Helix–Coil Stability Constants for the Naturally Occurring Amino Acids in Water. VII. Phenylalanine Parameters from Random Poly(hydroxypropylglutamine-*co*-L-phenylalanine)[1]

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ABSTRACT: Water-soluble random copolymers containing L-phenylalanine and N^5-(3-hydroxypropyl)-L-glutamine were prepared by copolymerization of the *N*-carboxy-α-amino acid anhydrides of L-phenylalanine and γ-benzyl L-glutamate, followed by amidation with 3-amino-1-propanol. The thermally induced helix–coil transitions in these copolymers in water were studied, and the Zimm–Bragg parameters σ and *s* for the transition in poly(L-phenylalanine) were deduced from an analysis of the melting curves using an approximate theory for random copolymers. The incorporation of L-phenylalanine was found to increase the helix content of the host polymers. Accordingly, the computed values of σ and *s* verify that L-phenylalanine is a helix-making residue in water in the temperature range 0–60°.

The evidence that short-range interactions dominate in determining the conformation of a polypeptide or protein has recently been reviewed.[3] Thus, the conformational preference of any naturally occurring amino acid residue is, in *first approximation*, independent of the nature of its neighbors, and conformational determinations from synthetic polymers and copolymers of amino acids are directly applicable to proteins. For reasons cited earlier,[4] random copolymers are best suited for experimental determination of the Zimm–Bragg parameters[5] σ and *s* which characterize the propensity of any amino acid residue to adopt the α-helical conformation. Thus, the host–guest technique (used in earlier papers of this series[4,6-10] to obtain σ and *s* for various naturally occurring amino acids) is applied here to obtain σ and *s* for L-phenylalanine. This guest residue is incorporated into a copolymer with an N^5-(3-hydroxypropyl)-L-glutamine host, and the thermally induced helix–coil transition in these copolymers in water is examined. Earlier experiments[11,12] had indicated that L-phenylalanine is a helix former in water, and this result is reflected in the numerical values of σ and *s* found here.

The synthesis of water-soluble random copolymers of L-phenylalanine with N^5-(3-hydroxypropyl)-L-glutamine is described in section I, and the experimental characterization of the 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[4] to determine the helix–coil stability parameters of L-phenylalanine in water.

I. Experimental Section: Preparation and Characterization of the Copolymers

The copolymers were prepared by first copolymerizing the *N*-carboxyanhydrides of L-phenylalanine and γ-benzyl L-glutamate in dioxane with triethylamine as an initiator. The benzyl blocking group was then replaced by reaction with 3-amino-1-propanol.

A. Materials. 3-Amino-1-propanol from Aldrich Chemical Co., Inc., was 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 from Fisher Scientific Co., absolute ethanol from U. S. Industrial Chemicals Co., and anhydrous methanol from A. R. Mallinckrodt Chemical Works, were used without further purification. Ether from Mallinckrodt Chemical Works was of analytical grade. 2,2,2-Trifluoroethanol obtained from Aldrich Chemical Co., Inc. was stirred over sodium bicarbonate and distilled. L-Phenylalanine and L-glutamic acid were purchased from Aldrich Chemical Co., Inc. Fisher Certified tetrahydrofuran was distilled over lithium aluminum hydride under nitrogen.

Using the L-glutamyl dipeptide method of Manning and Moore,[13] the starting L-amino acids were found to contain no detectable amounts (within 0.1%) of D residues.

Poly[N^5-(3-hydroxypropyl)-L-glutamine] fractions of degree of polymerization $\overline{DP}_w$ = 120 and 220 were fractions IIC and IIB, respectively, of paper II.[6]

B. Synthesis. *N*-Carboxyanhydrides. L-Phenylalanine *N*-carboxyanhydride was prepared by the action of phosgene on a suspension of the amino acid in H_4furan for 1–2 hr at 45–50° as described by Hirschmann *et al.*[14] Several recrystallizations from ether–hexane gave a product with a melting point of 92–93° (dec, cor). The reaction gave a yield of 45–50%.

γ-Benzyl L-glutamate *N*-carboxyanhydride was prepared as in paper IV[8] of this series, but using H_4furan as solvent.[14]

Poly(γ-benzyl L-glutamate$^{m'}$:L-phenylalanine$^{n'}$) Copolymers I–III. Random copolymers of L-phenylalanine and γ-benzyl L-glutamate containing from 5 to 15% phenylalanine were synthesized by polymerization of the *N*-carboxyanhydrides in dioxane with triethylamine as an initiator. The two *N*-carboxyanhydrides were dissolved in dioxane (at a concentration of about 10 mmol of total *N*-carboxyanhydride/80 ml of solvent) in the molar ratio desired for the copolymer product. Triethylamine initiator was added to give an A:I ratio of 25. The reaction flask was sealed with a "Drierite" drying tube and allowed to stand at room temperature for 1 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*

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(2) NIH Predoctoral trainee, 1970–1972.

(3) H. A. Scheraga, *Pure Appl. Chem.*, in press.

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

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

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

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

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

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

(10) J. E. Alter, G. T. Taylor, and H. A. Scheraga, *Macromolecules*, **5**, 739 (1972) (hereinafter called paper VI).

(11) H. J. Sage and G. D. Fasman, *Biochemistry*, **5**, 286 (1966).

(12) H. E. Auer and P. Doty, *Biochemistry*, **5**, 1716 (1966).

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

(14) 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).

Table I
Compositions and Chain Lengths of the Unfractionated (γ-Bzl-L-Glu$^{m'}$:L-Phe$^{n'}$) Copolymers

Polymer No.	L-Phe Content of Reaction Mixt (mol %)	L-Phe Content Found (mol %)	Av Mol Wt[a] ($\times 10^{-3}$)	$\overline{DP}$
I	5	4.9	130	595
II	10	10.1	145	685
III	15	14.2	150	740

[a] By viscometry, using the relation of Fujita *et al.*[15] for polymers in $CHCl_2COOH$.

vacuo. The yield ranged from 90 to 92%. The compositions and chainlengths (determined roughly with the relationship of Fujita *et al.*[15]) of these copolymers are given in Table I.

