) F. J. Joubert, N. Lotan, and H. A. Scheraga, *Biochemistry*, **9**, 2197 (1970).

(6) P. Doty, A. Wada, J. T. Yang, and E. R. Blout, *J. Polym. Sci.*, **23**, 851 (1957).

(7) K. Rosenheck and P. Doty, *Proc. Nat. Acad. Sci. U. S.*, **47**, 1775 (1961).

(8) S. Takashima, *Biopolymers*, **1**, 171 (1963).

(9) M. Nagasawa and A, Holtzer, *J. Amer. Chem. Soc.*, **86**, 538 (1964).

(10) G. Holzwarth and P. Doty, *ibid.*, **87**, 218 (1965).

(11) E. Iizuka and J. T. Yang, *Biochemistry*, **4**, 1249 (1965).

(12) R. McDiarmid and P. Doty, *J. Phys. Chem.*, **70**, 2620 (1966).

(13) N. Greenfield and G. D. Fasman, *Biochemistry*, **6**, 1630 (1967).

(14) N. Greenfield and G. D. Fasman, *ibid.*, **8**, 4108 (1969).

glutamic acid) and poly(L-lysine) should not be used as model random coils because they are proposed to assume partially ordered conformations under appropriate conditions.[15–18] It therefore becomes of interest to determine whether poly(N^5-ω-hydroxyethyl-L-glutamine) has the dimensional properties of a statistical coil; to compare the results to those obtained[19] with other polypeptides with $-CH_2R$ side chains, both charged and uncharged; and to determine what the implications are for the interpretation of the optical activity of polypeptides.

Materials and Methods

Materials. A slight modification of the method used to prepare poly(N^5-ω-hydroxypropyl-L-glutamine) from poly(γ-benzyl L-glutamate)[20] was utilized in the preparation of poly(N^5-ω-hydroxyethyl-L-glutamine). Poly(L-glutamic acid) was obtained from Sigma (Lot 120C-3140) as the sodium salt. Fractionation of the poly(N^5-ω-hydroxyethyl-L-glutamine) was accomplished using a 2.8 × 88 cm Sephadex G-100 column with water as the eluting agent. Polymer solutions were prepared in volumetric flasks using polypeptide samples which had been dried in a vacuum oven using a Dry Ice–diethylene glycol–monobutyl ether trap.

Viscosity. Viscosity measurements were carried out in the manner previously described.[21]

Circular Dichroism and Optical Rotatory Dispersion. Circular dichroism and optical rotatory dispersion were measured using a Durrum-Jasco Model J-20 recording spectropolarimeter. Calibration of the circular dichroism was based on $[\theta]_{291} = 7260$ (deg cm^2)/dmol for camphorsulfonic-d_{10} acid in water.[22] Temperature control was achieved by the circulation of water from a constant temperature bath through a hollow metal cell holder.

Osmometry. Osmotic pressures were determined in water using a Melabs CSM-2 osmometer equipped with an S & S B-20 membrane. Permeation of the polypeptide through the membrane was significant with the lowest molecular weight fraction.

Computations. Computation of the unperturbed dimensions of partially ordered polypeptides was accomplished using an IBM 360/65I computer. The necessary equations have been described by Flory.[23] They will be identified here by the chapter and equation numbers from ref 23. The mean-square end-to-end distance was computed from eq IV-28. Equation III-40 was used for the configuration partition function, and the matrices required in eq IV-28 were generated from eq IV-24, VII-27, and VII-40. The 2 × 2 identity matrix was used in place of eq VII-27 for the first peptide unit. The averaged transformation matrix for the random coil was taken from Brant and Flory,[24] and the transformation matrix for a particular helical geometry was evaluated from eq VII-11 using the appropriate values of the rotational angles ϕ and ψ, defined by the 1966 convention,[25] and standard values for the angles between bonds in the peptide unit.[26] The fraction of the amino acid residues in the helical state and the average number of amino acid residues in a helical sequence were calculated from s and σ, defined by Zimm and Bragg,[27] using eq VII-28, VII-34, and VII-36.

