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 ($\pm 1.4\%$),[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]

J. E. Alter,[2] G. T. Taylor, and H. A. Scheraga*

Department of Chemistry, Cornell University,
Ithaca, New York 14850. *Received June 12, 1972*

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.

---

(1) This work was supported by research grants from the National Science Foundation (No. GB-28469X1) and from the National Institute of Arthritis and Metabolic Diseases, U. S. Public Health Service (No. AM-08465).

(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).

TABLE I
COMPOSITIONS AND CHAIN LENGTHS OF THE UNFRACTIONATED P(BzG:Leu) COPOLYMERS

Polymer no.	L-Leucine content of reaction mixture, mol %	L-Leucine content found, mol %	Av mol wt[a] $\times 10^{-3}$	$\overline{\text{DP}}$
I	5	4.9	145	680
II	10	9.8	350	1670
III	15	14.9	160	790
IV	20	20.0	105	535
V	10	9.4	88	425

[a] By viscometry, using the relation of Fujita, *et al.*,[12] for polymers in DCA.

(I) Experimental Section. Preparation and Characterization of the Copolymers

The copolymers were prepared by first copolymerizing the *N*-carboxyanhydrides (NCA) L-leucine NCA and γ-benzyl L-glutamate NCA in the solvent dioxane with triethylamine as an initiator. The benzyl blocking group was then replaced by reaction with hydroxypropylamine or hydroxybutylamine.

(A) Materials. 3-Amino-1-propanol from Aldrich Chemical Co., Inc., and 4-amino-1-butanol from Chemical Procurement Laboratories, Inc., were dried over barium oxide and distilled under reduced pressure. Dioxane was purified shortly before use by refluxing and distilling over sodium. Hexane was dried over calcium sulfate and decanted just before use. Triethylamine was refluxed and distilled with acetic anhydride, and then dried and distilled over KOH. Ethyl acetate was dried over Linde Molecular Sieves (4A). Purified grade dichloroacetic acid (DCA) from Fisher Scientific Co., absolute ethanol from U. S. Industrial Chemicals Co., and anhydrous methyl alcohol from A. R. Mallinckrodt Chemical Works were used without further purification. Ether from Mallinckrodt Chemical Works was of analytical reagent grade. 2,2,2-Trifluoroethanol (TFE) obtained from Aldrich Chemical Co., Inc., was stirred over sodium bicarbonate and distilled. L-Leucine was purchased from Fisher Scientific Co., and L-glutamic acid from Aldrich Chemical Co., Inc. Fisher Certified Tetrahydrofuran (THF) was distilled from lithium aluminum hydride under nitrogen.

Using the L-leucyl dipeptide method of Manning and Moore,[10] the starting L-amino acids were found to contain no detectable amounts (within 0.1 %) of D residues.

PHPG of degree of polymerization, $\overline{\text{DP}}_w$ = 700 and PHBG of $\overline{\text{DP}}_w$ = 200 were fractions IIIB and VIB, respectively, of paper II.[4]

(B) Synthesis. *N*-Carboxyanhydrides. L-Leucine NCA was prepared by the action of phosgene on a suspension of the amino acid in THF for 1–2 hr at 45–50° as described by Hirschmann, *et al.*[11] Several recrystallizations from ether–hexane gave a product with a melting point of 77–78°. The reaction gave a yield of 60–65 %.

γ-Benzyl L-glutamate-NCA was prepared as in paper IV[6] of this series.

Poly(γ-benzyl L-Glu$^{m'}$:L-Leu$^{n'}$), [P(BzG:Leu)], Copolymers I–V. Random copolymers of L-leucine and γ-benzyl L-glutamate containing from 5 to 20 % leucine were synthesized by polymerization of the NCA's in dioxane with triethylamine as an initiator. The two NCA's were dissolved in dioxane (at a concentration of about 10 mmol of total NCA per 80 ml of solvent) in the molar ratio desired for the copolymer product. Triethylamine initiator was added to give an A/I ratio of 35. The reaction flask was sealed with a Drierite drying tube and allowed to stand at room temperature for a week. The viscous reaction mixture was then introduced into 400 ml of vigorously stirred absolute ethanol. The white precipitate was collected on a filter funnel, washed thoroughly with ethanol, and dried to constant weight over P_2O_5 *in vacuo*. The yield ranged from 57 to 88 %. The compositions and chain lengths (determined, roughly, with the relationship of Fujita, *et al.*[12]) of these copolymers are given in Table I.

Poly[*N*^{5}-(3-hydroxypropyl)-L-Gln^m:L-Leu^n], [P(HPG:Leu)], and Poly[*N*^{5}-(4-hydroxybutyl)-L-Gln^m:L-Leu^n], [P(HBG:Leu)], Copolymers VI–X. The γ-benzyl L-glutamate:L-leucine copolymers were treated with 3-amino-1-propanol or 4-amino-1-butanol to yield a series of water-soluble copolymers. The initial products I–V were treated, as previously described,[6] to yield samples VI–X. The yields ranged from 80 to 93 %.

(C) Spectral Analysis. The spectra of aqueous solutions of the copolymers VI–X showed no absorption at 257 mμ, indicating that the benzyl groups were completely removed (within 1.0 %) in the final stage of synthesis.

(D) Fractionation. The copolymers VI–X were fractionated with methanol and ether by the procedure described in paper II,[4] after which they were dissolved in water, lyophilized, and dried *in vacuo* over P_2O_5.

(E) Determination of Composition. The amino acid compositions of all copolymers were determined on a Technicon amino acid analyzer. Samples I–V were hydrolyzed in 12 *N* HCl at 105° for 72–96 hr in sealed ampoules, and fractions from samples VI–X were hydrolyzed in 12 *N* HCl at 105° for 4 days. From a study of the time dependence of the extent of hydrolysis (carried out on fraction IXC), it was found that 4 days was sufficient for complete hydrolysis of these polymers. Analysis of standards hydrolyzed under the same conditions showed that no correction for the destruction of the amino acids was necessary. The average experimental error in the determinations of the compositions is estimated to be ±5 %.

(F) Determination of Concentration. The concentrations of all polymer solutions were determined as described previously.[6] Each concentration was determined at least three times. The error in the determination was estimated to be ±3 %.