Poly[N^5-(3-hydroxypropyl)-L-glutamine^m:L-phenylalanine^n] Copolymers IV–VI. The γ-benzyl L-glutamate:L-phenylalanine copolymers were treated with 3-amino-1-propanol to yield a series of water-soluble copolymers. The initial products I–III were treated, as previously described,[8] to yield samples IV–VI. The yields were close to 75%.

C. Extent of Exchange. The absence of benzyl groups in these water-soluble copolymers generally can be confirmed by examining the ultraviolet absorption spectrum in the region ~257 mμ. However, because of the presence of phenylalanine in samples IV–VI, this was not feasible. Hence, in order to test that all the benzyl groups were removed (*i.e.*, exchanged with the propanolamine group) during the amidation, the following procedure was carried out. Roughly 50 mg of polymers I–VI were hydrolyzed in 12 *N* HCl for several days. In addition, about 25 mg of benzyl alcohol was treated in an identical manner. Since the hydrolysis of γ-benzyl L-glutamate polymers results in the production of benzyl alcohol, the hydrolysates of polymers I–III should yield benzyl chloride (because of the presence of the 12 *N* HCl), as verified with the control containing only benzyl alcohol and 12 *N* HCl. The absence of benzyl chloride in samples IV–VI, within the limits of detectability of the analysis, would ensure that the amidation had gone to completion.

Following this procedure, the presence of benzyl chloride in the hydrolysates was checked by gas chromatography with the Aerograph Model A-90-P instrument equipped with an 8 ft long, 0.25 in. wide, 5% Carbowax 20M on Gas Chrom Q glass column, 100–120 mesh. It was found that, with a column temperature of 180° and a flow rate of helium of about 4 cm^3/min, 5 μl of a standard solution containing 0.1 g of benzyl chloride in 100 ml of ether could be resolved into two distinct peaks (the smaller of these corresponding to benzyl chloride as demonstrated in a run in which ether was absent). Hence, roughly 5×10^{-6} g of benzyl chloride can be detected by this method. The hydrolysis of 50 mg of a fully blocked γ-benzyl glutamate polymer results in roughly 25 mg of benzyl chloride. If the benzyl group of this polymer were 99% exchanged in forming IV–VI, only 0.25 mg of benzyl chloride would result. By extracting any benzyl chloride formed during one of these hydrolyses into 25 ml of ether, reducing the volume of the ether to 5 ml by evaporation and injecting 100 μl of this solution onto the column (which would contain 5×10^{-6} g), it can be shown by the absence of the benzyl chloride peak that the polymer in question is indeed 99% free of benzyl groups. This procedure was carried out with polymers I–VI with the result that benzyl chloride was found in hydrolysates from polymers I–III, establishing the validity of the procedure, while no benzyl chloride (within 1% of the residues present) was found for polymers IV–VI. Hence, polymers IV–VI are at least 99% free of benzyl groups.

D. Fractionation. The copolymers IV–VI were fractionated on the basis of solubility with methanol and ether by the procedure described in paper II,[6] 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 Technion amino acid analyzer. Samples I–III were hydrolyzed in 12 *N* HCl at 105° for 72–96 hr in sealed ampoules, and fractions from samples IV–VI were hydrolyzed similarly for 48 hr. It was established from a study of the time dependence of the extent of the hydrolysis (carried out on fraction VIE) that 48 hr was sufficient time to ensure 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%.

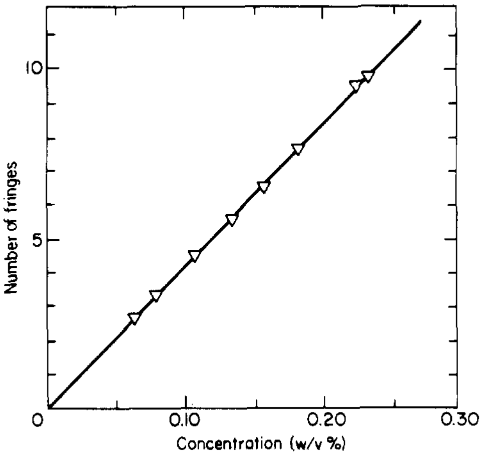

Figure 1. A plot of the number of fringes obtained from a synthetic boundary run *vs.* the concentration of the sample obtained from nitrogen analysis.

F. Determination of Concentration. The concentrations of all polymer solutions were determined as described previously.[8] Each concentration was determined at least four times. The error in these determinations was estimated to be ±3%.

G. Viscometry. The intrinsic viscosities of samples I–III were determined in $CHCl_2COOH$ at 25.0 ± 0.1° in a Cannon-Ubbelohde semimicro dilution viscometer as described earlier,[7] and the relation of Fujita *et al.*[15] was used to obtain only a rough molecular weight [using poly(γ-benzyl L-glutamate) as a model].

H. Optical Purity. Polymers IV–VI were checked for the presence of D residues which may have been formed during the amidation reaction. Again the L-glutamyl dipeptide method of Manning and Moore[13] was used. The L-Glu-L-Phe and L-Glu-D-Phe were separated on a Technicon amino acid analyzer with a sodium citrate elution buffer of pH 3.70. Dipeptide standards were prepared from L-phenylalanine and D-phenylalanine monomers.

I. Determination of Molecular Weights. Molecular weights for the fractions from samples IV–VI were determined by the conventional sedimentation equilibrium method using a Spinco Model E ultracentrifuge as reported earlier,[7] with the following exception. The determination of the concentration, c_0 in fringe units, from synthetic boundary runs was made, in turn, by calibrating the fringe shift against polymer concentration (assayed by nitrogen analysis). Figure 1 is a calibration curve obtained by plotting the fringe shift (extrapolated to zero time, as recommended by Chervenka[16]) against concentration. This procedure was adopted because occasional synthetic boundary runs exhibited a time dependence of the fringe shift across the cell which made extrapolation to zero time difficult. The calibration curve of Figure 1 was prepared only from those synthetic boundary runs in which there was little time dependence.

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. These molecular weights are accurate to within ±8%.

