chain, but up to now no classical reference spectra[16] could be used to interpret these CD results completely.

Conclusion

In this extension of our previous paper[1] we show that the mechanism of interaction in the polyanion–polycation complex formation can be relatively well interpreted by means of simple techniques such as conductimetry, potentiometry, and circular dichroism.

It is demonstrated that as soon as the DP of the glutamic acid of oligomer equals 6, a 1/1 uncharged complex is formed. With a DP ≥ 23 a β structure is cooperatively obtained, as shown by CD measurements. In fact, the problems of conformation of the complex can be solved easily when both interacting species are oligopeptides. The lack of information concerning the conformation of nonpeptide polyanions necessitates further investigation.

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Helix–Coil Stability Constants for the Naturally Occurring Amino Acids in Water. 19. Isoleucine Parameters from Random Poly[(hydroxypropyl)glutamine-*co*-L-isoleucine][1]

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ABSTRACT: The synthesis and characterization of water-soluble random copolymers containing L-isoleucine and N^5-(3-hydroxypropyl)-L-glutamine, and the thermally induced helix–coil transitions of these copolymers in water, are described. The incorporation of L-isoleucine was found to increase the helix content of the polymers in water at all temperatures in the range of 0–70 °C. The Zimm–Bragg parameters σ and s for the helix–coil transition in poly(L-isoleucine) in water were deduced from an analysis of the copolymer melting curves in the manner described in earlier papers. The computed values of s indicate that L-isoleucine stabilizes helical sequences at all temperatures in the range of 0–60 °C. The two nonpolar residues which are branched at their C^β atoms (L-isoleucine and L-valine) differ markedly in their helix–coil stability constants.

The "host–guest" technique is being used for the determination of the helix–coil stability constants for the naturally occurring amino acids in water. This paper is a continuation of the series of earlier papers[3–20] and extends the technique to L-isoleucine. In the "host–guest" technique, a water-soluble, α-helical host homopolymer with nonionizable side chains is selected, and a guest amino acid is incorporated at various compositions to form random copolymers. The Zimm–Bragg[21] helix–coil parameters σ and s for the guest residue are determined from the thermally induced helix–coil transitions of these copolymers by examining the effect of the guest residue on the helix–coil transition properties of the *host* homopolymer in water. These copolymers are prepared by incorporating L-isoleucine with the host N^5-(3-hydroxypropyl)-L-glutamine.

There has been no reported experimental determination of the helix–coil stability constants for L-isoleucine in aqueous solution, although the properties of homo- and copolymers involving this residue have been examined by a variety of techniques.[22–29] The present study indicates that L-isoleucine in water promotes helix–coil boundaries and enhances helix growth at all temperatures in the range of 0–60 °C.

The synthesis of water-soluble random copolymers of L-isoleucine with N^5-(3-hydroxypropyl)-L-glutamine is described in section I. The experimental characterization of these copolymers and their melting behavior in water are presented in section II. The data are analyzed in section III by means of an appropriate form of the theory[3] to determine the helix–coil stability parameters of L-isoleucine in water. The theory is based on evidence[30,31] that short-range interactions dominate in determining the local conformation of a polypeptide or protein. The parameters for L-isoleucine are compared with empirical observations on the behavior of this residue in proteins and with a theoretical analysis of these quantities.

I. Experimental Section

Preparation and Characterization of the Copolymers. With sodium methoxide as an initiator, the copolymers were synthesized by first copolymerizing L-isoleucine *N*-carboxyanhydride (NCA) and γ-benzyl L-glutamate NCA in dioxane. The γ-benzyl blocking groups were subsequently exchanged by reaction

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Table I
Synthesis of Copolymers 1 and 2, Poly[Glu(OBzl),Ile]

polymer	A/I[a]	species analyzed	composition[b] (mol % Ile) at the reaction time (min) indicated													$\overline{DP}$[c]
			0	19	20	21	39	40	41	59	60	61	69	70	79	80	
1	60	monomer pool	60	64	A[d]	56	65	A[d]	58	68	A[d]	59	63	Q[e]			1250
		growing polymer chain	-	12		10.8	10.9		10.2	11.3		10.0	9.6				
2	60	monomer pool	40	35	A[d]	-	35	A[d]	17	35	A[d]	34			39	Q[e]	1800
		growing polymer chain	-	-		-	9.6		7.2	4.9		4.9			5.3		

[a] Ratio of anhydride to initiator. [b] Reaction was monitored by quenching 0.5 mL of reaction mixture with 5 mL of 0.1 N HCl/ethanol. [c] By viscometry in DMF, using the relation of Fujita et al.[35] [d] Addition of aliquot of Glu(OBzl)-NCA. [e] Copolymerization reaction quenched.

with hydroxypropylamine to yield poly[N^5-(3-hydroxypropyl)-L-glutamine-*co*-L-isoleucine].

A. Materials. L-Isoleucine was purchased from Sigma Chemical Co. and was used without further purification. 3-Aminopropanol (Aldrich) was dried over Davison molecular sieves (4 Å), distilled under reduced pressure in a nitrogen atmosphere, and stored over molecular sieves. Constant-boiling 6 N hydrochloric acid (Mallinckrodt) was obtained by distillation. All other reagents and solvents were identical in quality and preparation to those used in paper IX of this series.[11]

Melting curves of poly[N^5-(3-hydroxypropyl)-L-glutamine], poly(HPG), of weight-average degree of polymerization $\overline{DP}_w$ = 300, 700, and 1000 were calculated from the values of σ and s given in paper II of this series.[4]

B. Synthesis. *N*-Carboxyanhydrides. L-Isoleucine *N*-carboxyanhydride was prepared by the action of phosgene on a suspension of the amino acid in dioxane for several hours at 55–65 °C as described by Hirschmann et al.[32] Recrystallization from ethyl acetate–hexanes gave a product with a melting point of 69.5–70.5 °C. Anal. Calcd for $C_7H_{11}NO_3$: C, 53.49; H, 7.05; N, 8.91. Found: C, 53.33; H, 7.19; N, 8.82.