(G) Viscosity. The viscosities of samples I–V were determined in DCA at 25.0 ± 0.1° in a Cannon-Ubbelohde semimicro dilution viscometer as described earlier,[5] and the relation of Fujita, *et al.*,[12] was used to obtain only a rough molecular weight (using poly(γ-benzyl L-glutamate), PBLG, as a model).

(H) Optical Purity. The starting materials as well as the final copolymers were checked for racemization by the L-leucyl dipeptide method of Manning and Moore.[10] The L-Leu-L-Leu and L-Leu-D-Leu were separated on a Technicon amino acid analyzer with a sodium citrate elution buffer of pH 3.90. Dipeptide standards were prepared from D,L-leucine.

(I) Determination of Molecular Weights. Molecular weights for the fractions from samples VI–X were determined by the conventional sedimentation equilibrium method using a Spinco Model E ultracentrifuge as reported earlier.[5] The concentration dependence of the weight-average molecular weight $\bar{M}_w$ was determined for each sample, and $\bar{M}_z$ was computed at the lowest concentration run made on each fraction. The molecular weights are accurate to within ±4 %.

The partial specific volumes ($\bar{v}$) of the P(HPG:Leu) or P(HBG:Leu) copolymers, required for the calculation of molecular weights, were determined from the amino acid content as described by Cohn and Edsall.[13] A value of $\bar{v}$ = 0.79[14] for PHPG and $\bar{v}$ = 0.816 for PHBG was used in the calculation of $\bar{v}$ for the copolymers. The latter value was obtained by adding the partial specific volume of a CH_2 group[15] to the value for PHPG.

---

(10) J. M. Manning and S. Moore, *J. Biol. Chem.*, **243**, 5591 (1968).

(11) R. Hirschmann, H. Schwam, R. G. Strachan, E. F. Schoenewaldt, H. Barkemeyer, S. M. Miller, J. B. Conn, V. Garsky, D. F. Veber, and R. G. Denkewalter, *J. Amer. Chem. Soc.*, **93**, 2746 (1971).

(12) H. Fujita, A. Teramoto, T. Yamashita, K. Okita, and S. Ikeda, *Biopolymers*, **4**, 781 (1966).

(13) E. J. Cohn and J. T. Edsall, "Proteins, Amino Acids, and Peptides," Reinhold, New York, N. Y., 1943, Chapter 16, p 371.

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

(15) C. De Loze, P. Saludjian, and A. J. Kovacs, *ibid.*, **2**, 43 (1964).

TABLE II
CHARACTERIZATION OF THE FRACTIONATED COPOLYMERS

Fraction[a]	L-Leucine content, mol %	$\bar{v}$, cm^3/g	$\bar{M}_w \times 10^{-3}$ [b]	$\bar{M}_z/\bar{M}_w$ [c]	$\overline{\mathrm{DP}}_w$
VIB	3.5	0.792	192	1.12	1050
VIF	3.8	0.793	57.2	1.03	312
VIIB	7.6	0.795	162	1.14	901
VIIIA	11.5				
VIIIB	12.3	0.799	111	1.08	630
VIIIC	12.3	0.799	85.6		483
VIIID	12.0	0.799	74.1		419
VIIIE	12.9	0.799	55.4		315
VIIIF	15.0	0.800	45.6	1.05	260
VIIIG	17.0				
IXC	15.3	0.823	31.6	1.03	170
XC	8.6	0.821	51.9		270

[a] Samples VI-X were obtained from unfractionated I-V, respectively. The host was HPG for all fractions except IXC and XC for which HBG was used. The letter corresponds to the fraction obtained in the fractionation procedure. [b] This value was determined by conventional sedimentation equilibrium (with an extrapolation to zero concentration). [c] These measurements were made only for those fractions whose melting curves were determined.

(J) Optical Rotatory Dispersion and Circular Dichroism Measurements. The optical rotatory dispersion (ORD) and circular dichroism (CD) measurements were made with a Cary Model 60 spectropolarimeter equipped with a Model 6001 CD attachment, as described in paper II.[4] Temperature control was maintained in all solutions to within ±0.2° with water-jacketed quartz cells. The concentrations used in these measurements were 0.03–0.35% (w/v). The experimental error in helix content, θ_h (which is defined as $-b_0/750$), results from (a) the error in the concentration (±3%), (b) the error in the values of b_0 for the complete helix and complete coil (±3%), and (c) the error in the slope of the Moffit-Yang plot (±2%).

(II) Results

(A) Characterization of the Copolymers. Table I summarizes the composition and the average degree of polymerization ($\overline{\mathrm{DP}}$) of the unfractionated P(BzG : Leu) copolymers, and Table II shows the data for the fractionated P(HPG : Leu) and P(HBG : Leu) copolymers that were investigated. The usual decrease in $\overline{\mathrm{DP}}_w$ attributed to transaminolysis,[4–7,14] upon conversion of these polymers to their hydroxyalkylglutamine derivatives, can be seen in the conversion of III to VIII.

In Table II, the various fractions from a given sample (especially sample VIII) seem to show an increase in leucine content with decreasing molecular weight. This behavior might arise if leucine were a better chain terminator than benzyl glutamate. Also, the leucine content seems to decrease when the benzyl group is replaced by the amino alcohol. This phenomenon could result from a preliminary fractionation (during the filtration prior to the lyophilization which *precedes* the fractionation with ether and methanol) in which part of the material (with higher leucine content) becomes insoluble. These analytical results do not seem to suggest that there are *gross* departures from randomness in the copolymers; in any case, it has been demonstrated in paper I[3] that the presence of relatively short blocks (of, say, leucine) does not influence the melting behavior of a random copolymer.