The partial specific volumes $\bar{v}$ of the poly[N^5-(3-hydroxypropyl)-L-glutamine^m:L-phenylalanine^n] copolymers required for the calculation of molecular weights were determined from the amino acid content as described by Cohn and Edsall.[17] A value of $\bar{v}$ = 0.79[18] was used for poly[N^5-(3-hydroxypropyl)-L-glutamine] in the calculation of $\bar{v}$ for the copolymers.

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

(16) C. H. Chervenka, "A Manual of Methods for the Analytical Ultracentrifuge," Spinco Division of Beckman Instruments, 1969, p 71.

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

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

Table II
Characterization of the Fractionated Copolymers

Fraction[a]	Wt of Fraction (mg)	L-Phe Content (mol %)	$\bar{v}$ (cm^3/g)	$\bar{M}_w \times 10^{-3}$ [b]	$\bar{M}_z/\bar{M}_w$	$\overline{DP}_w$
IVA	108	5.7				
IVB	203	5.8	0.789	254	1.20	1390
IVC	74	5.7				
IVD	86	5.7				
IVE	98	5.6				
IVF	75	5.4	0.789	79.4	1.29	432
IVG	96	5.3				
IVH	38	5.6				
VA	65	11.6				
VB	85	11.3				
VC	130	11.1	0.788	82.3	1.39	453
VD	85	11.3				
VE	140	10.6	0.788	28.9	1.13	159
VF	100	10.3	0.788	18.6	1.15	102
VIA	33	15.3				
VIB	77	14.3	0.787	49.6	1.25	275
VIC	67	15.2				
VID	53	14.0				
VIE	67	14.5				
VIF	75	14.9	0.787	15.7	1.01	87
VIG	32	15.0				

[a] Samples IV–VI were obtained from unfractionated I–III, respectively. The letters correspond to the fractions obtained in the fractionation procedure. [b] This value was determined by conventional sedimentation equilibrium (with extrapolation to zero concentration).

Table III
Values of b_0 in Trifluoroethanol and CHCl$_2$COOH

Fraction	L-Phe Content (mol %)	Solvent	Temp (°C)	b_0
VIC	15.2	Trifluoroethanol	1.0	−746
VD	11.3	Trifluoroethanol	2.9	−722
VID	14.0	CHCl$_2$COOH	23.0	48

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.[6] Temperature control was maintained to within ±0.2° with water-jacketed quartz cells. The concentrations used in these measurements were 0.03 to 0.30% (w/v). All solutions were filtered through 0.45 μ Millipore filters before measurement. The experimental error in the helix content, θ_h (which is defined as $-b_0/750$), results from (a) the error in the solution concentration, (b) the error in the values of b_0 for the complete helix and complete coil, and (c) the error in the slope of the Moffitt-Yang plot.

II. Results

A. Characterization of the Copolymers. Table I summarizes the composition and the average degree of polymerization ($\overline{DP}$) of the unfractionated poly(γ-benzyl L-glutamate$^{m'}$:L-phenylalanine$^{n'}$) copolymers, and Table II shows the data for the fractionated copolymers poly[N^5-(3-hydroxypropyl)-L-glutamine^m:L-phenylalanine^n] that were investigated. The usual decrease in $\overline{DP}_w$ attributed to transaminolysis[6-10,18] upon conversion of these polymers to their hydroxyalkylglutamine derivatives is apparent from a comparison of the two tables.

Two observations can be made from the data in Table II. First, the composition is essentially independent of chain length, within the experimental error, for a series of fractions from the same sample. In other words, the same relative amount of phenylalanine is incorporated into the short chains as into the long chains for a given sample of polymer. This indicates that there is little departure from randomness in these copolymers; in any case, it has been demonstrated in paper I[4] that the presence of relatively short blocks (of, say, phenylalanine) does not influence the melting behavior of a random copolymer to any significant extent. Second, the reaction mixtures of higher phenylalanine content lead to polymers of lower chain length. The small opposite trend indicated in Table I does not contradict this conclusion, since the data of Table I (obtained from viscosity measurements) are less accurate than those of Table II. The behavior indicated in Table II might arise if phenylalanine were a better chain terminator than benzyl glutamate.

The concentration dependence of the apparent molecular weights of the fractions studied, illustrated in Figure 2, is quite small, making it easy to extrapolate to infinite dilution to obtain $\overline{DP}_w$. The magnitude of $\bar{M}_z/\bar{M}_w$ shown in Table II indicates that the polymers used in this study were fairly homogeneous.

Samples of polymers IV–VI were hydrolyzed before fractionation and analyzed for the presence of D residues by the method of Manning and Moore.[13] Analysis of the dipeptides formed showed that 1.6, 1.7, and 1.9 mol % of the phenylalanine residues present in polymer lots IV–VI, respectively, were D residues. No racemization of the glutamic acid of the polymers was detected (within ±0.1%). The amount of racemization found here was considered to be too minor to affect the computed value of σ and s for L-phenylalanine.

B. ORD and CD Data for the Copolymers. To check that the optical properties examined here arise only from combinations of helical and coil conformations, the ORD and CD data shown in Figure 3 were obtained. The presence of a right-handed α-helical structure[19-21] (mixed

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

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

[21] N. Greenfield and G. D. Fasman, *Biochemistry*, 8, 4108 (1969).