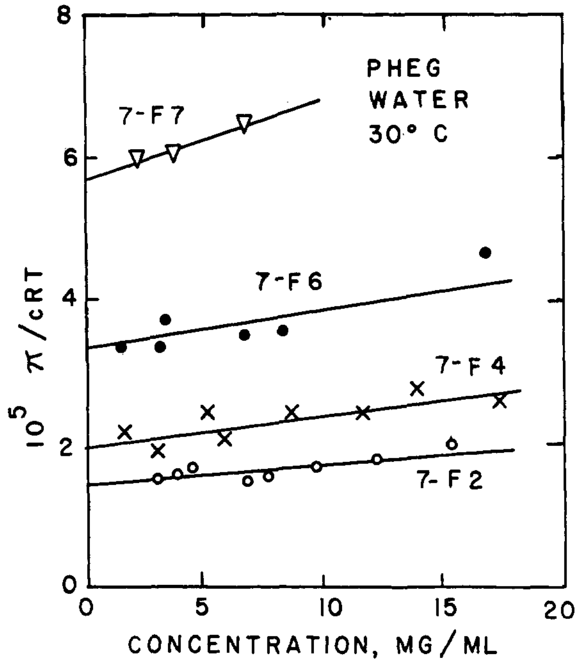

Figure 1. Reduced osmotic pressures of poly(N^5-ω-hydroxyethyl-L-glutamine) (PHEG) in water at 30°.

TABLE I
DATA FROM OSMOMETRY AT 30° IN WATER

Fraction	M_n	n_p	A_2, (cm^3 mol)/g^2
7-F7	17,500 ± 1000	101 ± 6	0.0011 ± 0.0001
7-F6	31,000 ± 2000	179 ± 12	0.00060 ± 0.00006
7-F4	51,000 ± 3000	296 ± 17	0.00043 ± 0.00005
7-F2	72,000 ± 3000	418 ± 17	0.00033 ± 0.00004

Results

Osmometry. The molecular weights for four fractions of poly(N^5-ω-hydroxyethyl-L-glutamine) were determined from the osmotic pressures in water at 30° using eq 1.[28] The osmotic pressure is given by π, c is the polymer concentration, R the gas constant, T the absolute temperature, M_n the number-average molecular weight, and A_2 the second virial coefficient.

$$\pi(cRT)^{-1} = M_n^{-1} + A_2c \qquad (1)$$

Figure 1 shows the reduced osmotic pressure for the four fractions plotted against concentration. Permeation was significant with the lowest molecular weight fraction. The number-average molecular weights obtained from the intercepts, the corresponding number of peptide bonds, and the second virial coefficients obtained from the slopes are presented in Table I. The uncertainties reflect reasonable alternative straight lines through the data. They do not include uncertainties arising from permeation of the lowest molecular weight fraction through the membrane. Therefore, the uncertainties for fraction 7-F7 represent a lower limit. The positive second virial coefficients show that water is a thermodynamically good solvent for poly(N^5-ω-hydroxyethyl-L-glutamine) at 30°. The second virial coefficients decrease

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(15) S. Krimm and J. E. Mark, *Proc. Nat. Acad. Sci. U. S.*, **60**, 1122 (1968).

(16) M. L. Tiffany and S. Krimm, *Biopolymers*, **6**, 1379 (1969).

(17) M. L. Tiffany and S., Krimm, *ibid.*, **8**, 347 (1969).

(18) S. Krimm, J. E. Mark, and M. L. Tiffany, *ibid.*, **8**, 695 (1969).

(19) D. A. Brant and P. J. Flory, *J. Amer. Chem. Soc.*, **87**, 2788 (1965).

(20) N. Lupu-Lotan, A. Yaron, A. Berger, and M. Sela, *Biopolymers*, **3**, 625 (1965).

(21) W. L. Mattice and L. Mandelkern, *Biochemistry*, **9**, 1049 (1970).

(22) J. Y. Cassim and J. T. Yang, *ibid.*, **8**, 1947 (1969).

(23) P. J. Flory, "Statistical Mechanics of Chain Molecules," Interscience, New York, N. Y., 1969.

(24) D. A. Brant and P. J. Flory, *J. Amer. Chem. Soc.*, **87**, 2791 (1965).

(25) J. T. Edsall, P. J. Flory, J. P. Kendrew, A. M. Liquori, G. Nemethy, G. N. Ramachandran, and H. A. Scheraga, *Biopolymers*, **4**, 130 (1966); *J. Biol. Chem.*, **241**, 1004 (1966); *J. Mol. Biol.*, **15**, 339 (1966).

(26) Reference 23, p 251.

(27) B. H. Zimm and J. K. Bragg, *J. Chem. Phys.*, **31**, 526 (1959).

(28) P, J. Flory, "Principles of Polymer Chemistry," Cornell University Press, Ithaca, N. Y., 1953, p 280.