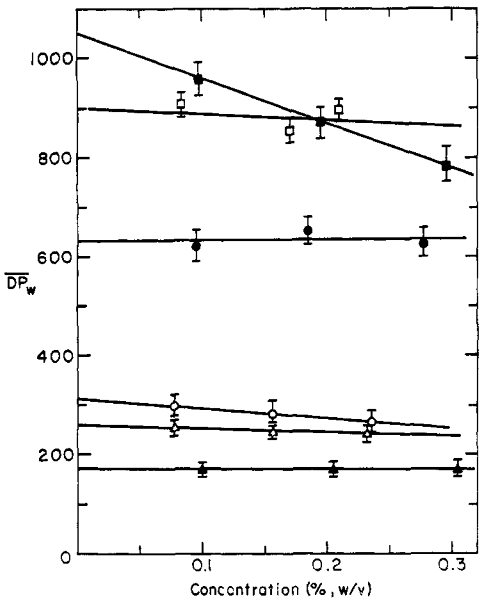

Figure 1. Concentration dependence of molecular weights for fractions used for analysis to obtain σ and s: (■) 3.5% Leu (with HPG), $\overline{\mathrm{DP}}_w$ = 1050 (fraction VIB); (□) 7.6% Leu (with HPG), $\overline{\mathrm{DP}}_w$ = 901 (fraction VIIB); (●) 12.3% Leu (with HPG), $\overline{\mathrm{DP}}_w$ = 630 (fraction VIIIB); (○) 3.8% Leu (with HPG), $\overline{\mathrm{DP}}_w$ = 312 (fraction VIF); (△) 15.0% Leu (with HPG), $\overline{\mathrm{DP}}_w$ = 260 (fraction VIIIF); (▲) 15.3% Leu (with HBG), $\overline{\mathrm{DP}}_w$ = 170 (fraction IXC). The error symbols represent the experimental error in each measurement.

From Figure 1, it can be seen that five of the six fractions used for the determination of σ and s (in section III) exhibit very little concentration dependence of their $\overline{\mathrm{DP}}_w$ values, with the exception of fraction VIB. If fraction VIB (and also fraction VIIB) were omitted from the analysis to obtain σ and s (see below), the value of σ dropped from 33×10^{-4} to 25×10^{-4}, which is within the error in σ, and the values of s changed very little; thus, fractions VIB and VIIB were retained in the analysis. From the values of $\bar{M}_z/\bar{M}_w$, it appears that the fractions used in the determination of σ and s were fairly homogeneous.

Using the Manning-Moore[10] dipeptide procedure, the starting materials, *i.e.*, L-leucine and L-glutamic acid, were found to contain no detectable D residues to within ±0.1%. Copolymer fractions VIIIE with 12.9 mol % leucine and a $\overline{\mathrm{DP}}_w$ of 315, and IXB (not shown in Table II) with 13.2 mol % leucine and a $\overline{\mathrm{DP}}_w$ of 209, were hydrolyzed. In the case of fraction VIIIE, the leucine in the hydrolysate was purified by separating it from the L-glutamic acid and 3-amino-1-propanol by preparative thin layer chromatography; in the case of fraction IXB, the hydrolysate was used without purification. The L-Leu dipeptides wre then prepared from the purified leucine of fraction VIIIE and from the total hydrolysate of fraction IXB. Analysis of the dipeptides showed that 1.9 mol % of the 41 leucines (or less than 0.8 residue) of fraction VIIIE and 0.79 mol % of the 28 leucines (or 0.2 residue) of fraction IXB were D residues. No racemization of the glutamic acid of the polymer (within ±0.1%) was detected by this procedure. We consider the amount of racemization found here to be too minor to affect the computed values of σ and s for L-leucine.

(B) ORD and CD Data for the Copolymers. The ORD and CD data for representative samples of fractions of P-(HPG:Leu) and P(HBG:Leu) in water at room temperature are shown in Figure 2. Both the ORD and the CD data clearly indicate the presence of a right-handed α-helical structure.[16–18] The helix content is seen to increase with increas-

---

(16) E. R. Blout, F. Schmier, and N. S. Simmons, *J. Amer. Chem. Soc.*, **84**, 3193 (1962).

(17) N. Greenfield, B. Davidson, and G. D. Fasman, *Biochemistry*, **6**, 1630 (1967).

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

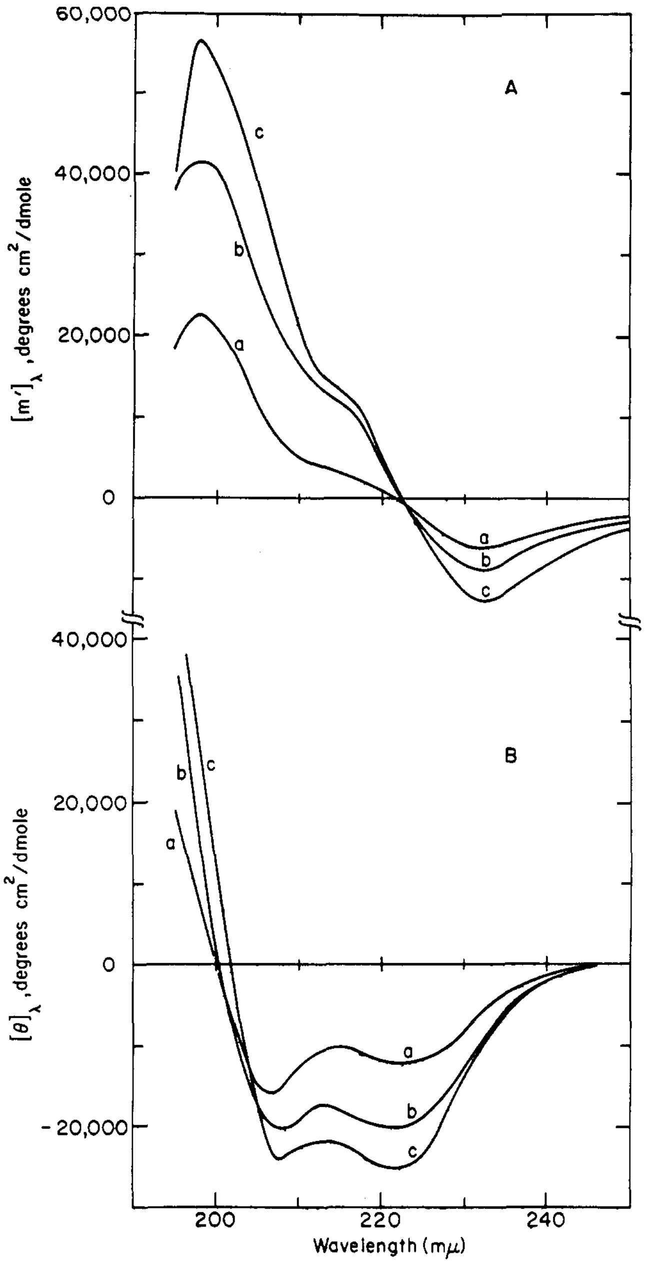

Figure 2. (A) ORD and (B) CD data in water at 25 ± 0.5° for representative samples of leucine copolymers: (a) 3.5% Leu (with HPG), $\overline{\mathrm{DP}}_w$ = 1050 (fraction VIB); (b) 12.3% Leu (with HPG), $\overline{\mathrm{DP}}_w$ = 630 (fraction VIIIB); (c) 15.3% Leu (with HBG), $\overline{\mathrm{DP}}_w$ = 170 (fraction IXC).

ing leucine content, as expected for a helix-forming residue. With increasing temperature, the ORD spectrum (not shown here) becomes characteristic of the random coil mixed with small amounts of helix, indicating that these copolymers undergo a thermally induced transition from the α helix to the random coil in water.