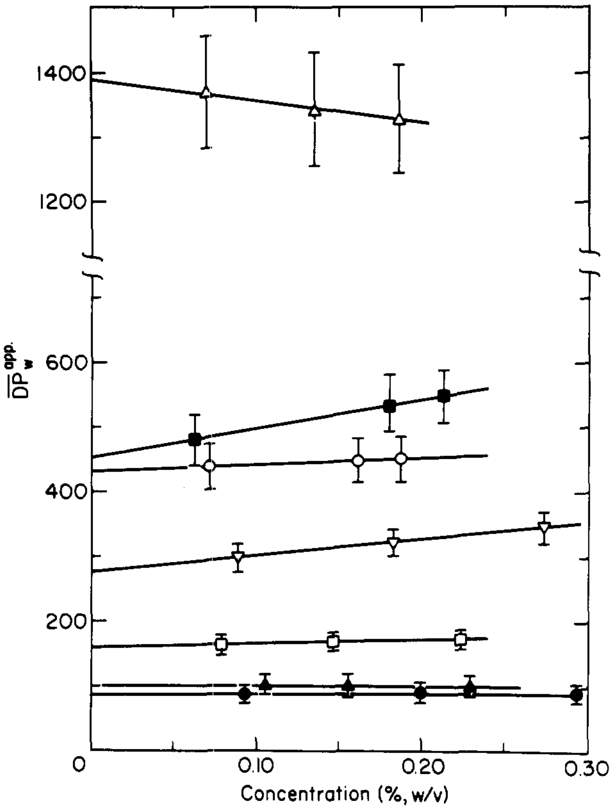

Figure 2. Concentration dependence of molecular weights for fractions used for analysis to obtain σ and s: (△) 5.8% Phe, $\overline{DP}_w$ = 1390 (fraction IVB); (■) 11.1% Phe, $\overline{DP}_w$ = 453 (fraction VC); (○) 5.4% Phe, $\overline{DP}_w$ = 432 (fraction IVF); (▽) 14.3% Phe, $\overline{DP}_w$ = 275 (fraction VIB); (□) 10.6% Phe, $\overline{DP}_w$ = 159 (fraction VE); (▲) 10.3% Phe, $\overline{DP}_w$ = 102 (fraction VF); (●) 14.9% Phe, $\overline{DP}_w$ = 87 (fraction VIF). The error symbols represent the experimental error in each measurement.

with random coil) is indicated, for example, by the trough at 233 mμ in the ORD curves and the double minimum at 222 and 208 mμ in the CD curves, all of which increase with increasing phenylalanine content and chainlength, in accordance with the expected helix-promoting properties of phenylalanine. 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. While the possibility that the ORD spectra shown in Figure 3 arise from overlapping helical and phenylalanyl Cotton effects cannot be eliminated, such an effect would be small because of the relatively small amount of phenylalanine present in the copolymers.

Measurements of b_0, shown in Figure 4 for the seven fractions studied over the range of λ = 270–430 mμ as a function of temperature, confirm the above conclusion. To exclude the possibility that the values of b_0 might be erroneous because of rotational contributions from side-chain phenylalanyl Cotton effects in the tail of the helical trough measured from 275 to 430 mμ, the CD spectra of several polymers of higher phenylalanine content were examined from 240 to 450 mμ. In no case were any dichroic bands distinguishable from the base lines. Hence, it appears that the b_0 values obtained are a true measure of the helix content of the polymers studied. The procedures used to obtain the curves of Figure 4 were the same as those in paper IV[8] except that here the solutions had a lower concentration range [from 0.1 to 0.3% (w/v)]. No concentration dependence was ever observed, and all curves were reproducible. The size of the error symbols in Figure 4 reflects the experimental errors in θ_h arising from errors in concentration (±3%), in b_0 for the full helix and coil (±3%) and in the slope of the Moffitt-Yang plot (±300/b_0%).

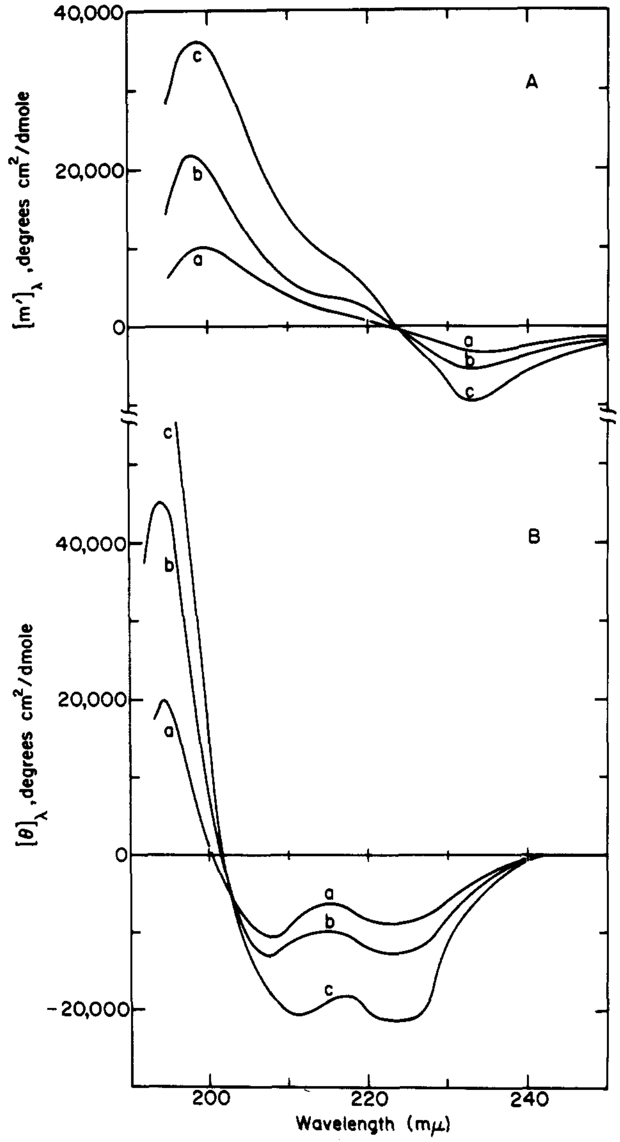

Figure 3. (A) ORD and (B) CD data in water at 25.0 ± 0.5° for representative samples of phenylalanine copolymers: (a) 5.4% Phe, $\overline{DP}_w$ = 432 (fraction IVF); (b) 10.6% Phe, $\overline{DP}_w$ = 159 (fraction VE); (c) 14.3% Phe, $\overline{DP}_w$ = 275 (fraction VIB).

These transitions were demonstrated to be reversible in all cases, except for the two dashed regions between 40 and 60° for fractions VIB and VIF shown in Figure 4. When these two polymers (of high phenylalanine content) were heated to 60° and cooled to room temperature, the resulting values of b_0 differed from those observed before heating. Similar results were obtained by Sage and Fasman[11] with random copolymers of L-glutamic acid and L-phenylalanine at pH 7.5. However, if the solutions of fractions VIB and VIF were heated only to 40°, then the transitions were reversible. Nevertheless, the same values of σ and s were obtained from the data of Figure 4 whether the data in the dashed curves at 50 and 60° were included in the analysis or not.