γ-Benzyl L-glutamate *N*-carboxyanhydride was prepared by treatment of γ-benzyl L-glutamate (synthesized according to the procedure of Prestige et al.[33]) with phosgene, using dioxane as a solvent as described by Blout and Karlson.[34]

Poly(γ-benzyl L-glutamate-*co*-L-isoleucine), Poly[Glu(OBzl),Ile], Copolymers 1 and 2. Random copolymers of γ-benzyl L-glutamate with up to 10% L-isoleucine were synthesized by polymerization of the NCA's in dioxane with sodium methoxide as initiator.[11] Since the NCA of γ-benzyl L-glutamate is more reactive than that of L-isoleucine under the polymerization conditions, the mole fractions of the two unreacted NCA's would be expected to vary during polymerization, resulting in higher incorporation of L-isoleucine toward the end of the polymerization. In order to prevent formation of the nonrandom polymers that would result from the different rates of incorporation, the following procedure was employed. The relative rates of reaction of the two NCA's were determined in a preliminary experiment. These relative reaction rates were then used to calculate the amount of γ-benzyl L-glutamate NCA that should be added during the course of polymerization to maintain the (initial) mole ratio of the two NCA's constant. Addition of aliquots of the more reactive NCA throughout the polymerization prevented the mole fraction of L-isoleucine NCA from varying more than 13% above its initial value.

The progress of the polymerization reaction was monitored by assaying for unreacted NCA's as described in paper X of this series.[12]. The randomness of the polymerization was assessed by following the composition of the unreacted monomer pool and the growing polymer chains by periodically quenching an aliquot of the reaction mixture in 0.1 N HCl/ethanol. The unreacted monomers were separated from the precipitated polymer by centrifugation, and their compositions were determined on the amino acid analyzer after hydrolysis. Table I summarizes the progress of the synthesis. To quench the polymerization and isolate the final copolymer, the reaction mixture was poured into absolute ethanol.[11] The yields of polymers 1 and 2, based on the number of moles of isoleucine, were 9.2 and 7.6%, respectively.

Table II
Characterization of Fractionated Copolymers

polymer	fraction	composition[a] (mol % Ile)	$\overline{M}_w$[b] × 10^{-3}	$\overline{M}_z/\overline{M}_w$[c]	$\overline{DP}_w$[b]
I	1	10.2			
	2	10.1	144.1	1.35	806
	3	10.3			
	4	10.2			
	5	9.7	124.2	1.15	698
	6	10.3			
	7	10.7	51.6	1.16	289
	8A[d]	10.3	27.2	1.18	152
	8B,C[d,e]	10.3	13.4	1.05	75
	8D[d]	10.3			
II	1	4.10			
	2	4.13			
	3	3.75			
	4	3.60	170.5	1.05	929
	5	3.86			
	6	4.15			
	7	4.23	98.7	1.05	539
	8	3.80			
	9	4.40			
	10	4.75	51.2	1.11	280
	11	4.71			
	12	4.56			

[a] Determined by hydrolysis in 6 N HCl at 110 °C for 96 h. [b] By conventional sedimentation equilibrium, with an extrapolation to zero concentration. [c] $\overline{M}_z/\overline{M}_w$ is reported for the run at the lowest concentration. [d] Fractions 8A, 8B,C, and 8D were derived from a refractionation of fraction 8. [e] Fraction 8B,C is the combination of fractions 8B and 8C.

With dimethylformamide (DMF) as a solvent, the polymer chain lengths were determined roughly with the viscosity–molecular weight relationship of Fujita et al.[35] and are given in Table I.

Poly[N^5-(3-hydroxypropyl)-L-glutamine-*co*-L-isoleucine], Poly(HPG,Ile), Copolymers I and II. Copolymers 1 and 2 were converted to the corresponding water-soluble copolymers poly(HPG,Ile), copolymers I and II, by treating them in dioxane with 3-amino-1-propanol at 50 °C under nitrogen as described in paper IV[6] of this series. The aminolysis of the benzyl ester groups was catalyzed[36] by adding 1-hydroxybenzotriazole (1.2 mol/mol of amino acid residues in the polymer) to the preswollen copolymer in dioxane. This method[18] reduced the time of exposure of the polymer to base and limited the extent of chain cleavage. The course of the aminolysis was monitored by assaying for unexchanged γ-benzyl ester groups, as described in paper X[12] of this series. The reaction was terminated when more than 99.5% of the benzyl ester groups had been exchanged. The reaction mixture was then poured into an excess of 1 N acetic acid and dialyzed against water at 20 °C until no amines could be detected by a ninhydrin test on 0.1 mL of the dialysate.[37] After lyophilization, a yield of about 70%, based on the number of moles of poly[Glu(OBzl),Ile], was obtained. The water-soluble copolymers I and II were fractionated with methanol and ether by the procedure

described in paper II[4] of this series. After fractionation, the polymers were dissolved in water, lyophilized, and dried in vacuo (and stored) over P_2O_5. Table II lists the fractions obtained. Only those fractions for which the molecular weights are listed were used in the analysis of their thermally induced helix–coil transitions.