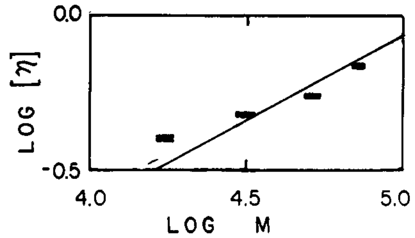

Figure 2. Log [η] *vs.* log molecular weight for poly(N^5-ω-hydroxyethyl-L-glutamine) in water at 30°.

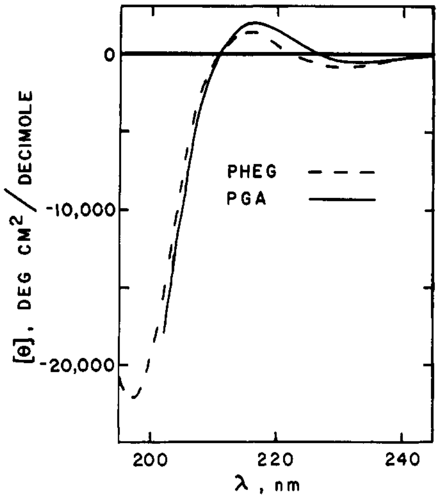

Figure 3. Circular dichroism of PHEG at 30° in water and of poly(L-glutamic acid) (PGA) at 37° in 0.3 *M* sodium phosphate, pH 7.85.

with increasing molecular weight, which is the anticipated result for a random-coil polymer.[29]

Intrinsic Viscosity. The intrinsic viscosities were obtained from the linear extrapolation of the reduced viscosity to zero concentration. The results obtained at 30° in water are presented in Figure 2 as the logarithm of the intrinsic viscosity *vs.* the logarithm of the number-average molecular weight. The exponent a in eq 2 is 0.50 for a random-coil polymer when the solution is ideal, *i.e.*, when the second virial coefficient is zero.[30] In good solvents, where the second virial coefficient is positive, a should be between 0.5 and 0.8 for a random-coil polymer.[30] The fractions used here do not cover a broad enough molecular weight range to define a precisely. The problem is made more difficult by the possibility of appreciable error in the molecular weight of the lowest molecular weight fraction due to permeation through the osmometer membrane. The line drawn in Figure 2 has a slope of 0.55. A line of approximately this slope will be a reasonably good representation of the data for the three highest molecular weight fractions, and signifies a random-coil conformation for poly(N^5-ω-hydroxyethyl-L-glutamine) in water at 30°.

$$[\eta] = K'M^a \tag{2}$$

Circular Dichroism and Optical Rotatory Dispersion. The circular dichroism and optical rotatory dispersion of poly(N^5-ω-hydroxyethyl-L-glutamine) and poly(L-glutamic acid) are shown in Figures 3 and 4. The spectra for poly(L-glutamic acid) were obtained at 37° in 0.3 *M* sodium phosphate, pH 7.85, corresponding to the conditions used by Brant and Flory[19] to measure the unperturbed dimensions. The spectra for poly(N^5-ω-hydroxyethyl-L-glutamine) were measured at 30° in water. The circular dichroism of poly(N^5-ω-hydroxyethyl-L-glutamine) in Figure 3 is taken from a recent study of the effects of temperature and several salts on the circular dichroism of poly(N^5-ω-hydroxyethyl-L-glutamine).[31]

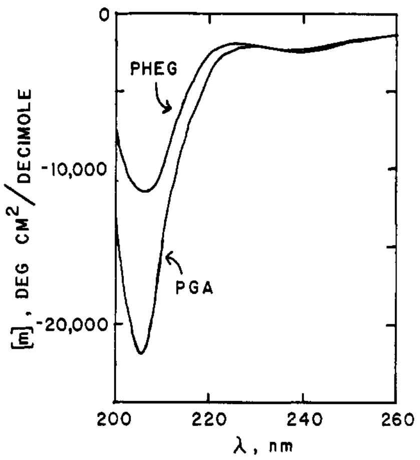

Figure 4. Optical rotatory dispersion of PHEG at 30° in water and of PGA at 37° in 0.3 *M* sodium phosphate, pH 7.85.