C. b_0 for Complete Helix and Complete Coil. For the homopolymers poly[N^5-(4-hydroxybutyl)-L-glutamine] and poly[N^5-(3-hydroxypropyl)-L-glutamine] studied in paper

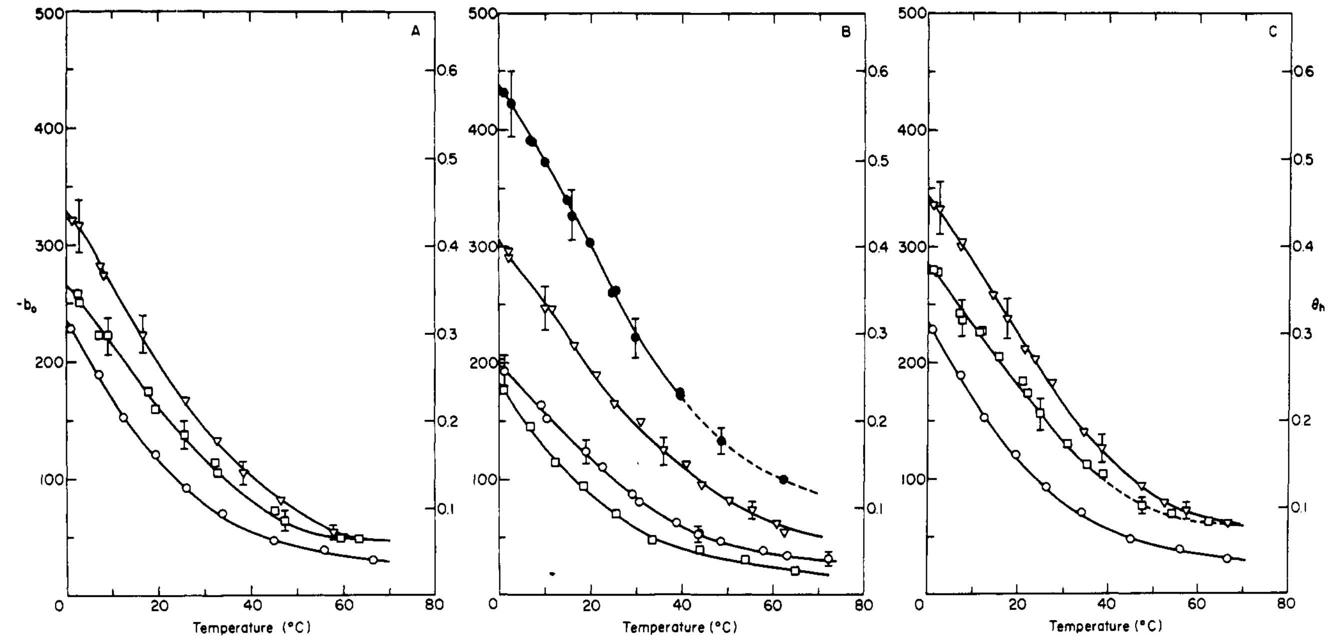

Figure 4. Temperature dependence of b_0 (and θ_h) for Phe copolymers in water: (A) (▽) 5.8% Phe, $\overline{DP}_w$ = 1390 (fraction IVB); (□) 5.4% Phe, $\overline{DP}_w$ = 432 (fraction IVF); (○) poly[N^5-(3-hydroxypropyl)-L-glutamine], $\overline{DP}_w$ = 220 (fraction IIB of paper II[4]); (B) (●) 14.3% Phe, $\overline{DP}_w$ = 275 (fraction VIB); (▽) 10.6% Phe, $\overline{DP}_w$ = 159 (fraction VE); (○) 10.3% Phe, $\overline{DP}_w$ = 102 (fraction VF); (□) poly[N^5-(3-hydroxypropyl)-L-glutamine], $\overline{DP}_w$ = 120 (fraction IIC of paper II[4]); (C) (▽) 11.1% Phe, $\overline{DP}_w$ = 453 (fraction VC); (□) 14.9% Phe, $\overline{DP}_w$ = 87 (fraction VIF); (○) poly[N^5-(3-hydroxypropyl)-L-glutamine], $\overline{DP}_w$ = 220 (fraction IIB of paper II[4]). The points are the experimental ones, and the lines represent the smoothed experimental curves. The size of the error symbols reflects the experimental errors affecting θ_h arising from the errors in the determination of the concentration, in the choice of values of b_0 for the full helix and coil conformations, and the slope of the Moffitt-Yang Plot.

Table IV
Comparison of the Values of θ_h, Calculated with Approximate and Exact Theories for Finite Chains[a]

L-Phe Content (mol Fraction)	$\overline{DP}_w$	Temp (°C)	$(\theta_h)_{exp}$	$(\theta_h)_{theor}$ Lifson[b]	Allegra[c]	Lehman-Mctague[c]
0.149	87	0	0.379	0.294	0.294	0.288
		30	0.176	0.169	0.169	0.164
		60	0.085	0.084	0.084	0.082
0.103	102	0	0.264	0.298	0.297	0.292
		30	0.112	0.153	0.152	0.149
		60	0.048	0.074	0.074	0.073
0.106	159	0	0.403	0.391	0.391	0.386
		30	0.197	0.192	0.192	0.189
		60	0.083	0.086	0.086	0.085

[a] The parameters used for poly[N^5-(3-hydroxypropyl)-L-glutamine] were those of Table II in paper II.[6] [b] The parameters used for L-phenylalanine were obtained by fitting the data to the Lifson theory, as shown in Table V. [c] The parameters used for L-phenylalanine were obtained by fitting the data to the Allegra theory, as shown in Table V.

II,[6] 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,[22] two of these fractions were examined in trifluoroethanol and one in $CHCl_2COOH$, with b_0 corrected for the dispersion of the refractive index of the solvent.[23] The results are shown in Table III. The high b_0 values of −746 and −722 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 +48 in $CHCl_2COOH$ 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-phenylalanine). 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-phenylalanine). This procedure has been discussed extensively in earlier papers of this series.[4,6-10] The approximations, corresponding to the theories of Lifson[24] and Allegra,[25] were both used to fit the data, and checked with the exact method of Lehman and McTague[26] in representative cases.[27] The results of these calculations are shown

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

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

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

(25) G. Allegra, *J. Polym. Sci., Part C*, **16**, 2815 (1967).