C. Analytical Methods. 1. Determination of Composition. The amino acid compositions of all copolymer fractions were determined on a Technicon TMS amino acid analyzer. Each copolymer fraction was hydrolyzed according to the procedure of Moore and Stein,[38] in 6 N HCl at 110 °C for 96 h in degassed sealed ampules. The choice of these (optimal) conditions for hydrolysis was based on a preliminary study of the concentration of isoleucine as a function of hydrolysis time for copolymer fraction I-3; this fraction was heated for 1–7 days to test for completeness of hydrolysis. In each case, the areas of the alloisoleucine peak (~3% after 4 days) and of the normal isoleucine peak were added to yield the total content of isoleucine. Analysis of amino acid standards under the same conditions showed that no correction for the destruction of these amino acids was required. In order to avoid formation of γ esters of glutamic acid with 3-amino-1-propanol,[15,39,40] the hydrolysates were titrated to pH 1–2 with 6 N NaOH at 0 °C before analysis. The average experimental error in the determination of the amino acid composition is estimated to be ±5%.

2. Determination of Concentration. The concentrations of all copolymer solutions were determined by micro-Kjeldahl nitrogen analysis, according to Lang's method[41] for digestion and the semiautomated method of detection of ammonia of Noel and Hambleton.[42] Six aliquots of each solution were analyzed. The error in this measurement was found to be ±3%.

3. Optical Purity. The stereoisomeric purity of the amino acids in the starting materials and in the copolymers, poly(HPG,Ile), was checked by regular amino acid analysis of monomers and by the dipeptide method of Manning and Moore.[43] Regular amino acid analysis separated monomers of D,L-alloisoleucine from D,L-isoleucine. The D and L diastereomers of glutamic acid and isoleucine were separated by synthesizing the L-leucyl and L-phenylalanyl dipeptides, respectively. The monomeric starting amino acids were found to contain less than 0.5% of any contaminating diastereomers.

Initially each hydrolyzed fraction was analyzed for monomeric D,L-alloisoleucine on a Technicon TMS amino acid analyzer. Less than 1% D,L-alloisoleucine was detected in each case, after correcting for 3% racemization during an identical hydrolysis of standard monomeric L-isoleucine. Racemization during hydrolysis would most likely occur at the C^α atom rather than at the less reactive C^β atom of the *sec*-butyl group of isoleucine. Racemization at C^α would yield D-alloisoleucine as the primary contaminating diastereomer. It was, however, also necessary to check for the presence of D-isoleucine; this was accomplished with the dipeptide procedure. Isoleucine and glutamic acid were isolated separately from acid hydrolysates of the copolymer fractions prior to derivatization. Ion-exchange column chromatography, using a modification of the method of Hirs et al.,[44] was used to achieve a separation of the amino acids. First, a hydrolysate of 6–12 mg of polymer was loaded on a 0.6 × 9 cm column (Pasteur pipet) of Dowex-50W (200–400 mesh) in the hydrogen form. Elution with 1 M pyridine separated isoleucine and glutamic acid from 3-amino-1-propanol, which was retained on the column. After evaporation of the pyridine, the isoleucine and glutamic acid were loaded on a 0.6 × 9 cm column (Pasteur pipet) of Dowex-1 (200–400 mesh) in the acetate form. Elution with water separated isoleucine from glutamic acid, which was retained until elution with 0.1 N acetic acid. At this point, the separated amino acids were individually assayed on the amino acid analyzer. No contamination of either amino acid was detected. The glutamic acid from this separation was coupled with L-Leu-NCA and the resulting diastereomeric dipeptides, L-Leu-L-Glu and L-Leu-D-Glu, were separated and determined quantitatively on the amino acid analyzer, using a 0.1 M citrate buffer, 20% (v/v) 1-propanol (0.2 N Na^+) at pH 2.9, 72 °C, on a 0.4 × 30 cm column of Chromobeads C.

The isoleucine from this separation was coupled with L-Phe-NCA, and the resulting diastereomeric dipeptides L-Phe-L-Ile and L-Phe-D-Ile were separated on a Spectra Physics SP 8000 high-performance liquid chromatography (LC) system using a Waters Associates reversed-phase μBondapak C_{18} column, 0.4 × 30 cm, and an isocratic elution with 90% 10 mM ammonium acetate buffer and 10% acetonitrile.[45]

By this technique, the glutamic acid and isoleucine residues in the polymers were found to contain less than 3% of the D isomers, after correcting for ≤5.5% racemization that may have occurred during hydrolysis or during the chromatographic separation (by carrying a mixture of L-isoleucine, L-glutamic acid, and 3-amino-1-propanol through the same procedure). The presence of less than 3% racemization of the isoleucine and glutamic acid residues in the copolymers was not considered to have any significant effect on the computed values of σ and *s*.

D. Viscosity, ORD, and CD Measurements. Viscosity, optical rotatory dispersion (ORD), and circular dichroism (CD) measurements were all carried out as described previously.[4]

E. Molecular Weight Determination. The molecular weights of fractions from polymers I and II were determined on aqueous solutions by the conventional sedimentation equilibrium method as reported earlier[4] and analyzed by the procedure described by Chervenka.[46] The initial concentration was determined by a calibration of fringe shift vs. polymer concentration as in paper VII of this series.[9] The concentration dependence of the weight-average molecular weight, $\bar{M}_w$, was determined for each sample, and $\bar{M}_z$ was computed from the run at the lowest concentration for each fraction. The estimated precision in the values of $\overline{DP}_w$ was ±5%.

The partial specific volumes ($\bar{v}$) of the copolymer fractions, required for the calculation of the molecular weights, were calculated from their amino acid compositions as described by Cohn and Edsall.[47] A value of $\bar{v}$ = 0.79 for PHPG was used in the calculation of $\bar{v}$ for the copolymers.[4]

II. Results

A. Synthesis and Characterization of the Copolymers. Table I summarizes the characteristics of the synthesis of the copolymers and the average degree of polymerization ($\overline{DP}$) of the unfractionated poly[Glu(OBzl),Ile] copolymers. Because of the relatively slow reaction rate of Ile-NCA, it was necessary to modify the synthetic procedure for this copolymer compared to that for the other copolymers in the series. Thus, Glu(OBzl)-NCA was added periodically in order to prevent initial nonrandom incorporation of blocks of Glu(OBzl) followed by incorporation of blocks of Ile. The adopted method of synthesis ensures that the copolymer will be a random one because the composition of the feed mixture remains within a narrow range of concentration throughout the polymerization. Only copolymers with feed compositions which were less than 13 mol % higher than the initial Ile composition were used in this study.