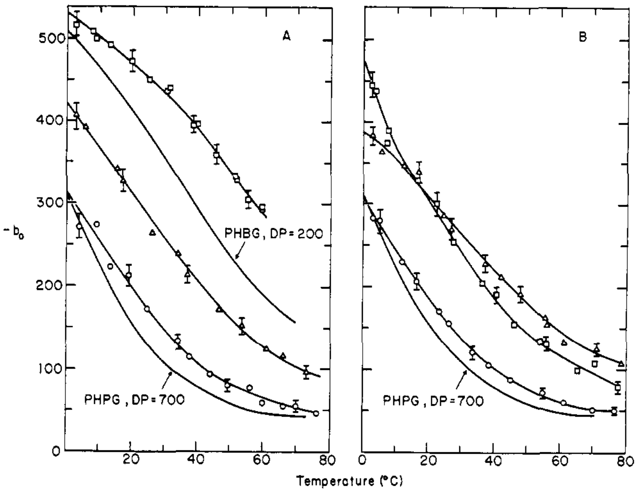

Figure 3. Temperature dependence of b_0 for Leu copolymers in water. PHPG of $\overline{\mathrm{DP}}_w$ = 700 (fraction IIIB of paper II[4]) and PHBG of $\overline{\mathrm{DP}}_w$ = 200 (fraction VIB of paper II[4]) are included for comparison: (A) (□) 15.3% Leu (with HBG), $\overline{\mathrm{DP}}_w$ = 170 (fraction IXC); (△) 12.3% Leu (with HPG), $\overline{\mathrm{DP}}_w$ = 630 (fraction VIIIB); (○) 3.5% Leu (with HPG), $\overline{\mathrm{DP}}_w$ = 1050 (fraction VIB); (B) (□) 7.6% Leu (with HPG), $\overline{\mathrm{DP}}_w$ = 901 (fraction VIIB); (△) 15.0% Leu (with HPG), $\overline{\mathrm{DP}}_w$ = 260 (fraction VIIIF); (○) 3.8% Leu (with HPG), $\overline{\mathrm{DP}}_w$ = 312 (fraction VIF). The points are the experimental ones, and the lines represent the smoothed experimental curves. The size of the error symbols reflects the experimental errors in θ_h arising from errors in the determination of the concentration, in the choice of values of b_0 for the full helix and coil conformations, and of the slope of the Moffitt-Yang plot.

TABLE III
b_0 IN TFE AND DCA AT 1°

Fraction	b_0 in TFE	b_0 in DCA
VIIIB ($\overline{\mathrm{DP}}_w$ 630)	−763	+37
IXC ($\overline{\mathrm{DP}}_w$ 170)	−733	

Measurements of b_0, shown in Figure 3 for the six fractions studied over the range of λ 280–420 mμ as a function of temperature, confirm the above conclusion. The procedures used to obtain these curves were the same as those in paper IV[6] except that here the solutions had a lower concentration range [from 0.1 to 0.3% (w/v)]. No concentration dependence was observed, and all curves were reproducible. The size of the error symbols in Figure 3 reflects the experimental errors in θ_h arising from errors in concentration, in b_0 for the full helix and coil, and in the slope of the Moffitt-Yang plot (see section I, part J, for the magnitudes of these errors). The transition was demonstrated to be reversible in all cases.

These thermally induced melting curves demonstrate several things. First, the curves are somewhat S shaped, and the sharpness of the transition increases with increasing molecular weight, as predicted earlier.[19] Secondly, the helix content depends not only on the chain length, but also on the leucine content; *e.g.*, fraction VIIIF with 15.0% leucine and DP = 260 lies above fraction VIB with 3.5% leucine and DP = 1050. Third, the dependence of b_0 on temperature, leucine content, and molecular weight is complex and produces the crossover effect seen with fractions VIIB and VIIIF. Fourth, the fact that the curves for fractions VIB and VIF are close to each other indicates that a DP of 312 is essentially infinite for this leucine content. Lastly, the fact that the melting cuve of fraction IXC does not extend beyond 60° is due to precipitation which will be discussed in section IIID.

(C) b_0 for Complete Helix and Complete Coil. For the homopolymers PHBG and PHPG studied in paper II, the value of b_0 for the complete helix was taken to be −750 and for the complete coil as zero. Because these values vary with the nature of the side chain,[20] two fractions of the leucine co-

---

(19) H. A. Scheraga, *Proteins*, 1, 477 (1963).

(20) J. N. Vournakis, J. F. Yan, and H. A. Scheraga, *Biopolymers*, 6, 1531 (1968).

TABLE IV

COMPARISON OF THE VALUE OF θ_h, CALCULATED WITH THE APPROXIMATE AND EXACT THEORIES[a] FOR FINITE CHAINS

L-Leucine content, mol fraction	$\overline{DP}_w$	Temp, °C	$(\theta_h)_{exptl}$	$(\theta_h)_{theor}$ Lifson[b]	Allegra[c]	Allegra[d]	Lehman-McTague[c]
0.153	170	0	0.708	0.695	0.699	0.708	0.699
		30	0.577	0.567	0.571	0.600	0.572
		60	0.380	0.378	0.379	0.410	0.380
0.150	260	0	0.515	0.533	0.534	0.525	0.534
		30	0.347	0.363	0.363	0.360	0.363
		60	0.192	0.196	0.196	0.179	0.196
0.038	312	0	0.405	0.445	0.444	0.435	0.442
		30	0.181	0.187	0.186	0.170	0.185
		60	0.081	0.086	0.086	0.075	0.086

[a] The parameters used for PHPG and PHBG were those of Table II in paper II.[4] [b] The parameters used for L-leucine were obtained by fitting the data by the Lifson theory, as shown in Table V ($\sigma = 30 \times 10^{-4}$). [c] The parameters used for L-leucine were obtained by fitting the data by the Allegra theory, as shown in Table V ($\sigma = 33 \times 10^{-4}$). [d] Same as footnote *c*, but for $\sigma = 12 \times 10^{-4}$.

polymers were examined in trifluoroethanol and one in DCA, with b_0 corrected for the dispersion of the refractive index of the solvent.[21] The results are shown in Table III. The high b_0 values of -763 and -733 for the two fractions in trifluoroethanol indicate that the value of -750 is a reasonable one for the complete helix in these copolymers. Similarly, the value of $+37$ in DCA demonstrates that zero is not an unreasonable one for the complete coil. As already stated, the error in these values has been taken into account in the error analysis.