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

(27) All computer programs used in these calculations are available and can be obtained as directed in footnotes 26 and 27 of paper I.[4]

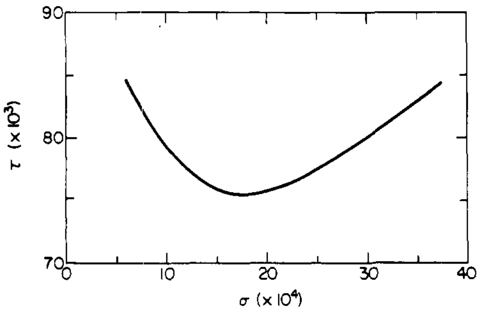

Figure 5. Determination of the best temperature-independent value of σ as the one which corresponds to the lowest value of τ for the phenylalanine copolymers, using the Allegra theory.

Table V
Values of the Zimm–Bragg Parameter *s* for Poly(L-phenylalanine) in Water from 0 to 60°

Temp (°C)	*s*	
	Lifson[a]	Allegra[a]
0	1.05_6	1.06_1
10	1.07_6	1.08_4
20	1.07_8	1.08_6
30	1.06_2	1.06_9
40	1.04_1	1.04_7
50	1.01_2	1.01_6
60	1.00_0	1.00_3

[a] Calculated for $\sigma = 0.0018$.

in Table IV along with some original 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 phenylalanine parameters.

As pointed out in paper VI,[10] the approximate theories appear to hold here for phenylalanine for two reasons. First, the values of s for poly[N^5-(3-hydroxypropyl)-L-glutamine] and phenylalanine are close to unity over the range of the study and, second, σ for phenylalanine is moderately large, 18×10^{-4}. As was shown in paper I,[4] these two factors cause the approximations to converge very rapidly to the exact result. In fact, as shown in Table IV, 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.[6] The best fit for all copolymer melting data was obtained by minimizing τ. Figure 5 shows that the second-order Allegra theory gives a minimum in τ at $\sigma = 18 \times 10^{-4}$.

The error in σ was determined by refitting the experimental data which were altered so that θ_h^{exptl}, $\overline{\text{DP}}_w$, and X_{Phe} (the mole per cent of phenylalanine] took on all combinations of their maximum and minimum values (corresponding to the respective experimental errors in these quantities); *i.e.*, the minimum in the τ *vs.* σ plot was redetermined. By fitting various combinations of these maximum and minimum values, the largest variation in σ was found to be 0.0006. This error in σ arises almost exclusively from the error in $\overline{\text{DP}}_w$; while σ is relatively insensitive to errors in θ_h^{exptl} and X_{Phe}, this is not true for the quantity s. Finally, since the minimum in Figure 5 is somewhat shallow, an additional estimated uncertainty of 0.0002 in σ was allowed for. Hence, the best temperature-independent value of σ was found to be 0.0018 ± 0.0008.

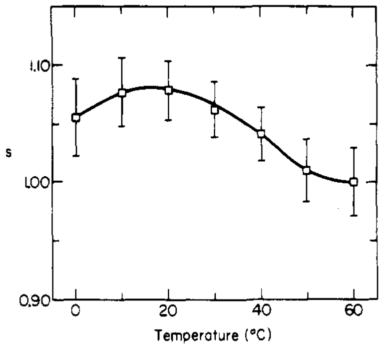

Figure 6. A plot of s *vs.* T for poly(L-phenylalanine) in water for $\sigma = 0.0018$. The error symbols are described in section IIIA.

Table VI
Thermodynamic Parameters for L-Phenylalanine[a]

$\Delta G_{20°}$, cal/mole	-44 ± 12
ΔH, cal/mole	-170 ± 80
ΔS, eu	-0.46 ± 0.28

The values of s, computed from both the Lifson and Allegra theories, are listed in Table V. Figure 6 shows the temperature dependence of s together with error symbols. The magnitudes of the errors in s were computed by a procedure similar to that used to obtain the error in σ. The maximum possible value of s at a given temperature was that which fit $\theta_h^{\text{exptl}} + \Delta\theta_h^{\text{exptl}}$, $X_{\text{Phe}} - \Delta X_{\text{Phe}}$ and $\overline{\text{DP}}_w - \Delta(\overline{\text{DP}}_w)$. Since σ and s are not independent, this fitting was carried out for values of σ in the range of $\sigma + \Delta\sigma$ to $\sigma - \Delta\sigma$, taking that value of σ which yielded the maximum value of s. In a similar way, the minimum possible value of s was computed at each temperature. The maximum errors in s, obtained by this procedure, agreed well (in representative cases) with a more complex treatment which involved analytical differentiation of the expression relating θ_h to s, X, and $\overline{\text{DP}}_w$. It should be noted that no account was taken of possible errors in the Zimm–Bragg parameters for poly[N^5-(3-hydroxypropyl)-L-glutamine]. It is obvious from Table V that both the Lifson and Allegra theories give values of s at each temperature which agree well with each other within the experimental error.

Figure 7 shows the computed melting curves (using the Allegra best-fit data) along with the experimental points. The error symbols on the computed curves arise from the previously described errors in σ and s. The agreement between the calculated and experimental values of θ_h is only moderately good for most of the fractions. It may be that, even though short-range interactions dominate in determining the comformation, there is a small longer-range interaction present, possibly a stacking of the phenyl side chains. Such a side-chain–side-chain interaction could be taken into account by an adjustable (free-energy) parameter.[28] However, because of the use of a temperature-independent value of σ, it was not felt justified to introduce this additional parameter. The values of σ and s in Table V certainly account for the major part of the helix-coil transition in poly(L-phenylalanine).

The thermodynamic quantities ΔG (the free energy), ΔH (the enthalpy), and ΔS (the entropy) for the conversion of a coil residue of L-phenylalanine to a helical one at the end of a long helical sequence can be obtained from

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(28) D. Poland and H. A. Scheraga, "Theory of Helix-Coil Transitions in Biopolymers," Academic Press, New York, N. Y., 1970, p 140.