Table II summarizes the molecular weight and composition data for the corresponding exchanged and fractionated copolymers poly(HPG,Ile). Only those fractions for which sedimentation equilibrium molecular weights are listed were used to determine the Zimm–Bragg parameters σ and *s*. The usual decrease in $\overline{DP}_w$ attributed to transamidation upon conversion to the (hydroxypropyl)glutamine copolymers[48] is apparent by a comparison of Tables I and II.

The isoleucine compositions of the fractions of copolymers I and II are independent of chain length for a given copolymer parent. This indicates that there is little departure from randomness in these copolymers. While this criterion for near-randomness has been used in previous papers in this series, it was verified (in the case of copolymers containing methionine as the guest residue) by determining the distribution of methionine in the fragments obtained from a degradation of the copolymer with CNBr.[49] In this study, random copolymers were obtained by using the periodic addition method of syn-

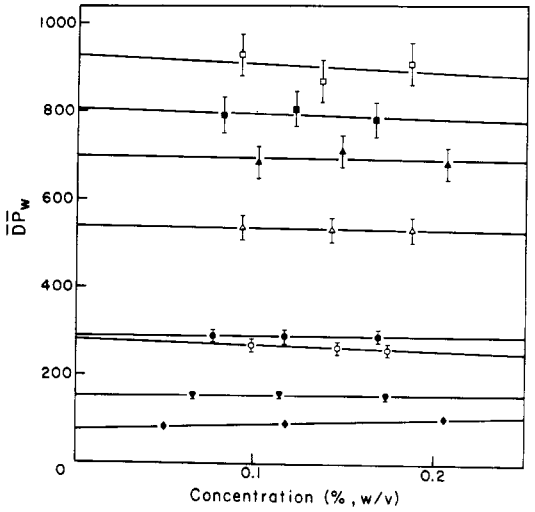

Figure 1. Concentration dependence of molecular weights for fractions used in analysis to obtain σ and s: (□) 3.6% Ile, $\overline{DP}_w$ = 929 (fraction II-4); (■) 10.1% Ile, $\overline{DP}_w$ = 806 (fraction I-2); (▲) 9.7% Ile, $\overline{DP}_w$ = 698 (fraction I-5); (△) 4.2% Ile, $\overline{DP}_w$ = 539 (fraction II-7); (●) 10.7% Ile, $\overline{DP}_w$ = 289 (fraction I-7); (○) 4.8% Ile, $\overline{DP}_w$ = 280 (fraction II-10); (▼) 10.3% Ile, $\overline{DP}_w$ = 152 (fraction I-8A); (♦) 10.3% Ile, $\overline{DP}_w$ = 75 (fraction I-8B,C). The error symbols represent the experimental error in each measurement.

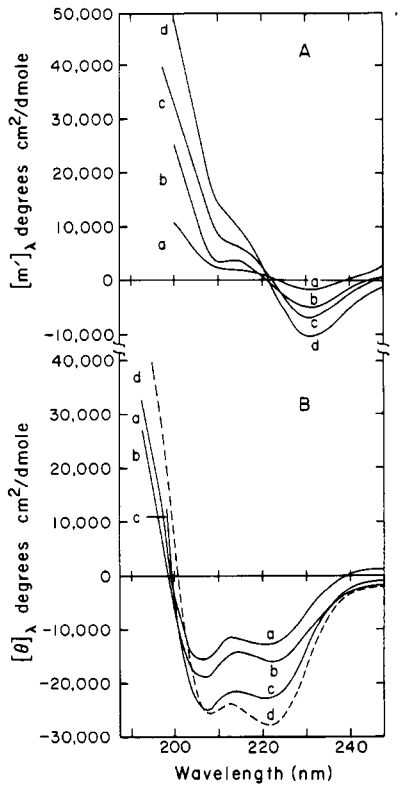

Figure 2. (A) ORD and (B) CD data in water for representative fractions of isoleucine copolymers: (a) 10.3% Ile, $\overline{DP}_w$ = 152 at 25 °C (fraction I-8A); (b) 3.6% Ile, $\overline{DP}_w$ = 929 at 25 °C (fraction II-4); (c) 10.2% Ile, $\overline{DP}_w$ = 800 at 25 °C (fraction I-4); (d) 3.6% Ile, $\overline{DP}_w$ = 929 at 1 °C (fraction II-4).

thesis in order to compensate for the disparate rates of reaction of the host and guest monomers. In addition, it has been shown theoretically that small deviations from randomness (blocks of guest residues ~10–15% of $\sigma^{-1/2}$) do not influence the melting behavior of these copolymers.[3]

Figure 1 shows the concentration dependence of the apparent molecular weights for the eight fractions used in computing σ and s. The data have been extrapolated to infinite dilution to obtain $\overline{DP}_w$. The values of $\bar{M}_z/\bar{M}_w$ for these fractions do not depart significantly from unity, indicating that they are relatively homogeneous.

