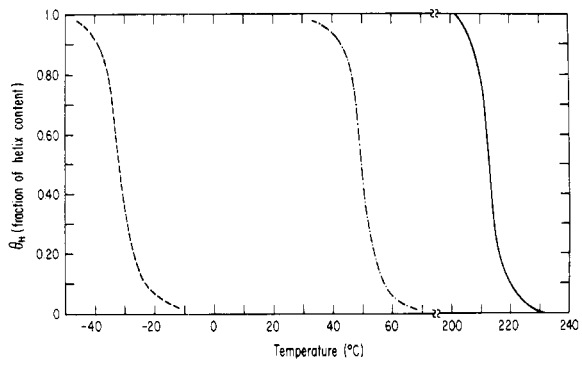

Figure 5. Calculated helix-to-coil melting in chloroform for: (- - -) poly(L-alanyl-L-lactic acid); (– • –) poly(L-alanyl-L-lactic acid); (—) poly(L-alanine).

on polypeptide helix stability, we compare in Figure 5 helix-to-coil melting curves calculated for poly(L-Ala-L-Lac), poly[(L-Ala)$_2$-L-Lac], and poly(L-Ala) in an inert solvent. Klotz and Franzen[16] have measured the enthalpy of formation of the peptide hydrogen bond in chloroform. We use their value of −4.2 kcal/mol for $\Delta H°_t$ in the calculations for Figure 5. For v we use the value of 0.012 determined by Ingwall et al.[17] for poly(L-Ala). Because of their similarity, we set $\sigma = v$ for the calculations of Figure 5. The melting temperature of poly(L-Ala), $T°_p$, was calculated from eq 12 and the melting temperature $T°_d$ = 323 K was observed here for poly[(L-Ala)$_2$-L-Lac] in chloroform. Although these parameters are approximate, they serve our purpose in illustrating hydrogen-bonding effects shown by the curves in Figure 5. Calculations based entirely on experimentally observed parameters will be reported in the future when more extensive measurements have been completed.

The curves of Figure 5 clearly reveal the profound effect of hydrogen bonding on helix stability. Melting temperatures of −32, 50, and 214 °C are indicated for poly(L-Ala-L-Lac), poly[(L-Ala)$_2$-L-Lac], and poly(L-Ala), respectively. The calculations of Figure 5 are consistent with the observed conformational properties of these polymers. The large differences in melting temperatures are dramatic indicators of the importance of hydrogen bonding in the determination of helix stability. The melting of poly(L-Ala-L-Lac) is predicted to occur at an experimentally accessible temperature. We are currently performing experiments with a specially designed low-temperature cell to test the calculations of Figure 5. Because of its extremely high melting temperature (>200 °C), it is now clear why the thermally induced helix-to-coil transition of poly(L-Ala) in an inert organic solvent has not been observed.

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References and Notes

(1) L. Mathias, W. D. Fuller, D. Nissen, and M. Goodman, *Macromolecules*, preceding paper in this issue.
(2) R. T. Ingwall and M. Goodman, *Macromolecules*, **7**, 598 (1974).
(3) R. T. Ingwall, C. Gilon, and M. Goodman, *Macromolecules*, **9**, 802 (1976).
(4) G. D. Fasman, "Poly-Amino Acids", Vol. 1, G. D. Fasman, Ed., Marcel Dekker, New York, N.Y., 1967.
(5) P. J. Flory, *Macromolecules*, **7**, 381 (1974).
(6) S. Lifson, *J. Chem. Phys.*, **40**, 3705 (1964).
(7) D. Nissen, G. Gilon, and M. Goodman, *Makromol. Chem., Suppl.*, **1**, 23 (1975).
(8) W. Radding, N. Ueyama, C. Gilon, and M. Goodman, *Biopolymers*, **15**, 591 (1976).
(9) (a) R. T. Ingwall, C. Gilon and M. Goodman, *J. Am. Chem. Soc.*, **97**, 9356 (1975); (b) unpublished results.
(10) V. Madison and J. Schellman, *Biopolymers*, **11**, 1041 (1972).
(11) A. Elliott, "Infrared Spectra and Structure of Organic Long-Chain Polymers", Edward Arnold, Ltd., London, 1963.
(12) R. Zbinden, "Infrared Spectroscopy of High Polymers", Academic Press, New York, N.Y., 1964, pp 201 and 202.
(13) B. H. Zimm and J. K. Bragg, *J. Chem. Phys.*, **31**, 526 (1959).
(14) S. Lifson and A. Roig, *J. Chem. Phys.*, **34**, 1963 (1961).
(15) D. Poland and H. A. Scheraga, "Theory of Helix-Coil Transition in Biopolymers", Academic Press, London, 1970.
(16) I. M. Klotz and J. S. Franzen, *J. Am. Chem. Soc.*, **84**, 3461 (1962).