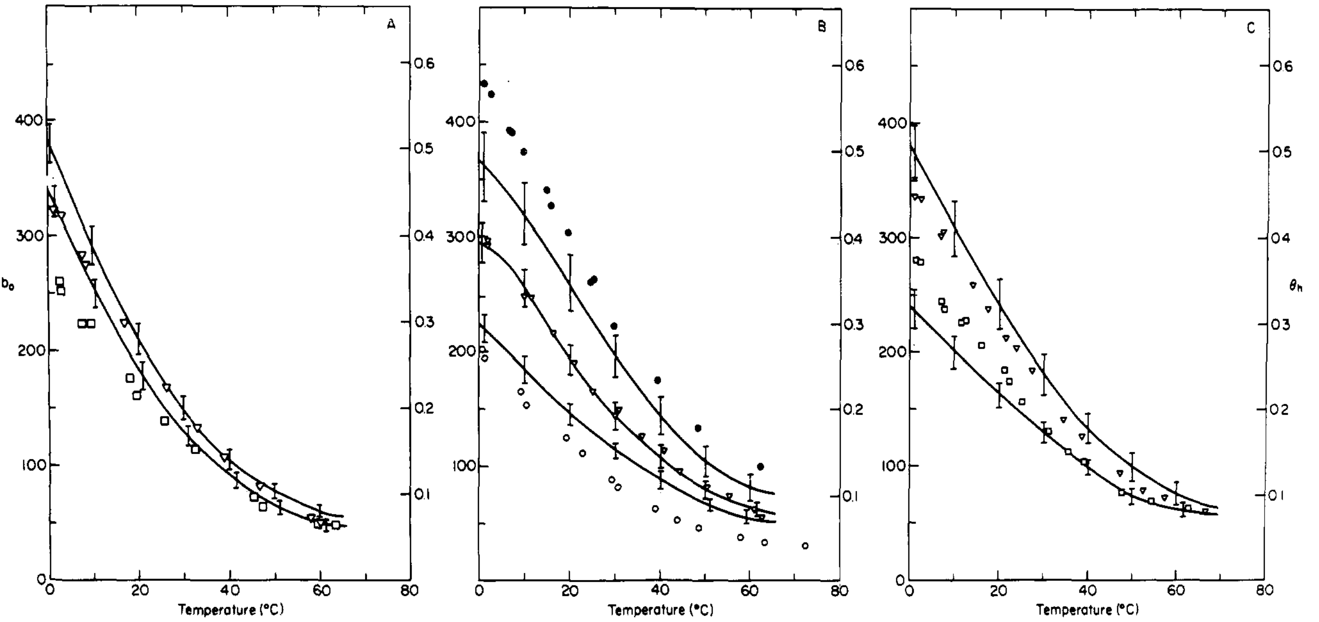

Figure 7. Comparison of the calculated melting curves obtained from the parameters of the Allegra theory for L-phenylalanine with the experimental points: (A) (▽) 5.8% Phe, $\overline{DP}_w$ = 1390 (fraction IVB); (□) 5.4% Phe, $\overline{DP}_w$ = 432 (fraction IVF); (B) (●) 14.3% Phe, $\overline{DP}_w$ = 275 (fraction VIB); (▽) 10.6% Phe, $\overline{DP}_w$ = 159 (fraction VE); (○) 10.3% Phe, $\overline{DP}_w$ = 102 (fraction VF); (C) (▽) 11.1% Phe, $\overline{DP}_w$ = 453 (fraction VC); (□) 14.9% Phe, $\overline{DP}_w$ = 87 (fraction VIF). The error symbols indicate errors in the calculated values of θ_h arising from errors in the values of σ and s used. These errors are explained in section IIIA.

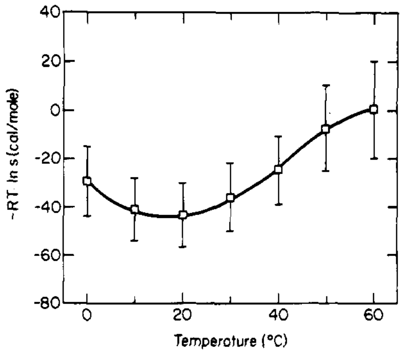

Figure 8. A plot of $-RT \ln s$ (*i.e.*, ΔG) *vs.* T for poly(L-phenylalanine) in water for σ = 0.0018. The error symbols were calculated as described in section IIIA.

the values of s and its temperature dependence. Figure 8 shows a plot of ΔG (= $-RT \ln s$) *vs.* temperature with error symbols calculated as in Figure 6. The data were fit to a straight line by a weighted least-squares method, using the deviations in Figure 6 as weighting factors[29] as described in paper IV.[8] The resulting least-squares straight line is not shown in Figure 8. The parameters calculated from the Allegra s values of Table V are listed in Table VI. The errors in the values of the thermodynamic quantities ΔH and ΔS were found in a straightforward manner by utilizing the expressions for the errors in the slope ($-\Delta S$) and intercept (ΔH) of a weighted least-squares straight line fit of ΔG to T, as given in ref 29. These errors reflect both the uncertainties in the original data and also the deviations of the data from a straight line. As seen in Table VI, these errors are relatively large. For this reason, little meaning can be attached to the value of $\Delta H/\Delta S$ as a transition temperature.

B. Comparison with Other Results. Although no other authors have assigned numerical values to the helix–coil stability constants for L-phenylalanine in water, some qualitative results have been reported. Sage and Fasman,[11] working with random copolymers of L-glutamic acid and L-phenylalanine, found that the incorporation of L-phenylalanine promoted helix formation in the copolymers, in qualitative agreement with our result that L-phenylalanine is a helix former ($s > 1$). Auer and Doty[12] studied two copolymers in which blocks of poly(L-phenylalanine) and poly(L-leucine), respectively, were incorporated between blocks of poly(D,L-glutamic acid). They found that both of the nonpolar blocks formed stable α-helical regions, but further, that the poly(L-leucine) blocks were thermally more stable than those of poly(L-phenylalanine). Quantitatively, the results presented in this study on L-phenylalanine and the work presented in the previous paper of this series on L-leucine[10] confirm this result in that the computed values of s at all temperatures are higher for L-leucine than L-phenylalanine.