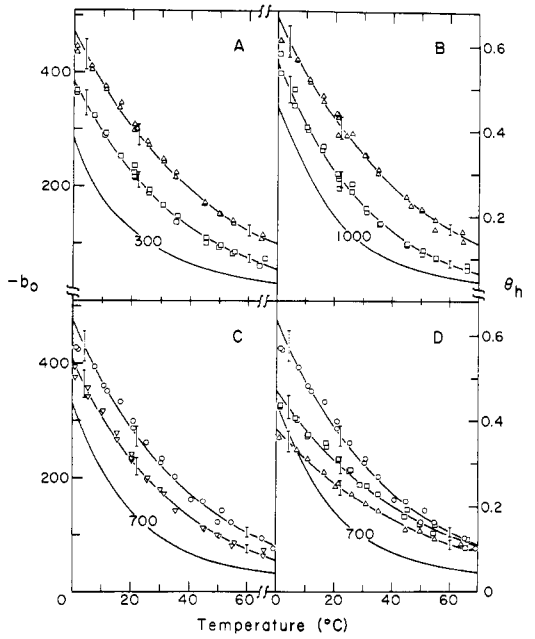

Figure 3. Temperature dependence of $-b_0$ for poly(HPG,Ile) copolymers in water. The melting curves of poly(HPG) (lines without experimental points, obtained by interpolation of data from ref 4) of $\overline{DP}_w$ = 300, $\overline{DP}_w$ = 700, and $\overline{DP}_w$ = 1000 are included for comparison: (A) (△) 10.7% Ile, $\overline{DP}_w$ = 289 (fraction I-7), (□) 4.8% Ile, $\overline{DP}_w$ = 280 (fraction II-10); (B) (△) 10.1% Ile, $\overline{DP}_w$ = 806 (fraction I-2), (□) 3.6% Ile, $\overline{DP}_w$ = 929 (fraction II-4); (C) (○) 9.7% Ile, $\overline{DP}_w$ = 698 (fraction I-5), (▽) 4.2% Ile, $\overline{DP}_w$ = 539 (fraction II-7); (D) (○) 9.7% Ile, $\overline{DP}_w$ = 698 (fraction I-5), (□) 10.3% Ile, $\overline{DP}_w$ = 152 (fraction I-8A), (△) 10.3% Ile, $\overline{DP}_w$ = 75 (fraction I-8BC). The points are the experimental ones and the lines repesent the smoothed data used in subsequent calculations. The error symbols reflect the errors in b_0 arising from errors in the determination of solution concentration, in the slope of the Moffitt–Yang plot, and in the choice of the values of b_0 for the fully helical and fully coil conformations.

A calibration curve of polymer concentration, C_0 in fringe units, from synthetic boundary runs in the ultracentrifuge, vs. polymer concentration, assayed by nitrogen analysis, was determined. This was done to ensure internal consistency in the various physical measurements which might be subject to error as a result of micro-Kjeldahl analyses. The curve, constrained to pass through the origin, had a least-squares slope of 38.90 fringe/% (w/v).

B. ORD and CD Data for the Copolymers. The ORD and CD spectra for representative fractions of poly(HPG,Ile) in water are shown in Figure 2. Both sets of curves can be described in terms of polymers composed of varying amounts of right-handed α helix and random coil; however, the presence of a small amount of β structure cannot be excluded.[50-52] The relative contribution of each conformation is a function of both temperature and composition.

The incorporation of L-isoleucine in a copolymer with HPG increases the helix content (compare curve D of Figure 3 of paper II[4] with curves a and b of Figure 2B). From Figure 2 it is evident that the helix content for poly(HPG,Ile) in water increases as the temperature decreases, the $\overline{DP}_w$ increases, and the composition of L-isoleucine increases, by comparing curves b and d, a and c, and b and c, respectively. Poly(HPG,Ile) undergoes a

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

composition (mol % Ile)	$\overline{DP}_w$	temp, °C	$(\theta_h)_{exptl}$	$(\theta_h)_{theor}$ Lifson[b]	Allegra[c]	Lehman-McTague[c]
10.1	806	0	0.667	0.642	0.645	0.645
		30	0.336	0.328	0.328	0.328
		60	0.169	0.160	0.159	0.159
9.7	698	0	0.637	0.633	0.636	0.636
		30	0.299	0.320	0.320	0.320
		60	0.140	0.155	0.155	0.155
10.7	289	0	0.630	0.601	0.604	0.604
		30	0.323	0.311	0.311	0.311
		60	0.166	0.155	0.155	0.155
10.3	152	0	0.470	0.530	0.530	0.530
		30	0.249	0.270	0.271	0.271
		60	0.132	0.138	0.138	0.139
10.3	75	0	0.379	0.387	0.386	0.381
		30	0.209	0.198	0.199	0.197
		60	0.115	0.109	0.110	0.109
3.60	929	0	0.560	0.553	0.551	0.551
		30	0.223	0.217	0.215	0.215
		60	0.088	0.097	0.097	0.097
4.23	539	0	0.545	0.545	0.543	0.542
		30	0.235	0.223	0.221	0.221
		60	0.101	0.101	0.101	0.101
4.75	280	0	0.517	0.510	0.507	0.505
		30	0.226	0.216	0.215	0.214
		60	0.099	0.101	0.101	0.101

[a] The parameters used for PHPG were those of Table II in paper II.[4] [b] The parameters used for L-isoleucine were obtained by fitting the data using the Lifson theory with $\sigma = 5.1 \times 10^{-3}$. [c] The parameters used for L-isoleucine were obtained by fitting the data using the Allegra theory with $\sigma = 5.5 \times 10^{-3}$.

thermally induced helix-coil transition in water.

The ORD spectra in the range of 280–450 nm were used to calculate the values of the Moffitt-Yang parameter b_0 at various temperatures as described in paper VII.[9] The thermally induced helix-coil transition curves for eight representative fractions and for PHPG[4] are shown in Figure 3. The curves exhibited no concentration dependence and were found to be reversible and reproducible. A representative check for thermal reversibility is indicated in Figure 4. The size of the error symbols in Figure 3 indicates two standard deviations in b_0 (or in helix content θ_h) and reflects the errors in the determination of concentration ($\pm 0.03 b_0$), the slope of the Moffitt-Yang plot (± 3, in b_0 units), and the choice of the values of b_0 for the fully helical and fully coil conformations ($\pm 0.027 b_0$).[6]

Figure 3 shows that incorporation of isoleucine increases the helix content of all copolymers at all temperatures studied relative to that of a PHPG homopolymer of comparable chain length.