The circular dichroism for both polypeptides exhibits a small negative band at 231–233 nm, a larger positive band at 215–216 nm, and a much more intense negative band at lower wavelengths. The wavelength at which the maximum negative circular dichroism is obtained could not be measured with poly(L-glutamic acid) due to the absorption of light by the buffer. The maximum signal is obtained at 198 nm with poly(N^5-ω-hydroxyethyl-L-glutamine). The positive circular dichroism is more intense for poly(L-glutamic acid) than for poly(N^5-ω-hydroxyethyl-L-glutamine). While the maximum intensity of the large negative circular dichroism band in the poly(L-glutamic acid) spectrum is not directly observable, the relative intensities of the negative band near 200 nm for both polypeptides can be inferred from the optical rotatory dispersion spectra. The optically active spectral transition responsible for the negative circular dichroism at 198 nm will give rise to a trough in the optical rotation at slightly longer wavelengths.[32] The trough observed at 204–205 nm in Figure 4 is much more intense for poly(L-glutamic acid) than for poly(N^5-ω-hydroxyethyl-L-glutamine), and implies that the negative circular dichroism band just below 200 nm is also more intense for poly(L-glutamic acid) than for poly(N^5-ω-hydroxyethyl-L-glutamine). Previous comparisons of the circular dichroism of poly(N^5-ω-hydroxyethyl-L-glutamine) and ionized poly(L-glutamic acid) in solvents which transmit light in the appropriate spectral region confirm these conclusions.[4,31]

Discussion

The calculation of the experimental characteristic ratio of poly(N^5-ω-hydroxyethyl-L-glutamine) in water at 30° can be carried out from the measurements reported here using eq 3–5.[19,33,34] The intrinsic viscosity in a Θ solvent is $[\eta]_\Theta$, M_0

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(29) Reference 28, p 536.

(30) Reference 28, p 622.

(31) W. L, Mattice, J. T. Lo, and L. Mandelkern, *Macromolecules*, **6**, 729 (1972).

(32) L. Velluz, M. Legrand, and M. Grosjean, "Optical Circular Dichroism," Academic Press, New York, N. Y., 1965, p 11.

(33) P. J. Flory, *J. Chem. Phys.*, **17**, 303 (1949).

(34) T. A. Orofino and P. J. Flory, *ibid.*, **26**, 1067 (1957).

$$\{r^2\}_0/n_pl_p{}^2 = ([\eta]_\Theta/\Phi M^{1/2})^{2/3}M_0l_p{}^{-2} \quad (3)$$

$$[\eta]_\Theta = [\eta]/\alpha^3 \quad (4)$$

$$A_2M/[\eta] = 2^{5/2}\pi N_0(27\Phi)^{-1} \ln [1 + 0.5\pi^{1/2}(\alpha^2 - 1)] \quad (5)$$

is the molecular weight of an ω-hydroxyethyl-L-glutamine residue, α is the expansion coefficient, N_0 is Avogadro's number, and Φ is a universal constant. This procedure has been used previously to determine the experimental characteristic ratio of random coil polypeptides from data obtained in good solvents.[19, 35–38] Since Φ may depend slightly upon the strength of solvent–polymer interactions,[39, 40] expansion coefficients and characteristic ratios were calculated using $\Phi = 0.0021$ and $\Phi = 0.0026$, where the intrinsic viscosity is expressed in deciliters per gram and the dimensions are in ångströms. These values encompass the range in Φ which has been used in calculating the unperturbed dimensions of random-coil polypeptides[19, 35–38] and analogous polyesters.[41]

The expansion coefficients and characteristic ratios calculated from eq 3–5 are presented in Table II. Since the expansion coefficients are only slightly greater than unity, the correction for the nonideality of the solvent is small. Any trend in the expansion coefficient with molecular weight, in the range covered, does not exceed the experimental uncertainty. The characteristic ratios also exhibit no trend within the molecular weight range covered. This observation justifies the use of eq 3–5 to calculate the characteristic ratio. The result for the highest molecular weight fraction, where the accuracy of the determination is greatest, is a characteristic ratio of 10 ± 1.