(III) Discussion

(A) Helix-Coil Parameters for Poly(L-leucine). The melting curves of the copolymers described in section II were analyzed according to the LAPS (Lifson-Allegra-Poland-Scheraga) hierarchy to obtain σ and s for poly(L-leucine). This procedure has been discussed extensively in earlier papers of this series.[3-7] The approximations, corresponding to the theories of Lifson[22] and Allegra,[23] were both used to fit the data, and checked with the exact method of Lehman and McTague[24] in representative cases.[25] The results of these calculations are shown in Table IV along with some original experimental data for comparison. Both the second-order (Allegra) and the first-order (Lifson) approximations give results which agree well with those from the Lehman-McTague method. The higher order (Allegra) approximation will be used in all subsequent discussion of the leucine parameters.

The approximate theories used above for leucine appear to hold for two reasons. First, the values of s for PHBG or PHPG and leucine are close to unity over the range of the study and, second, σ for leucine is rather large, 33×10^{-4}. As was shown in paper I,[3] these two factors cause the approximations to converge very rapidly to the exact result. In fact, as we have seen, even the first-order theory is adequate for fitting the data.

The copolymer melting data were analyzed with σ taken as independent of temperature. The best value of σ was obtained by application of the "goodness of fit" criterion, expressed in terms of the parameter τ defined in paper II.[4] The best fit for all copolymer melting data was obtained by minimizing τ. Figure 4 shows that the second-order Allegra theory gives a minimum in τ at $\sigma = 33 \times 10^{-4}$. The error in σ was estimated by computing a quantity τ_{exptl}, which is defined as follows

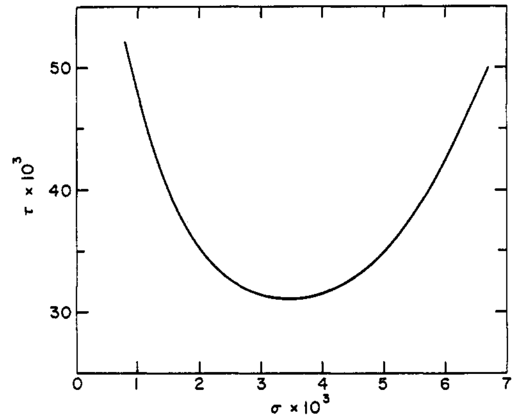

Figure 4. Determination of the best temperature-independent value of σ as the one which corresponds to the lowest value of τ for the leucine copolymers, using the Allegra theory.

$$\tau_{exptl} = \sum_i \sum_T [(\Delta\theta_{h,calcd})_{i,T} + (\Delta\theta_{h,exptl})_{i,T}]^2 (\theta_{h,exptl})_{i,T}^2$$

where $(\Delta\theta_{h,calcd})_{i,T}$ is a fractional quantity which contains all the errors in composition and chain length and $(\Delta\theta_{h,exptl})_{i,T}$ is a fractional quantity which contains all the errors in the estimate of the maximum value of b_0, concentration, and slope of the Moffit-Yang plot. This value of τ_{exptl} defines the extreme values of σ (using Figure 4); the possible deviation in σ was found to be large, with σ extending to a lower limit of 12 $\times 10^{-4}$ and an upper limit of 60 $\times 10^{-4}$. It should be noted that no account was taken of possible errors in the Zimm-Bragg parameters for PHBG and PHPG.

The values of s, computed from both the Lifson and Allegra theories (with the lower limit and the best values, respectively, of σ in the case of the Allegra theory) are listed in Table V. Figure 5 shows the temperature dependence of s with error symbols computed in two different ways. The first method involved the use of $\theta_{h,max}$ and $\theta_{h,min}$ as described in paper IV,[6] with σ as the best-fit Allegra value to generate maximum and minimum values of s at each temperature (triangles). In the second method, each melting curve was

---

(21) J. R. Partington, "An Advanced Treatise on Physical Chemistry," Vol. IV, Longmans, Green and Co., New York, N. Y., 1960, pp 92, 99.

(22) S. Lifson, *Biopolymers*, 1, 25 (1963).

(23) G. Allegra, *J. Polym. Sci., Part C*, No. 16, 2815 (1967).

(24) G. W. Lehman and J. P. McTague, *J. Chem. Phys.*, 49, 3170 (1968).

(25) All computer programs used in these calculations are available and can be obtained as directed in footnotes 26 and 27 of paper I.[3]

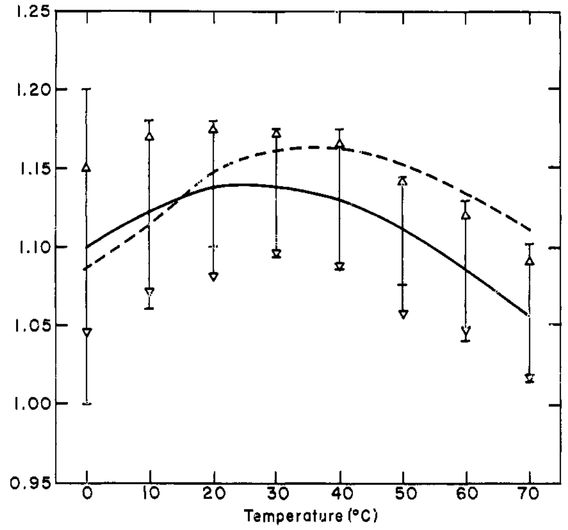

Figure 5. A plot of *s vs. T* for poly(L-leucine) in water. The solid line shows the value obtained from the Allegra analysis with σ = 0.0033, and the dashed line with σ = 0.0012. The two sets of error symbols are described in section IIIA.