Helix–Coil Stability Constants for the Naturally Occurring Amino Acids in Water. 17. Threonine Parameters from Random Poly(hydroxybutylglutamine-*co*-L-threonine)[1]

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ABSTRACT: The synthesis and characterization of water-soluble random copolymers containing L-threonine with N^5-(4-hydroxybutyl)-L-glutamine and the thermally induced helix–coil transitions of these copolymers in water are described. The incorporation of L-threonine was found to decrease the helix content of the copolymers in water at all temperatures. The Zimm–Bragg parameters σ and s for the helix–coil transition of poly(L-threonine) in water were deduced from an analysis of the melting curves of the copolymers in the manner described in earlier papers. The computed values of s indicate that L-threonine destabilizes helical sequences at all temperatures in the range of 0–70 °C.

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, the latest of which was paper 16.[3] In the present paper, this approach is extended to L-threonine. In the "host–guest" technique, a water-soluble α-helical host homopolymer with nonionizable side chains is selected, and various amounts of a guest residue are incorporated into it to form random

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copolymers. The use of random copolymers, in which L-threonine is the minor component, allows the determination of the helix–coil transition properties of L-threonine residues. Thus, it is possible to calculate the Zimm–Bragg[4] parameters σ and s for the guest residues by examining their influence on the helix–coil transition properties of the host homopolymers. L-Threonine residues are incorporated into a copolymer with an N^5-(4-hydroxybutyl)-L-glutamine host, and the thermally induced helix–coil transition in these copolymers in water is examined.

The synthesis of water-soluble random copolymers of L-threonine with N^5-(4-hydroxybutyl)-L-glutamine (HBG) is described in section I, and the experimental characterization of these copolymers and their melting behavior in aqueous solution are presented in section II. Finally, in section III, the data are analyzed by means of an appropriate form of the theory[5] to determine the helix–coil stability parameters of L-threonine in water. The theory is based on evidence[6] that short-range interactions dominate in determining the local conformation of a polypeptide or protein. The parameters for L-threonine are compared with empirical observations on the behavior of this residue in proteins.

I. Experimental Section

Preparation and Characterization of the Copolymers. The synthesis of the copolymers was achieved by first copolymerizing the *N*-carboxyanhydrides (NCA's) of *O*-trimethylsilyl-L-threonine and γ-benzyl L-glutamate in dioxane, using sodium methoxide as an initiator. The resulting copolymers were then converted to the 4-hydroxybutylglutamine derivatives by treatment with 4-amino-1-butanol, and, afterwards, the *O*-trimethylsilyl groups were removed by treatment with mild aqueous acid.

A. Materials. L-threonine (allo-free) was purchased from Sigma Chemical Co., D,L-*allo*-threonine was purchased from Nutritional Biochemicals Co., and D,L-threonine (allo free) from Scwartz-Mann Co. All were used without further purification. Trimethylchlorosilane was purchased from Aldrich and used without purification. All other reagents and solvents were identical in quality and preparation to those used in paper 9 of this series.[7]

B. Synthesis. *N*-Carboxyanhydrides. L-threonine was reacted with silver oxide in aqueous solution to yield silver threoninate.[8] The silver threoninate was suspended in dioxane (freshly distilled from sodium) and the calculated amount of phosgene was introduced to produce white crystals of L-threonine NCA[8] which decomposed at 90–95 °C (lit.[8] 94–98 °C). Anal. Calcd for $C_5H_7NO_4$: C, 41.38; H, 4.86; N, 9.65; Found: C, 41.38; H, 5.53; N, 9.61. This product was dissolved in tetrahydrofuran and treated with trimethylchlorosilane. Anhydrous pyridine was added, and the precipitated pyridine hydrochloride was removed by filtration. The filtrate was concentrated to an oil in vacuo and taken up in hexanes–ethyl acetate (3:1 v/v). After several hours at 3 °C, the clear supernatant was decanted and concentrated in vacuo to yield a crude semicrystalline substance which was triturated twice with hexanes and dried to yield white crystals which decomposed at 68–72 °C (lit.[8] 76 °C). (This procedure was adapted from that of Hirschmann et al.[8]) Anal. Calcd for $C_8H_{15}NO_4Si$: C, 44.22; H, 6.96; N, 6.44; Si, 12.92. Found: C, 44.16; H, 6.84; N, 6.51; Si, 12.91.