C. b_0 for Complete Helix and Complete Coil. In all of the previous papers of this series, the magnitude of b_0 for the complete helix (assumed to be temperature independent) has been taken as −750 and for the complete coil as zero, following the assignments of paper II.[4] Because these values are a function of the identity of the side chain,[53] several fractions were checked in trifluoroethanol (TFE) and dichloroacetic acid (DCA), with b_0 corrected for the dispersion of the refractive index of the solvent.[54] Typical results were obtained with fractions I-2 in TFE at 1 °C and II-10 in DCA at 25 °C which gave b_0 values of −740 and +10, respectively. In light of this, b_0 extrema of −750 and 0 were used in this study.

III. Discussion

Helix-Coil Parameters for Poly(L-isoleucine). The procedure used to analyze the melting curves of the copolymers has been described in earlier papers of this series.[3-5] Initially the first approximation in the LAPS (Lifson-Allegra-Poland-Scheraga) hierarchy of approximations, corresponding to the theory of Lifson,[55] was used.

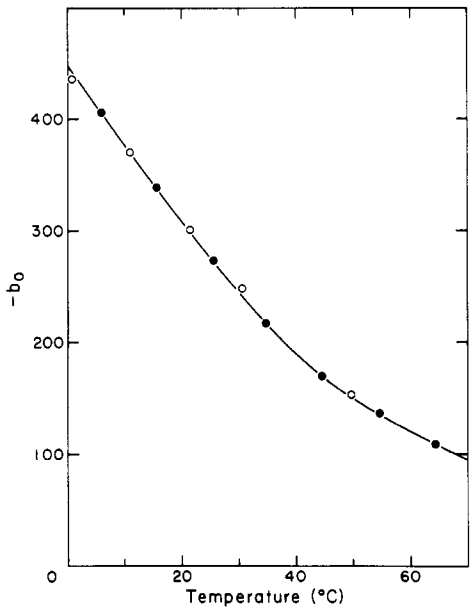

Figure 4. Plot of $-b_0$ vs. temperature to demonstrate the reversibility of the helix-coil transition. The filled symbols were determined while heating the solution and the open symbols were determined while cooling the solution, for a 10.7% Ile copolymer, $\overline{DP}_w = 289$ (fraction I-7).

This procedure generated values of σ and s and economized computer time, after which improved values were obtained with the second level of approximation, corresponding to the theory of Allegra.[56] This approximation was compared with results from the exact theory of Lehman and McTague[57] at three temperatures for each fraction. (All computer programs used in these calculations can be obtained as directed in footnotes 26 and 27 of paper I.[3]) Table III shows the results of these calculations. Both the first-order and second-order approximations yield results in good agreement with those calculated by the Lehman-McTague method. The following discussion is based on

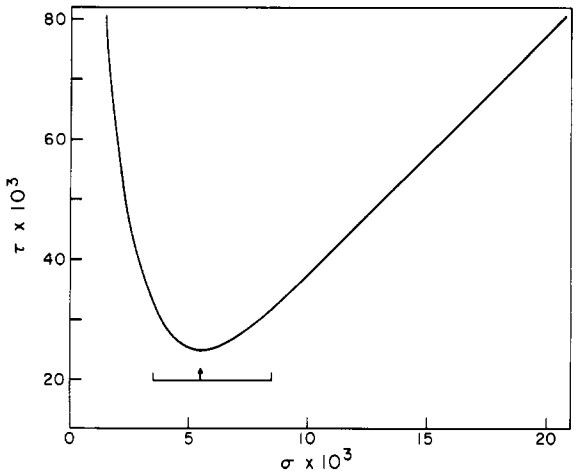

Figure 5. Determination of the best temperature-independent value of σ as the one which corresponds to the lowest value of τ for the isoleucine copolymers, using the Allegra approximation. The arrow represents this best value (5.5×10^{-3}), while the bracket represents the limits of error in σ (discussed in section III). The Lifson theory yielded a slightly lower value of σ, 5.1×10^{-3}.

Table IV
Values of the Zimm-Bragg Parameter s for Poly(L-isoleucine) in Water from 0 to 70 °C

temp, °C	s	
	Lifson[a]	Allegra[b]
0	1.20_8	1.25_8
5	1.17_2	1.21_0
10	1.14_7	1.17_9
15	1.13_0	1.15_6
20	1.11_5	1.14_0
25	1.10_3	1.12_4
30	1.09_2	1.11_1
35	1.08_2	1.09_7
40	1.07_0	1.08_3
45	1.05_7	1.06_8
50	1.04_4	1.05_1
55	1.02_8	1.03_5
60	1.01_2	1.01_5
65	0.99_5	0.99_5
70	0.97_5	0.97_4

[a] Computed with the Lifson theory, using $\sigma = 5.1 \times 10^{-3}$. [b] Computed with the Allegra theory, using $\sigma = 5.5 \times 10^{-3}$.

the data from the Allegra approximation.