TABLE V
VALUES OF THE ZIMM–BRAGG PARAMETER *s* FOR POLY(L-LEUCINE) IN WATER FROM 0–70°

	s		
Temp, °C	Lifson (σ = 30 × 10^{-4})	Allegra (σ = 33 × 10^{-4})	Allegra (σ = 12 × 10^{-4})
0	1.09	1.10	1.09
10	1.11	1.12	1.12
20	1.12	1.14	1.15
30	1.12	1.14	1.16
40	1.12	1.13	1.16
50	1.10	1.11	1.15
60	1.08	1.09	1.14
70	1.06	1.06	1.11

analyzed *separately* using the best Allegra σ to determine the deviation of *s*; then the deviations for all curves at a given temperature were averaged (these average deviations are indicated by I). It is obvious from a comparison with Table V that both theories give values of *s* at each temperature which agree with each other well within the experimental error, even if the lower value of σ is used. Figure 6 shows the computed melting curves [using the Allegra best-fit data (with σ = 33 × 10^{-4})] along with the experimental points. Each set of data listed in Table V gives melting curves which are indistinguishable from those shown in Figure 6. The error symbols on the computed curves arise from errors in the amino acids analysis and molecular weight. The agreement between the calculated and experimental values is excellent, except at 0°, where a large deviation can be seen. This manifests itself in a large error symbol on *s* (in Figure 5) at 0°, and therefore also on ΔG at this temperature. Because of the errors mentioned above, it was not felt justified to attemp to fit the data with a temperature-dependent σ.

The thermodynamic quantities ΔG (the free energy), ΔH (the enthalpy), and ΔS (the entropy) for the conversion of a coil residue of L-leucine to a helical one at the end of a long helical sequence can be obtained from the values of *s* and its temperature dependence. Figure 7 shows a plot of ΔG ($= -RT \ln s$) *vs.* temperature with error symbols calculated as in Figure 5. The data were fit by a weighted least-squares method, using the deviations in Figure 7 as weighting factors[26] as described in paper IV.[6] The resulting least-squares straight line is not shown in Figure 7. The parameters calculated from the Allegra *s* values of Table V (for two different values of σ) are listed in Table VI. It is difficult to estimate the errors in these computed thermodynamic parameters (except ΔG) because of the interdependence of ΔS and ΔH. The ΔG *vs.* temperature curve has a slight minimum (zero slope), and thus any small change in the value of ΔG on either side of the minimum has a large effect on ΔH and ΔS. Therefore, the values presented in Table VI should be looked upon only as rough estimates of these quantities, and no meaning can be attached to the value of the ratio $\Delta H/\Delta S$ as a transition temperature.

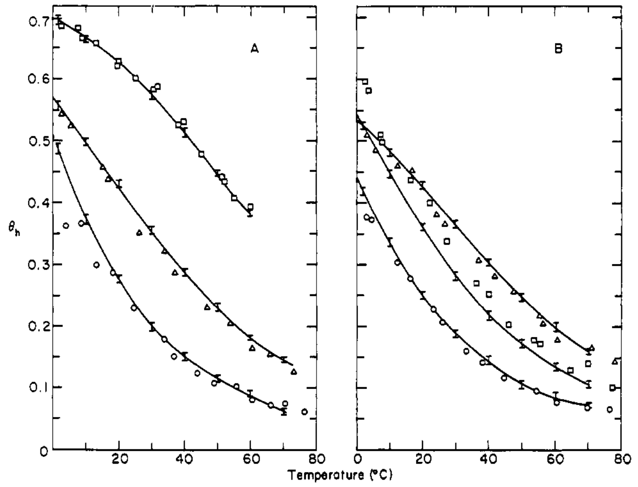

Figure 6. Comparison of the calculated melting curves, obtained from the parameters of the Allegra theory (with σ = 33 × 10^{-4}) for L-leucine given in Table V and those of PHPG and PHBG of Table II in paper II,[4] with the experimental points: (A) (□) 15.3% Leu (with HBG), $\overline{DP}_w$ = 170 (fraction IXC); (Δ) 12.3% Leu (with HPG), $\overline{DP}_w$ = 630 (fraction VIIIB); (○) 3.5% Leu (with HPG), $\overline{DP}_w$ = 1050 (fraction VIB); (B) (□) 7.6% Leu (with HPG), $\overline{DP}_w$ = 901 (fraction VIIB); (Δ) 15.0% Leu (with HPG), $\overline{DP}_w$ = 260 (fraction VIIIF); (○) 3.8% Leu (with HPG), $\overline{DP}_w$ = 312 (fraction VIF). The error symbols indicate errors in the calculated values of θ_h arising from errors in composition and chain length (see Figure 3 for additional errors in the experimental points).

TABLE VI
THERMODYNAMIC PARAMETERS FOR L-LEUCINE[a]

$\Delta G_{20°}$, cal/mol	−76.0	−81.5
ΔH, cal/mol	−149.2	−23.8
ΔS, eu	−0.267	+0.189
σ	33 × 10^{-4}	12 × 10^{-4}

[a] For two different (temperature independent) values of σ.

(B) Comparison with Other Results. It is of interest to compare these results with those of other experiments[9,27,28] on copolymers containing leucine. Qualitatively, all results indicate that leucine may be classified as a helix former. In one previous paper,[27] in which quantitative estimates of parameters

---

(26) P. R. Bevington, "Data Reduction and Error Analysis for the Physical Scientist," McGraw Hill, New York, N. Y., 1969, Chapter 6, p 92.

(27) R. E. Nylund and W. G. Miller, *J. Amer. Chem. Soc.*, 87, 3542 (1965).

(28) G. D. Fasman, C. Lindblow, and E. Bodenheimer, *Biochemistry*, 3, 155 (1964).