γ-Benzyl L-glutamate *N*-carboxyanhydride was prepared by treatment of γ-benzyl L-glutamate (synthesized according to the procedure of Prestidge et al.[9]) with phosgene, as described by Hirschmann et al.[8]

Poly[γ-benzyl L-glutamate-*co*-*O*-trimethylsilyl-L-threonine], Poly[Glu(OBzl),Thr(OTMS)], Copolymers 1–4. Random copolymers of γ-benzyl L-glutamate and up to 13% of *O*-trimethylsilyl-L-threonine were prepared by polymerizing mixtures of the two *N*-carboxyanhydrides in dioxane with sodium methoxide as an initiator. The progress of the polymerization reactions was monitored by assaying for unreacted *N*-carboxyanhydrides as described in paper 10 of the series,[10] and the polymers were isolated by pouring the reaction mixture into absolute ethanol as usual.[7] The chain lengths of these polymers were determined roughly with the viscosity–molecular weight relationship of Fujita et al.[11] and are given in Table II.

Table I
Time Dependence of Incorporation of Threonine in Copolymer 2

Reaction time, min	L-Thr content of monomer pool, mol %
0	10[a]
90	12
180	24
240	38

[a] Composition of reaction mixture prior to initiation.

Two early attempts to synthesize copolymers containing L-threonine as the guest residue (copolymers 1 and 2) showed that the *N*-carboxyanhydrides were not incorporated quantitatively into the copolymers. For copolymer 1, the monomer pool consisted of 10% OTMS-L-threonine NCA, and 90% γ-benzyl L-glutamate NCA. The initiator was added with an anhydride to initiator molar ratio (A/I) of 40, and the reaction was terminated after it had gone 90% to completion (340 min). The resulting copolymer contained only 2.5% threonine instead of the 10% that was expected. For copolymer 2, the monomer pool consisted of 16.6% of the OTMS-L-threonine NCA and 83.4% of the γ-benzyl L-glutamate NCA. The A/I ratio was 40, as above, and the reaction was stopped after it had gone 86% to completion (390 min). The resulting polymer contained only 3.1% threonine instead of the expected 16.6%.

This behavior is similar to that observed earlier when L-valine was the guest residue,[12] and it was assumed that the low incorporation of threonine into the copolymers was due to the fact that threonine (like valine) is branched at the β carbon. The OTMS group is close enough to the reactive site of the threonine NCA so that it presents steric hindrance. Consequently, the more reactive γ-benzyl L-glutamate NCA is used up preferentially, and thus the polymers contain more glutamic acid, and less threonine, than the monomer pool from which they were synthesized. This was demonstrated in the synthesis of copolymer 2, by monitoring the composition of the monomer pool. Aliquots of the reaction mixture were treated with 6 N HCl to precipitate the polymer. The supernatants which contained the unreacted monomers were then analyzed for their amino acid content. The results are shown in Table I.

Since copolymers 1 and 2 contained so much less threonine than was expected, and since the reaction mixtures from which they were synthesized deviated significantly from their original composition, neither of these copolymers was used to study the thermally induced helix–coil transitions. For copolymers 3 and 4, the amount of OTMS-L-threonine NCA in the reaction mixture was increased, and the reaction was stopped earlier in order to prevent the monomer pool from becoming enriched in OTMS-L-threonine NCA.

The conditions for the synthesis of all four copolymers as well as their compositions and chain lengths are summarized in Table II.

Poly[N^5-(4-hydroxybutyl)-L-glutamine-*co*-L-threonine], Poly[HBG,Thr], Copolymers I–IV. The protected copolymers 1–4 were dissolved in dioxane and treated with

Table II
Synthetic Conditions, Compositions, and Chain Lengths of Unfractionated Poly[Glu(OBzl),Thr(OTMS)]

Polymer No.	L-Thr content of reaction mixture, mol %	A/I	% completion of reaction	L-Thr content[a] of copolymer, mol %	Av mol wt[b] x 10^{-3}	$\overline{DP}$
1	10.0	40	90	2.5	300	1420
2	16.6	40	86	3.1	360	1670
3	50.0	40	52	13.0	184	900
4	33.0	100	57	6.5	250	1180

[a] Corrected for 33% L-threonine destruction relative to L-leucine in 12 N HCl hydrolysis. [b] By viscometry using the relationship of Fujita et al.[11] for polymers in dichloroacetic acid.