The value of σ which best described the copolymer melting curves was obtained by calculating the "goodness of fit" parameter, τ, defined in paper II.[4] This parameter was minimized assuming σ to be temperature independent. A plot of τ vs. σ for the approximation corresponding to the Allegra theory is shown in Figure 5. The best value of σ was taken as 5.5×10^{-3}. (The value of σ obtained for the Lifson theory was 5.1×10^{-3}.) Using the values of s corresponding to the best value of σ, the uncertainty in the best value of σ was determined by calculating the value of this parameter for each fraction at each temperature individually. The resulting distribution of values of σ displays a skewness about the best value of σ ($\sigma = 5.5 \times 10^{-3}$). This skewness was determined quantitatively by computing an arithmetic average of all values of σ greater than the best value and an arithmetic average of all values of σ less than the best value. These two arithmetic averages are shown as error limits in Figure 5. The temperature dependence of σ was computed by finding the "best" value of σ at each temperature individually for *all* fractions. The resulting variation of σ with temperature was found to be well within the error limits of a temperature-*in*dependent σ. Therefore, in the determination of $s(T)$ for L-isoleucine, σ was taken to be independent of temperature. Table IV lists the values of s at each temperature derived from both the Lifson and Allegra theories, using the appropriate "best" value of σ. The agreement between the two approximate theories is very good, particularly at higher temperatures.

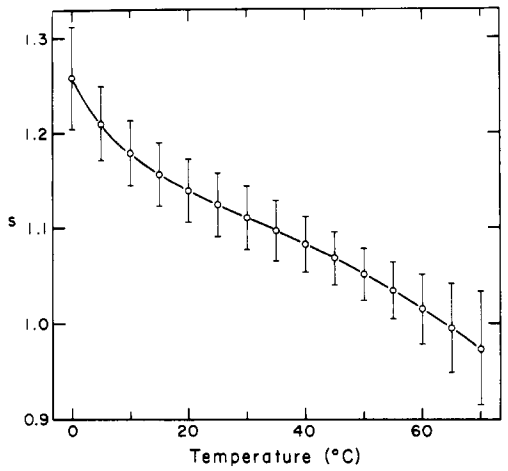

Figure 6. Plot of s vs. T for poly(L-isoleucine) in water. The error symbols are described in section III. The solid line is drawn to pass through all the points.

Table V
Thermodynamic Parameters for L-Isoleucine

ΔG°_{20}, cal/mol	-76.1 ± 17.3
ΔH°, cal/mol	-571 ± 21
ΔS°, eu	-1.68 ± 0.07
σ	5.5×10^{-3}

The temperature dependence of s is shown for the Allegra theory in Figure 6. The error symbols on the computed values of s are standard deviations in s at a given temperature as calculated from the values of s (with σ fixed at 5.5×10^{-3}) found when each fraction was fit individually at a given temperature.

The melting curves, computed with the best-fit Allegra values for σ and s, are shown in Figure 7 along with the experimental data. The error symbols on the theoretical curves represent errors in the determination of molecular weight (±5%) and amino acid composition (±5%); no allowance has been made for possible errors in the Zimm–Bragg parameters for PHPG. The agreement between the calculated and experimental values of θ_h is reasonably good in all cases.

Figure 8 shows a plot of $-RT \ln s$ (ΔG°) vs. temperature with error symbols calculated from the standard deviation in s and the straight line calculated by the weighted least-squares method described in paper IV.[6,58] Assuming that ΔH° and ΔS° are independent of temperature, values of these parameters can be computed from the temperature dependence of ΔG°. Table V summarizes the thermodynamic parameters for the conversion of one residue of L-isoleucine from a coil to a helical conformation at the end of a long helical sequence. The tabulated uncertainty in ΔG°_{20} follows directly from the error in s at 20 °C. The uncertainties in ΔH° and ΔS° are derived from the procedure used to fit the calculated points of Figure 8 to a straight line.[58]

Comparison with Other Results. There have been no other determinations of the values for the helix–coil stability constants for L-isoleucine in water. Studies of thin films of poly(L-isoleucine) using ORD, UV, IR,[22] and X-ray diffraction[23] indicate that, in the solid state, the preferred

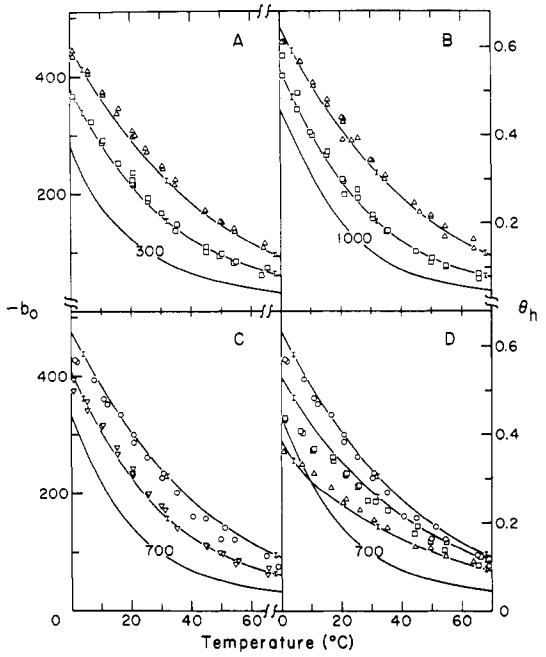

Figure 7. Comparison of the calculated melting curves obtained from the parameters of the Allegra theory (with $\sigma = 5.5 \times 10^{-3}$) for L-isoleucine given in Table V and those of poly(HPG) of Table II in ref 4, with the experimental points for the copolymer in water. The curves for poly(HPG) $\overline{DP}_w = 300$, $\overline{DP}_w = 700$, and $\overline{DP}_w$ 1000 (see ref 4) are included for comparison: (A) (△) 10.7% Ile, $\overline{DP}_w = 289$ (fraction I-7), (□) 4.8% Ile, $\overline{DP}_w = 280$ (fraction II-10); (B) (△) 10.1% Ile, $\overline{DP}_w = 806$ (fraction I-2), (□) 3.6% Ile, $\overline{DP}_w = 929$ (fraction II-4); (C) (○) 9.7% Ile, $\overline{DP}_w = 698$ (fraction I-5), (▽) 4.2% Ile, $\overline{DP}_w = 539$ (fraction II-7); (D) (○) 9.7% Ile, $\overline{DP}_w = 698$ (fraction I-5), (□) 10.3% Ile, $\overline{DP}_w = 152$ (fraction I-8A), (△) 10.3% Ile, $\overline{DP}_w = 75$ (fraction I-8B,C). The error symbols indicate errors in the calculated values of θ_h arising from the errors in composition and chain length. See Figure 3 for additional errors in experimental points.