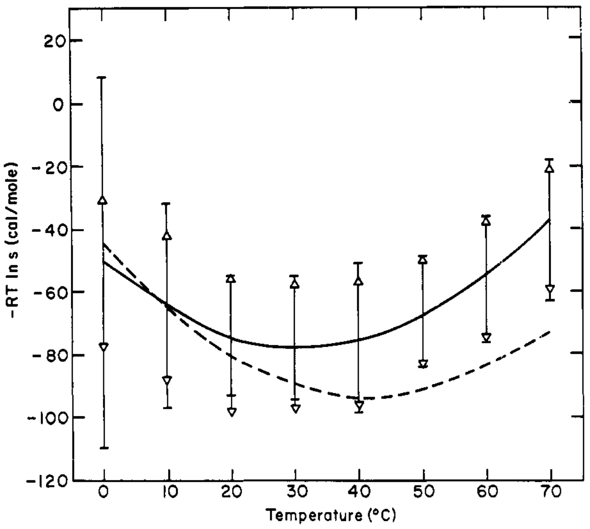

Figure 7. A plot of $-RT \ln s$ (*i.e.*, ΔG) *vs.* T for poly(L-leucine) in water. The solid line shows the values obtained from the Allegra analysis with $\sigma = 0.0033$, and the dashed line with $\sigma = 0.0012$. The two sets of error symbols were calculated as described in section IIIA.

were made, the chain lengths were rather short, of the order of DP ~75, and the samples were not fractionated. Since heterogeneity in chain length, especially at such low DP, affects the parameters very strongly, meaningful comparison cannot be made with the data presented here.

In the experiments of Ostroy, *et al.*,[9] the chain lengths were very short, and an attempt was made to avoid heterogeneity by using the Merrifield synthesis. Despite these precautions, the resulting polymers were probably heterogeneous to some extent. We have reanalyzed the original experimental data (from Figure 6 of ref 9) of Ostroy, *et al.*,[9] with a reestimate of the experimental errors in the data; the latter arise primarily from errors in DP (this low DP) which affect θ_{calcd}, and from the value of $(b_0)_{\max}$ that must be used for such short chains. No account was taken of possible electrostatic repulsions between the poly(D,L-lysine) portions of the block copolymers of Ostroy, *et al.*[9] With our lower limit value of $\sigma = 12 \times 10^{-4}$, we obtain values of s *vs.* T which are indistinguishable from the computed curves of Figure 6 and, at the same time, fit the data of Ostroy, *et al.*,[9] as shown in Figure 8. Considering the differences between the block copolymers of Ostroy, *et al.*,[9] and the random copolymers studied here, the parameters of Table V appear to describe the behavior of L-leucine in both types of polymer, and presumably also in a protein.

(C) Implications. From a comparison of the values of s for L-leucine with those for L-alanine,[6] we see that leucine is the stronger helix former, since s is greater than unity for leucine throughout the whole range of 0–70°, whereas s is smaller for alanine and becomes less than unity near 70°. The primary source of the difference is the more positive value of ΔS for leucine (−0.27 eu; see Table VI) compared to that (−0.70 eu) for alanine,[6] outweighing the enthalpy effect which favors alanine. This behavior is reminiscent of that of hydrophobic bonding,[29] and presumably arises from the formation of some type of near-neighbor (*e.g.*, side chain to backbone) interaction (involving a reduction of ordered water structure) when a leucyl residue (more so than an alanyl residue) is transformed from the coil to the helix state; such interactions, involving the side chain, are absent in polyglycine.[5,9,30] It is interesting to note that such an effect of the side chain had already been demonstrated[30] in statistical mechanical calculations of the helix–coil transition in aqueous solutions of polyglycine, poly(L-alanine), and poly(L-leucine), respectively. It was found[30] that the temperature dependence of s for leucine had a similar shape (with a maximum at 20°) to that indicated in Table V. Such interactions can also be expected for L-leucyl residues in proteins.

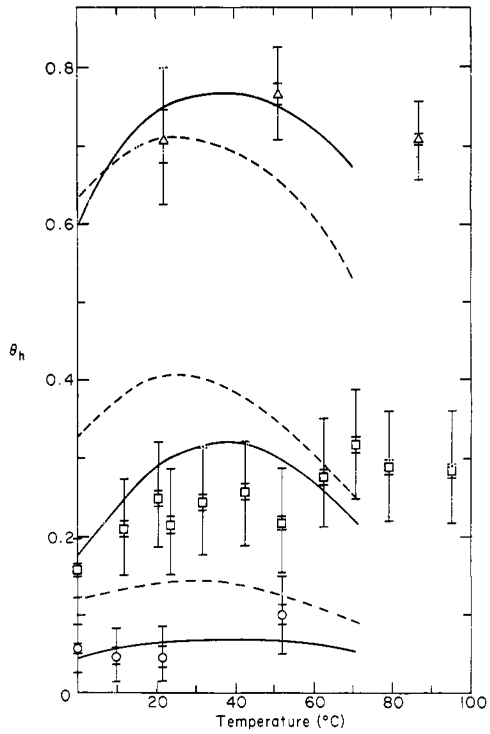

Figure 8. A comparison of the melting curves (computed with parameters deduced from the random copolymers using the Allegra theory) with the experimental points of Figure 6 of ref 9 (which were obtained with block copolymers of the type $(\text{D,L-lysine})_m$–$(\text{L-leucine})_n$–$(\text{D,L-lysine})_m$]. The solid lines were computed with $\sigma = 0.0012$ and the dashed lines with $\sigma = 0.0033$. The error symbols (and inner limit lines) are those of Figure 6, ref 9, and represent errors in the Moffitt–Yang plot. The outer-limit error lines are those calculated with the methods of section IIIA: (○) poly(L-leucine), $\overline{\text{DP}}_w = 11$; (□) poly(L-leucine), $\overline{\text{DP}}_w = 21$; (△) poly(L-leucine), $\overline{\text{DP}}_w = 56$; the errors in $\overline{\text{DP}}_w$ at these very low values of $\overline{\text{DP}}_w$ affect θ_h significantly.

(D) Reversible Heat Precipitation of Copolymers. Fractions IXC and XC; both of which were prepared with HBG as the host, exhibited visible precipitation on heating above 60 and 35°, respectively. Thus, melting data for fraction IXC were not collected above 60°, and no melting data are reported here for fraction XC; however, the melting curves for these fractions were reversible even if the samples were heated above their precipitation temperatures. This reversible heat precipitation was independent of concentration in the range of 0.05–0.3% (w/v). While this effect was not investigated further here, it is of interest to note that such reversible heat precipitation occurs in some proteins and in poly(L-proline).[31]

---

(29) G. Nemethy and H. A. Scheraga, *J. Phys. Chem.*, **66**, 1773 (1962).