Brant and Flory have shown that four polypeptides with $-CH_2R$ side chains have the same characteristic ratios, regardless of whether they are ionized polypeptides in aqueous solvents or nonionic polypeptides in organic solvents. The characteristic ratios they obtained were 9.6 (±10%) for poly(β-benzyl L-aspartate) in *m*-cresol; 8.8 (±10%) for poly(L-glutamic acid) in 0.3 *M* sodium phosphate, pH 7.85; 8.6 (±10%) for poly(L-lysine) in 1.0 *M* sodium bromide, pH 4.54; and 8.8 for poly(γ-benzyl L-glutamate) in dichloroacetic acid.[19]

According to Krimm and coworkers, the circular dichroism of a random-coil polypeptide should exhibit a single weak negative band near 200 nm.[16–18] Short left-handed helices, stabilized by the repulsions between the charged side chains, were considered to be responsible for the enhanced negative circular dichroism near 200 nm and the appearance of a positive circular dichroism band near 215 nm with ionized poly(L-glutamic acid) and poly(L-lysine) under conditions of low to moderate ionic strength.[15–18] A similar proposal has been advanced by Hiltner, *et al.*[42]

In the case of poly(N^5-ω-hydroxyethyl-L-glutamine), it is observed that the experimental characteristic ratio is independent of molecular weight, the characteristic ratio has the numerical value anticipated for a random-coil polypeptide with a $-CH_2R$ side chain,[24] and there cannot be special effects arising from the interaction of charged side chains because the side chains are not ionized. The circular dichroism of poly(N^5-ω-hydroxyethyl-L-glutamine) can legitimately be associated with a random-coil polypeptide. The rotational states which occur in the statistical coil must lead to the positive circular dichroism band, a situation which also occurs in poly(L-proline).[43] Calculations have shown that consideration of nearest-neighbor interactions, weighted according to a conformational map[44] similar to that of Brant and Flory, can lead to a positive circular dichroism band at 218 nm.[45]

TABLE II
EXPANSION COEFFICIENTS AND CHARACTERISTIC RATIOS AT 30° IN WATER

Fraction	Expansion coefficient $\Phi = 0.0021$	Expansion coefficient $\Phi = 0.0026$	Characteristic ratios $\Phi = 0.0021$	Characteristic ratios $\Phi = 0.0026$
7-F7	1.16 ± 0.03	1.18 ± 0.04	11.2 ± 1.0	9.5 ± 1.0
7-F6	1.12 ± 0.03	1.15 ± 0.03	11.3 ± 1.0	9.8 ± 0.9
7-F4	1.13 ± 0.02	1.16 ± 0.03	10.2 ± 0.7	8.9 ± 0.6
7-F2	1.12 ± 0.01	1.13 ± 0.02	10.7 ± 0.5	9.5 ± 0.5

Figures 3 and 4 show that the optical activity of poly(N^5-ω-hydroxyethyl-L-glutamine) and poly(L-glutamic acid) are qualitatively similar. However, there are some quantitative differences in the spectra. The positive band at 215–216 nm and the negative band near 200 nm are both more intense in the case of poly(L-glutamic acid). It is necessary to inquire whether this result with poly(L-glutamic acid) should be interpreted as another circular dichroism spectrum which is caused by random-coil polypeptide or whether it might be due to the presence of ordered regions stabilized by the interaction of the charged side chains, as suggested by Krimm and coworkers.[15–18] If the latter is the case, it would be necessary that $\{r^2\}_0/n_pl_p{}^2$ for the partially ordered poly(L-glutamic acid) should be unchanged from the characteristic ratio for the random coil.

The equivalence of the characteristic ratio of a random coil and $\{r^2\}_0/n_pl_p{}^2$ for a partially ordered polypeptide would not be unprecedented. The calculation of $\{r^2\}_0/n_pl_p{}^2$ for polypeptides with a $-CH_2R$ side chain, which are partially in the α-helical conformation, has been carried out by Miller and Flory.[46] They showed that when σ is 0.003, $\{r^2\}_0/n_pl_p{}^2$ for a polypeptide with $n_p = 1027$ decreases as the helical content increases, reaches a minimum at about 35% helix, and then increases to very large values with further increases in helical content. Consequently, when the ordered structure in question is an α helix, it is possible that $\{r^2\}_0/n_pl_p{}^2$ for a partially ordered polypeptide could be less than, equal to, or greater than the characteristic ratio of the random coil, depending upon the extent of ordering. The contraction in the unperturbed dimensions at intermediate helical contents was attributed to the compactness of the α helix,[46] which has a translation along the helix axis of 1.50 Å per residue.[47]

The mathematical framework used by Miller and Flory[46] to calculate the dimensional properties of partially α-helical polypeptides can be utilized to estimate the effect of the left-

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(35) W. G. Miller, D. A. Brant, and P. J. Flory, *J. Mol. Biol.*, **23**, 67 (1967).

(36) S. Lapanje and C. Tanford, *J. Amer. Chem. Soc.*, **89**, 5030 (1967).