C. Implications. A comparison of the values of s for L-alanine,[8] L-phenylalanine, and L-leucine[10] shows that L-phenylalanine lies intermediate between L-alanine and L-leucine in its helix-promoting properties over almost the whole temperature range studied. It can be seen from the thermodynamic parameters for these transitions that, as pointed out in paper VI,[10] the primary source of this effect is the increasingly more positive values of ΔS for the transitions in going from L-alanine to L-phenylalanine to L-leucine. The trend in the enthalpies would reverse the order of helical stabilities, but it is outweighed by the entropy effect. This trend could be interpreted in terms of increased hydrophobic bonding[30] in the helical state when going from L-Ala to L-Phe to L-Leu. This presumably, arises from the formation of some type of near-neighbor (*i.e.*, side-chain to backbone) interaction (involving a reduction of ordered water structure) when a nonpolar amino acid residue is transformed from the coil to the helix state. Finally, the increasing values of σ in going from L-alanine to L-phenylalanine to L-leucine indicate

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

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

that those residues which tend to form more stable helical sequences also tend to nucleate the formation of these regions better.

IV. Conclusions

Water-soluble random copolymers containing L-phenylalanine and N^5-(3-hydroxypropyl)-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-phenylalanine) were deduced. The values for s show that L-phenylalanine is a moderately strong helix former, stronger than L-alanine, but weaker than L-leucine. These results are in good agreement with the earlier qualitative results of other workers. The greater stability of the α-helical form of poly(L-phenylalanine) compared to poly(L-alanine) and of poly(L-leucine) compared to both poly(L-phenylalanine) and poly(L-alanine) has been attributed to hydrophobic interactions.

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Ring Closure in Chain Molecules with C_n, I, or S_{2n} Symmetry[1]

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ABSTRACT: Mathematical methods are developed for the exact generation of conformations of cyclic chain molecules with C_n, I, or S_{2n} symmetry, when bond lengths and bond angles are maintained fixed. The methods are illustrated for the case of cyclohexane. An explicit expression is given for the dihedral angles of the flexible boat form of cyclohexane. Application of these methods to conformational energy calculations of cyclic oligopeptides is discussed.

There is considerable interest in the conformations of cyclic oligopeptides, which may or may not have symmetry. In order to study their conformations, it is necessary to generate ring structures of such molecules. The problem of the exact ring closure of a chain molecule with fixed bond lengths and bond angles has already been solved without assuming any symmetry,[3-5] and differs from that in which symmetry is assumed. This paper is concerned with solving the mathematical problem of finding a set of dihedral angles in a cyclic chain molecule whose conformation has C_n, I, or S_{2n} symmetry. Although the methods have been developed with applications to cyclic oligopeptides in mind, they are applicable to any cyclic chain molecule with symmetry. Therefore, the methods are illustrated for the simple case of cyclohexane. The origin of the rigidity and flexibility of the chair and boat forms, respectively, of cyclohexane is discussed from both the point of view of the theory developed earlier[3] and that presented here.

I. Nature of Symmetry in Cycle Structures

In general, a chain molecule can be regarded as a series of *units* linked by single bonds about which rotation can take place (see Figure 1). The ith bond (with dihedral angle for rotation around it being denoted as ω_i) between atom B_{i-1} in the $(i - 1)$th unit and atom A_i in the ith unit links these two units. The ith *structure*, consisting of the ith unit and atoms B_{i-1} and A_{i-1}, is a rigid one; *i.e.*, the relative positions of the atoms in the structure do not change even when ω_i and ω_{i+1} do. Each unit may consist either of a single atom or of two or more atoms. In the case of a polyethylene-type molecule, atoms A_i and B_i are a single identical atom. When atoms B_{i-1}, A_i, B_i, A_{i+1} lie in a single plane, the structure is said to have a *planar backbone* (as in the polypeptide chain); this definition of a planar backbone includes the trivial case in which atoms A_i and B_i are a single identical atom. It also includes an important special case where the ith structure has a plane of symmetry in which atoms B_{i-1}, A_i, B_i, A_{i+1} (and any other atoms that define the backbone of the chain) lie.

For symmetry to exist in a molecule, there must, first of all, be symmetry in the covalent structure. In a molecule with C_n symmetry, rotation about the symmetry axis by $2\pi/n$ leads to the same structure, and the following relation holds among its dihedral angles

$$\omega_{i+km} = \omega_i \quad (i = 1, 2, \ldots, m;\ k = 1, 2, \ldots, n - 1) \tag{1}$$

where n is the number characterizing the symmetry, and m is the number of dihedral angles in a *symmetry unit* [a succession of units (in the sense defined above) whose conformation (or its mirror image) repeats in a manner specified by the symmetry]. In the case of I symmetry, inversion through a point leads to the same structure, and the following relation holds

$$\omega_{i+m} = -\omega_i \quad (i = 1, 2, \ldots, m) \tag{2}$$

In the case of S_{2n} symmetry, rotation about the symmetry axis by $\pi \pm \pi/n$, and then inversion through a point on the symmetry axis, leads to the same structure, and the following relation holds

$$\omega_{i+2km} = \omega_i \quad (i = 1, 2, \ldots, m;\ k = 1, 2, \ldots, n - 1) \tag{3}$$

$$\omega_{i+(2k+1)m} = -\omega_i$$

$$(i = 1, 2, \ldots, m;\ k = 0, 1, 2, \ldots, n - 1)$$

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(2) On leave of absence from the Department of Physics, Faculty of Science, University of Tokyo, Tokyo, Japan, Summer, 1971, and from the Department of Physics, Faculty of Science, Kyushu University, Fukuoka, Japan, Summer 1972.

(3) N. Gō and H. A. Scheraga, *Macromolecules*, **3**, 178 (1970); this paper will be referred to hereinafter as A, with equation numbers cited as (A-x).

(4) The solution is a trivial one when bond lengths and bond angles are allowed to vary. The necessity (or the advantage) of treating bond lengths and bond angles as fixed was discussed earlier[5] and reviewed briefly in paper A.

(5) N. Gō and H. A. Scheraga, *J. Chem. Phys.*, 51, 4751 (1969).