(30) D. C. Poland and H. A. Scheraga, *Biopolymers*, **3**, 335 (1965).

(31) A. Ciferri and T. A. Orofino, *J. Phys. Chem.*, **70**, 3277 (1966).

(IV) Conclusions

Water-soluble random copolymers containing L-leucine and either N^5-(3-hydroxypropyl)-L-glutamine or N^5-(4-hydroxybutyl)-L-glutamine were synthesized and characterized. From an analysis of the thermally induced helix–coil transitions of these copolymers, the Zimm–Bragg parameters σ and s for poly(L-leucine) were deduced. The values for s show that L-leucine is a strong helix former, stronger than L-alanine. Comparison with earlier experiments using the block copolymer method indicates similar quantitative results and shows that the state of a residue in a polypeptide or protein, in first approximation, is essentially independent of the chemical nature of its neighbors. The greater stability of the α-helical form of poly(L-leucine) compared to those of poly(L-alanine) and polyglycine has been attributed to hydrophobic interactions.

Acknowledgment. We are indebted to Dr. R. H. Andreatta, Mr. H. Tjan, and Mr. G. Davenport for technical assistance and to Mrs. P. H. Von Dreele and Mrs. K. E. B. Platzer for helpful discussions.

Dipole Moments and the Conformations of Polypeptides.
I. Some Compounds Related to Poly(β-benzyl L-aspartate)[1]

Eric H. Erenrich[2] and Harold A. Scheraga*

Department of Chemistry, Cornell University,
Ithaca, New York 14850.
Received June 21, 1972

ABSTRACT: Dipole moment measurements have been made to obtain information about the orientation of polar side chains with respect to the backbone in several α-helical poly(amino acids). The polymers studied were poly(β-*o*-, *m*-, and *p*-chlorobenzyl L-aspartate) as well as poly(β-benzyl L-aspartate) and poly(γ-benzyl L-glutamate) in both ethylene dichloride and methylene chloride. Dipole moments per residue and rotational relaxation times (for the whole molecule) were determined by measurement of the dielectric constant and dielectric loss in the frequency range from 2 MHz to 50 Hz. The polymers behave like rigid α helices in both solvents, with the relaxation times being consistent with those expected from an application of the Perrin equation to a molecule having the known geometry of the α helix. All of the polymers were found to have the same dipole moment, 3.9 ± 0.2 D/residue, in both solvents. These results suggest that the side chains are in random orientation (equivalent to freely rotating side chains) with respect to each other.

For many years, synthetic poly(α-amino acids) have been used as simple models in theoretical and experimental studies of the interactions which determine the three-dimensional conformations of proteins. From a theoretical point of view, conformational energy calculations have provided insight into the factors responsible for the stability of various polypeptide structures, notably the α helix;[3-5] for example, it has been possible to identify the factors which determine whether a given poly(amino acid) will form a right- or left-handed α helix. A variety of experimental techniques has also been used to examine several aspects of the conformations of such polymers; these include optical rotatory dispersion (ORD),[6] circular dichroism (CD),[7] nuclear magnetic resonance (nmr) spectroscopy,[8-11] infrared[12] and laser Raman[13,14] spectroscopy, linear dichroism,[15] and dipole moment measurements.[16,17]

The conformational energy calculations[3-5] provide additional information about the minimum-energy structure, in particular the orientation of the side chains with respect to the α-helical backbone. Thus, it is of interest to determine the conformations of the side chains of an α-helical polymer experimentally, and the dipole moment study reported here was undertaken for this purpose. This technique is useful because the dipole moments of the peptide groups of an α helix are almost parallel to the helix axis, leading to an extremely large (backbone) dipole moment of the helical polymer. In fact, the existence of this large dipole moment is additional evidence for the presence of a rigid helical structure. The contribution from the backbone dipole moment will be enhanced or diminished, depending on the orientations of the side chains, if the latter also possess a permanent dipole moment. Thus, the overall dipole moment of the α-helical polymer provides information about the orientation of the side chains. However, it should be emphasized that the con-

---

(1) This work was supported by research grants from the National Institute of General Medical Sciences of the National Institutes of Health, U. S. Public Health Service (Grant No. GM-14312), and from the National Science Foundation (Grant No. GB-28469X1).

(2) NIH predoctoral trainee, 1965–1966; Predoctoral Fellow of the National Institute of General Medical Sciences, National Institutes of Health, 1966–1971.

(3) T. Ooi, R. A. Scott, G. Vanderkooi, and H. A. Scheraga, *J. Chem. Phys.*, **46**, 4410 (1967).

(4) J. F. Yan, F. A. Momany, and H. A. Scheraga, *J. Amer. Chem. Soc.*, **92**, 1109 (1970).

(5) M. Gō, N. Gō, and H. A. Scheraga, *J. Chem. Phys.*, **54**, 4489 (1971).

(6) J. T. Yang in "Poly-α-Amino Acids," G. D. Fasman, Ed., Marcel Dekker, New York, N. Y., 1967, p 239.

(7) S. Beychok in ref 6, p 293.

(8) J. L. Markley, D. H. Meadows, and O. Jardetzky, *J. Mol. Biol.*, **27**, 25 (1967).

(9) E. M. Bradbury, B. G. Carpenter, C. Crane-Robinson, and H. Goldman, *Macromolecules*, **4**, 557 (1971).

(10) D. N. Silverman and H. A. Scheraga, *Biochemistry*, **10**, 1340 (1971).

(11) D. N. Silverman, G. T. Taylor, and H. A. Scheraga, *Arch. Biochem. Biophys.*, **146**, 587 (1971).

(12) T. Miyazawa in ref 6, p 69.

(13) P. Sutton and J. L. Koenig, *Biopolymers*, **9**, 615 (1970).

(14) A. Lewis and H. A. Scheraga, *Macromolecules*, **4**, 539 (1971).

(15) T. C. Troxell and H. A. Scheraga, *ibid.*, **4**, 519, 528 (1971).

(16) J. Applequist and T. G. Mahr, *J. Amer. Chem. Soc.*, **88**, 5419 (1966).

(17) A. Wada in ref 6, p 369.