(37) W. L. Mattice and L. Mandelkern, *ibid.*, **93**, 1769 (1971).

(38) W. L. Mattice and L. Mandelkern, *Biochemistry*, **10**, 1934 (1971).

(39) W. R. Krigbaum and D. K. Carpenter, *J. Phys. Chem.*, **59**, 1166 (1955).

(40) D. McIntyre, A. Wims, L. C. Williams, and L. Mandelkern, *ibid.*, **66**, 1932 (1962).

(41) A. E. Tonelli and P. J. Flory, *Macromolecules*, **2**, 255 (1969).

(42) W. A, Hiltner, A. J. Hopfinger, and A. G. Walton, *Biophys. Soc. Abstr.*, **12**, 159a (1972).

(43) L. Mandelkern and W. L. Mattice, Proceedings of the Fifth Jerusalem Symposium on the Conformation of Biological Molecules and Polymers, in press.

(44) D. A. Brant, W. G. Miller, and P. J. Flory, *J. Mol. Biol.*, **23**, 47 (1967).

(45) A. E. Tonelli, *Macromolecules*, **2**, 635 (1969).

(46) W. G. Miller and P. J. Flory, *J. Mol. Biol.*, **15**, 298 (1966).

(47) G. N. Ramachandran and V. Sasisekharan, *Advan. Protein Chem.*, **23**, 283 (1968).

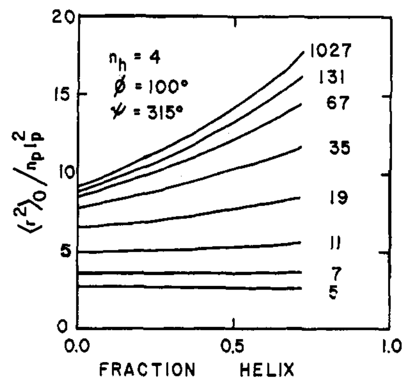

Figure 5. The effect of helices with ϕ, ψ = 100°, 315° and an average length of four residues on $\{r^2\}_0/n_pl_p^2$. The degree of polymerization for each curve is entered at the right-hand side of the figure.

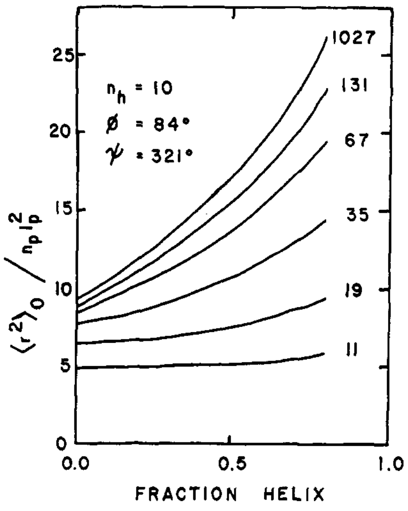

Figure 6. The effect of helices with ϕ, ψ = 84°, 321° and an average length of ten residues on $\{r^2\}_0/n_pl_p^2$. The degree of polymerization for each curve is entered at the right-hand side of the figure.

handed helices proposed by Krimm and Mark[15] on the dimensions of polypeptides. While Krimm and Mark[15] do not predict values of σ and s for their proposed ordered structures, they do predict the number of residues in a helical segment for various helix geometries. Helices of four to seven units in lengths were considered most likely.[15] The effect of such helices on $\{r^2\}_0/n_pl_p^2$ can be predicted by calculating the mean-square end-to-end distance for pairs of s and σ which give the proposed average number of amino acid residues in a helical segment, as described in the section on Materials and Methods.

The result of these calculations is shown in Figures 5–7. Figure 5 shows $\{r^2\}_0/n_pl_p^2$ when the average length of a helical segment is four residues and the helix has the rotational angles ϕ = 100° and ψ = 315°, the values suggested by Krimm and Mark[15] for a helix four units long. The results are shown for various fractions of the residues in the helical state and for polypeptide chains of various degrees of polymerization. Figure 6 shows the results when the average length of a helical segment is ten and the helix has ϕ = 84° and ψ = 321°, the geometry proposed by Hiltner, *et al.*[42] In each case $\{r^2\}_0/n_pl_p^2$ increases as the fraction of residues in the helical state increases throughout the entire range of helix content. Figure 7 shows the result at a degree of polymerization of 1027 for the case where the average length of a helical segment is seven. Two of the calculations are for helices with ϕ, ψ = 100°, 315° and ϕ, ψ = 45°, 355°, corresponding to the geometries considered most likely[15] when the average length of a helical segment is four and sixteen, respectively. The helix with ϕ, ψ = 66°, 340° is an estimate of the geometry for the case when the average number of residues in a helical segment is seven, based on interpolation of the predictions of Krimm and Mark[15] for higher and lower helix lengths. While the precise helix geometry does affect the dimensions slightly, it is again seen that $\{r^2\}_0/n_pl_p$ will increase with increasing helical content for any helix of this type.