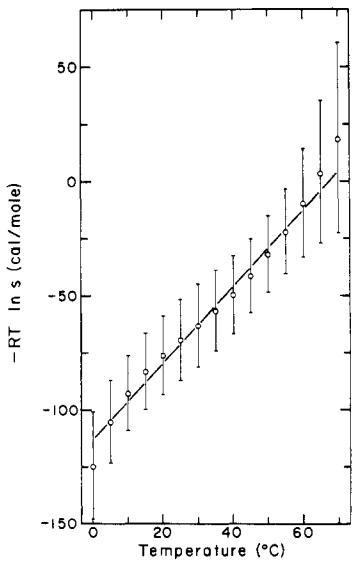

Figure 8. Plot of $-RT \ln s$ (i.e., $\Delta G°$) vs. temperature for poly(L-isoleucine) in water. The straight line is a weighted least-squares fit, constructed as described in section III. The error symbols were calculated as in Figure 6.

conformation is a β structure. Protected homooligopeptides of isoleucine from dimer to octamer, studied in TFE, using UV,[24,25] ORD,[24] CD,[25-27] and in the solid state, using X-ray diffraction,[27] were found to assume a β structure, beginning at the hexamer and heptamer. Studies of block copolymers of L-lysine and L-isoleucine in aqueous solution, using IR,[28] potentiometric titration,[29] and CD,[28,29] indicate that isoleucine has a very high potential for formation of a β sheet.

The tendency for an amino acid to promote helix formation may be determined from X-ray data of proteins of known three-dimensional structure. Isoleucine is a helix maker on the basis of these conformational analyses.[59-73] The frequency of occurrence of isoleucine in these sets of proteins is less than 5%; therefore, a more representative analysis was obtained, as in paper 18,[20] using the procedure of Isogai et al.[66] The normalized relative frequency[66] for the occurrence of helical isoleucine was found to be 1.10, based on 8 proteins,[67] 1.00, based on 15 proteins,[68] 1.12, based on 16 proteins,[69] 1.20, based on 20 proteins,[70] 1.08, based on 29 proteins,[71] 0.89, based on 16 proteins,[72] 1.61, based on 26 proteins,[73] and 1.02, based on 23 proteins (unpublished data in connection with ref 66). A value greater than 1 for the normalized relative frequency indicates that the relative frequency of occurrence of helical isoleucine is greater than the relative frequency of occurrence of all helical conformations in the protein sample. Several factors which may account for the variation in the values of the normalized relative frequencies include the protein sample size and kind and the limits of the dihedral angles (ϕ,ψ) used to assign a particular residue to the α-helical state.

The interpretation of these normalized relative frequencies in terms of the statistical weight s is not quite correct[31] because of the omission of the effect of cooperativity (it is assumed that $\sigma = 1$ for computations of the frequencies) and the effects of solvents on these parameters.[69,73]

A value of $s = 1.0_5$ for L-isoleucine (assumed to pertain to 20 °C) was obtained from a theoretical analysis by Finkelstein et al.[74] The experimental value found in this study at the same temperature is $s = 1.14_0$.

Implications. Copolymers of poly(HPG,Ile) exhibit greater helicity than the corresponding homopolymer PHPG of comparable chain length. L-Isoleucine promotes the formation of a very stable helix as indicated by the values of s obtained here. The value of σ for L-isoleucine is comparable in size to that found for uncharged L-glutamic acid, charged and uncharged L-aspartic acid, L-tyrosine, L-methionine, and L-tryptophan and indicates that L-isoleucine (compared to other amino acids) promotes the formation of boundaries between helix and coil states.

Table V lists the thermodynamic parameters for the conversion of an L-isoleucine residue in the coil state to a helix state at the end of a long helical sequence. For L-isoleucine, this conformational change is favored enthalpically, but is unfavorable entropically, below about 60 °C. The magnitudes of $\Delta H°$ and $\Delta S°$ are similar to those observed for uncharged L-glutamic acid, charged and uncharged aspartic acid, L-tyrosine, L-methionine, and L-tryptophan and they reflect changes in specific interaction energies and hydrophobic bonding in going from the coil to the helix conformation.

It appears as though the two β-branched amino acids L-valine and L-isoleucine are distinctly different in their abilities to promote helix formation. L-Valine, studied previously by the "host–guest" technique,[10] has been shown to have a relatively low value of $\sigma = 1 \times 10^{-4}$ and to decrease the helix content of the polymer at low temperatures and increase it at high temperatures. Since the previous valine-containing copolymers[10] might not have been sufficiently random, because of a low rate of polymerization

of Val-NCA, as Ile-NCA, a new set of valine-containing copolymers was prepared[75] by the synthetic method used in this paper. The resulting copolymers were random. The results of both studies[10,75] of L-valine agree qualitatively; thus, the difference between L-valine and L-isoleucine in their abilities to promote helix formation is a real one. The existence of distinctly different helix–coil parameters for these two β-branched amino acids suggests that there are differences in hydrophobic bonding as well as other interactions in their helix and/or coil conformations.[76]

IV. Conclusions

Water-soluble random copolymers of L-isoleucine and N^5-(3-hydroxypropyl)-L-glutamine have been prepared and characterized. From an analysis of the thermally induced helix–coil transitions of these copolymers, the Zimm–Bragg parameters σ and s were determined. On the basis of the value of s, L-isoleucine can be classified as a helix stabilizer, while the value of σ indicates that L-isoleucine is also an efficient helix initiator.

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