Table III
Characterization of Fractionated Copolymers

Fraction[a]	L-Thr[b] content, mol %	$\overline{M}_w \times 10^{-3}$[c]	$\overline{DP}_w$	$\overline{M}_z/\overline{M}_w$[d]
IIIB	9.5	69.9	366	1.21
IIIE	12.4	25.0	133	1.08
IVB	4.8	132.5	678	1.05
IVD	5.1	85.9	440	1.01
IVG	5.0	67.4	345	0.89

[a] Copolymers III and IV were derived from unexchanged copolymers **3** and **4**, respectively. The letters correspond to the fractions obtained in the fractionation procedure. [b] Corrected for 2.2% L-threonine destruction relative to L-leucine in 6 N HCl hydrolysis. [c] This value was obtained by conventional sedimentation equilibrium (with exptrapolation to zero concentration). [d] $\overline{M}_z/\overline{M}_w$ is reported for the run at the lowest concentration.

4-amino-1-butanol to convert them into the corresponding water-soluble copolymers, as described previously.[13] The progress of the aminolysis was monitored as described in paper 10,[10] and the reaction was terminated when more than 99.5% of the benzyl ester groups had been exchanged. The reaction mixture was poured into an excess of cold 1 N acetic acid to neutralize the base and, simultaneously, cleave the very labile OTMS ether.[8,14] It is even likely that some of the OTMS protecting groups had already been cleaved during the treatment with ethanol[14] in the workup of the parent copolymer, but this would not be expected to interfere with the exchange reaction or cause any side reactions of the threonine side chains with hydroxybutylamine during aminolysis.

The copolymers were then dialyzed against water until amines could no longer be detected by a ninhydrin test[15] on 0.1 mL of the dialyzate. The water-soluble poly[HBG,Thr] was recovered by lyophilization, and the crude product was fractionated by the procedure described in paper 2[16] of this series. The compositions and chain lengths of the fractions analyzed for their thermally induced helix–coil transitions are summarized in Table III. Data for copolymers derived from copolymers 1 and 2 are not shown in Table III since, as described above, these two copolymers were not considered suitable for helix–coil transition studies.

C. Analytical Methods. Check for Deprotection of Threonine. As described above, the trimethylsilyl group was removed by exposure to 1 N acetic acid and water. The absence of silicon (<±0.4%) from the water-soluble copolymers served as evidence that the removal of the OTMS group from the threonine side chains was complete. Analysis for silicon was performed by Galbraith Laboratories in Knoxville, Tenn. The error in this analysis is ±0.4%.[14]

Galbraith reported 0.16% silicon for a sample from exchanged copolymer III. However, they also reported 0.70 and 0.04% silicon for two samples of homopolymer (PHPG), for which no silicon-containing compounds were involved in the synthesis. The large experimental error involved in these analyses is probably due to contamination from silicon-containing glassware.

Determination of Composition. The copolymer fractions were hydrolyzed in evacuated, degassed, sealed ampules for 24 h in 12 N HCl at 130 °C (γ-benzyl glutamate copolymers) or 6 N HCl at 105 °C (exchanged copolymers). The samples were then treated by the procedure of Spitz[17] in order to prevent formation of artifacts.[18] A Technicon TSM amino acid analyzer was used for the analyses. For those fractions which were used to obtain melting curve data, two separate samples were hydrolyzed and a total of at least five replicates were analyzed. All results were corrected for the degradation of threonine during 6 or 12 N hydrolysis. The average experimental error in the determination of the composition for the water soluble fractions was ±1.8%.

Determination of Concentration. The concentrations of all copolymer solutions which were used for circular dichroism and optical rotatory dispersion measurements were determined from nitrogen analyses by a combination of Lang's method for digestion[19] and the semiautomated method of detection of ammonia of Noel and Hambleton.[20] The error in concentration was ±3%.