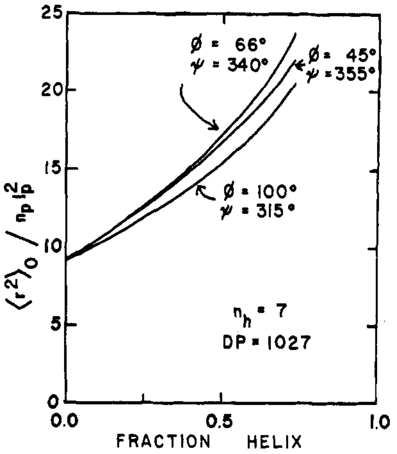

Figure 7. The effect of helices with an average length of seven residues and with ϕ, ψ = 45°, 355°; 66°, 340°; and 100°, 315° on $\{r^2\}_0/n_pl_p^2$. All curves are for a degree of polymerization of 1027.

The conclusion from Figures 5–7 is that the formation of left-handed helices of the type which have been proposed[15–18, 42] to occur in ionized homopolypeptides will necessarily bring about an increase in $\{r^2\}_0/n_pl_p^2$. The left-handed helical structures used in Figures 5–7 have translations along the helix axis of 3.12 to 3.55 Å per residue, values which are more than twice the result obtained with the α helix.[47] The high extension of the left-handed helices causes the unperturbed dimensions to increase with increasing helix content throughout the entire composition range.[48]

If the observation that poly(L-glutamic acid) exhibits a stronger negative circular dichroism band near 200 nm than does poly(N^5-ω-hydroxyethyl-L-glutamine) under the conditions used in Figure 3 were to be attributed to the existence of short extended left-handed helices, it would require that $\{r^2\}_0/n_pl_p^2$ be larger for poly(L-glutamic acid) than for poly(N^5-ω-hydroxyethyl-L-glutamine). The experimental results under these conditions are 8.8 (±10%) for poly(L-glutamic acid)[19] and 10 ± 1 for poly(N^5-ω-hydroxyethyl-L-glutamine), which are identical within experimental error. If there is any small difference in the results for these two polypeptides, it would appear to be in the opposite direction to that which would be required if extended helices of the type proposed by Krimm and Mark were present in the poly(L-glutamic acid).

We can conclude that the qualitatively similar circular di-

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(48) A referee suggested an alternation computation based upon the assumption of noncooperative formation of the local helices. The computation in this case is carried out by setting σ = 1 and choosing s to obtain the appropriate average length of a helical segment. When this procedure was applied to the geometry suggested by Krimm and Mark[15] for helices with lengths of 4 and 16 residues and to the geometry suggested by Hiltner, *et al.*,[42] with a length of seven residues, $\{r^2\}_0/n_pl_p^2$ was calculated to be in the range 18–35 for a degree of polymerization of 1027. The computation according to this procedure also leads to the conclusion that the formation of short segments of extended left-handed helices will lead to an increase in $\{r^2\}_0/n_pl_p^2$.

chroism spectra in Figure 3 are both characteristic of random-coil polypeptides. A possible explanation for the quantitative differences in the circular dichroism spectra may reside in the different interaction of the polypeptides with the solvent. The expansion coefficient for poly(L-glutamic acid) at 37° in 0.3 *M* sodium phosphate, pH 7.85, is 1.34 (±1.4%),[19] which is decidedly greater than the result in Table II for poly(N^5-ω-hydroxyethyl-L-glutamine) in water at 30°. The spectra in Figures 3 and 4 could be explained in a qualitative manner if the greater expansion of poly(L-glutamic acid) is accompanied by more heavily weighting values of ϕ and ψ which lead to positive circular dichroism near 215 nm and intense negative circular dichroism near 200 nm.

The characteristic ratios of poly(γ-benzyl L-glutamate), poly(β-benzyl L-aspartate), and poly(L-lysine) were also determined to be close to nine by Brant and Flory.[19] Conclusions regarding their circular dichroism under the conditions used are not possible because the solvents (dichloroacetic acid, *m*-cresol, and 1 *M* sodium bromide) do not transmit light in the relevant spectral range.

Helix–Coil Stability Constants for the Naturally Occurring Amino Acids in Water. VI. Leucine Parameters from Random Poly(hydroxypropylglutamine-*co*-L-leucine) and Poly(hydroxybutylglutamine-*co*-L-leucine)[1]

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ABSTRACT: The synthesis and characterization of water-soluble random copolymers containing L-leucine with either N^5-(3-hydroxypropyl)-L-glutamine or N^5-(4-hydroxybutyl)-L-glutamine are described, and the thermally induced helix–coil transitions of these copolymers in water have been studied. The incorporation of L-leucine was found to increase the helix content of the polymer. The Zimm–Bragg parameters σ and s for the helix–coil transition in poly(L-leucine) in water were deduced from an analysis of the melting curves of the copolymers in the manner described in earlier papers. Within the limits of error of both methods, the values of σ and s for poly(L-leucine) were found to be in good agreement with those obtained earlier using sandwich-type block copolymers of D,L-lysine and L-leucine in water, thus giving additional weight to the view that the conformational state of an amino acid residue in a polypeptide or protein, in first approximation, is essentially independent of the chemical nature of its neighbors.

The use of the "host–guest" technique for the evaluation of the helix–coil stability constants of various amino acids in water has been illustrated in earlier papers of this series.[3–7] In this method, a water-soluble, α-helical host homopolymer is selected, and various amounts of a guest residue are incorporated into it to form random copolymers. By examining the influence of the guest residues on the helix–coil transition of the host homopolymer, it is possible[3–7] to determine the Zimm–Bragg[8] parameters σ and s for the guest residues. Two host homopolymers, poly[N^5-(4-hydroxybutyl)-L-glutamine] (PHBG) and poly[N^5-(3-hydroxypropyl)-L-glutamine] (PHPG) have been used in this work.[4–7] The first is quite helical at room temperature, and the second much less so. The choice of host is determined primarily by the anticipated helix-forming or helix-breaking propensity of the guest residue. In the present study, we have used both of these hosts for the evaluation of the parameters for L-leucine, although most of the work was done with PHPG, since L-leucine was known to act as a helix former.[9] This behavior was further demonstrated by this work.

The values of σ and s for poly(L-leucine) had been determined earlier[9] by the use of block copolymers containing poly(L-leucine) of various chain lengths flanked on either side by poly(D,L-lysine) which made the copolymers water soluble (presumably without affecting the values of σ and s of the central poly(L-leucine) block). The absence of charges and ease of synthesis in the random copolymers studied here make the host–guest technique a more attractive one. However, if the basic assumptions of both techniques are correct, then both should give the same results for poly(L-leucine) in water. As we will show below, the results from both methods are in good agreement.

The synthesis of water-soluble random copolymers of L-leucine with either HPG or HBG is described in section I, and the experimental characterization of these copolymers and their melting behavior in water are presented in section II. Finally, in section III, the data are analyzed by means of an appropriate form of the theory[3] to determine the helix–coil stability parameters of L-leucine in water.

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(2) NIH Predoctoral Trainee, 1969–1972.

(3) P. H. Von Dreele, D. Poland, and H. A. Scheraga, *Macromolecules*, **4**, 396 (1971) (hereinafter called paper I).

(4) P. H. Von Dreele, N. Lotan, V. S. Ananthanarayanan, R. H. Andretta, D. Poland, and H. A. Scheraga, *ibid.*, **4**, 408 (1971) (hereinafter called paper II).

(5) V. S. Ananthanarayanan, R. H. Andreatta, D. Poland, and H. A. Scheraga, *ibid.*, **4**, 417 (1971) (hereinafter called paper III).

(6) K. E. B. Platzer, V. S. Ananthanarayanan, R. H. Andreatta, and H. A. Scheraga, *ibid.*, **5**, 177 (1972) (hereinafter called paper IV).

(7) L. J. Hughes, R. H. Andreatta, and H. A. Scheraga, *ibid.*, **5**, 187 (1972) (hereinafter called paper V).

(8) B. H. Zimm and J. K. Bragg, *J. Chem. Phys.*, **31**, 526 (1959).

(9) S. E. Ostroy, N. Lotan, R. T. Ingwall, and H. A. Scheraga, *Biopolymers*, **9**, 749 (1970).