Assay for D-Amino Acids. The water-soluble copolymers were checked for the presence of D residues as follows. A small sample of copolymer (10–15 mg) was hydrolyzed in 6 N HCl. Separation of the two amino acids in the hydrolyzate was achieved with a modified version of an ion-exchange column described by Hirs et al.[21] A column approximately 30 cm long and 0.75 cm in diameter was filled with Dowex AG1-X8 (200–400 mesh) in the acetate form. This column was equilibrated with 0.5 N acetic acid, and the sample was then added and subsequently eluted with water. The separated amino acids were then reacted with L-leucine-NCA according to the method of Manning and Moore.[22] The resulting dipeptides were separated on a Technicon NC-1 automated amino acid analyzer, with a column that was 120 cm long and 0.6 cm in diameter. Elution conditions had to be developed which would separate standard solutions of L-Leu dipeptides containing L-Thr, L-*allo*-Thr, D-Thr, and D-*allo*-Thr, respectively. Using a column packing of Technicon Chromo Beads type B, the column was eluted at 62 °C with a 0.2 N sodium citrate buffer (pH 3.15) that contained 15% (v/v) methanol. The elution volumes were 220, 231, 244, and 257 mL for the four isomers, with the peak at 244 mL being identified as that corresponding to L-Thr (allo-free), by adding L-Leu-L-Thr to the original racemic mixture. After correcting for any racemization that may have occurred during hydrolysis, or during the column separation (by carrying a mixture of L-threonine, L-glutamic acid, and aminobutanol through the same procedure), it was found that there was no racemization within an estimated experimental error of ±4%.

D. Viscosity, ORD, and CD Measurements. Viscosity, optical rotary dispersion (ORD), and circular dichroism (CD) measurements were all carried out as described in paper 2[16] of this series.

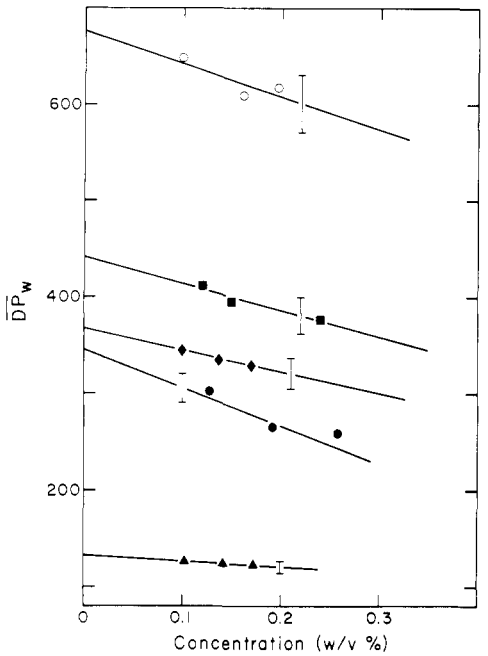

Figure 1. Concentration dependence of molecular weights for fractions used in analysis to obtain σ and s: (○) fraction IVB (4.8% Thr, $\overline{DP}_w$ = 678); (■) fraction IVD (5.1% Thr, $\overline{DP}_w$ = 440); (♦) fraction IIIB (9.5% Thr, $\overline{DP}_w$ = 366); (●) fraction IVG (5.0% Thr, $\overline{DP}_w$ =345); (▲) fraction IIIE (12.4% Thr, $\overline{DP}_w$ = 133).

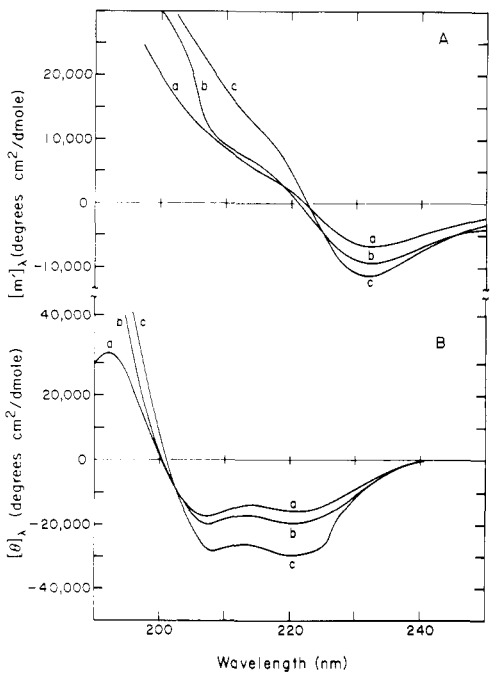

Figure 2. (A) ORD and (B) CD data in water at 20 °C for representative fractions of L-threonine copolymers: (a) fraction IIIE (12.4% Thr, $\overline{DP}_w$ = 133); (b) fraction IIIB (9.5% Thr, $\overline{DP}_w$ = 366); (c) fraction IVB (4.8% Thr, $\overline{DP}_w$ = 678).

E. Molecular Weights. The molecular weights of the water-soluble fractions were determined by the conventional sedimentation equilibrium method as reported in paper 2.[16] The concentration dependence of the weight-average molecular weight, $\overline{M}_w$, was determined for each sample, and $\overline{M}_z$ 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 several fractions, required for the calculation of molecular weights, were determined from the amino acid content as described by Cohn and Edsall.[23] A value of $\bar{v}$ = 0.81 for PHBG was used in the calculation of $\bar{v}$ for the copolymers.[16]

II. Results

A. Characterization of the Copolymers. Table II summarizes the composition of the reaction mixtures and the composition and average degree of polymerization ($\overline{DP}$) of the unfractionated poly[Glu(OBzl),Thr(OTMS)] copolymers. Table III summarizes the composition, $\overline{DP}_w$, and $\overline{M}_z/\overline{M}_w$ data for those fractions of the corresponding exchanged copolymers poly[HBG,Thr] which were used to determine the Zimm–Bragg parameters σ and s. The usual decrease in $\overline{DP}$, attributed to transamidation upon conversion of γ-benzyl glutamate to the hydroxybutylglutamine copolymers, is apparent from a comparison of the two tables.

As can be seen from an inspection of Table II, only a small fraction (~¼) of the threonine in the reaction mixture appeared in the copolymer. This behavior suggests that OTMS-threonine *N*-carboxyanhydride may polymerize at a slower rate than γ-benzyl glutamate *N*-carboxyanhydride, leading to copolymers which become rich in threonine only near the end of the reaction. Therefore, the rate of incorporation of threonine during polymerization was measured, and the results are shown in Table I. It can be seen that the amount of threonine incorporated varies with reaction time. It thus appears that the threonine is not incorporated randomly in the copolymer. In order to minimize these departures from randomness, copolymers 3 and 4 were prepared under the different conditions cited in section IB (with the polymerization reaction terminated after only ~50% completion). Subsequent analysis showed that, for copolymer 3, the deviations from randomness were not severe in that the threonine content did not differ very much from one fraction of the exchanged polymer to the next. Copolymer 4 seems to have even smaller departures from randomness since the compositions of the various fractions of the exchanged copolymers were almost independent of chain length. Furthermore, it has been demonstrated in paper 1[5] and discussed in paper 11[24] of this series that the presence of fairly large size blocks (i.e., small deviations from randomness) does not influence the melting behavior of these copolymers. While this criterion has been used in all previous papers in this series to indicate that the copolymers were sufficiently random for the purpose required here, a more rigorous proof was provided for copolymers containing methionine as the guest residue, by analyzing the distribution of fragments from a CNBr digest of the copolymer.[25] Thus, we have analyzed the melting data of the fractions with a theory[5] based on a random distribution of the host and guest residues in the copolymers.

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

Samples from combined fractions of copolymer III were hydrolyzed and analyzed for optical purity using the method of Manning and Moore.[22] No racemization (beyond an estimated experimental error of ±4%) was found for threonine after the results had been corrected for racemization during the hydrolysis and the workup procedure. All samples were therefore considered to be optically pure enough for use in the computation of the values of σ and s for L-threonine.

B. b_0 for Complete Helix and Complete Coil. For the host homopolymer, poly[HBG], studied in paper 2,[16] the value of b_0 corresponding to the complete helix was assigned as −750 and that corresponding to the complete coil was taken as zero.

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

L-Thr, mol fraction	$\overline{DP}_w$	Temp °C	$(\theta_h)_{exptl}$	$(\theta_h)_{theor}$ Lifson[b]	Allegra[c]	Lehman–McTague[c]
0.048	678	0	0.696	0.706	0.698	0.698
		30	0.495	0.465	0.464	0.463
		60	0.269	0.252	0.252	0.252
0.095	366	0	0.544	0.561	0.554	0.550
		30	0.335	0.364	0.364	0.362
		60	0.179	0.204	0.204	0.204
0.124	133	0	0.409	0.376	0.383	0.378
		30	0.245	0.254	0.256	0.255
		60	0.136	0.153	0.154	0.153

[a] The parameters used for hydroxybutylglutamine were taken from Table II of paper 2.[16] [b] The parameters used for L-threonine were obtained by fitting the experimental data with the Lifson theory using $\sigma = 1 \times 10^{-5}$. [c] The parameters used for L-threonine were obtained by fitting the experimental data with the Allegra theory using $\sigma = 1 \times 10^{-5}$.

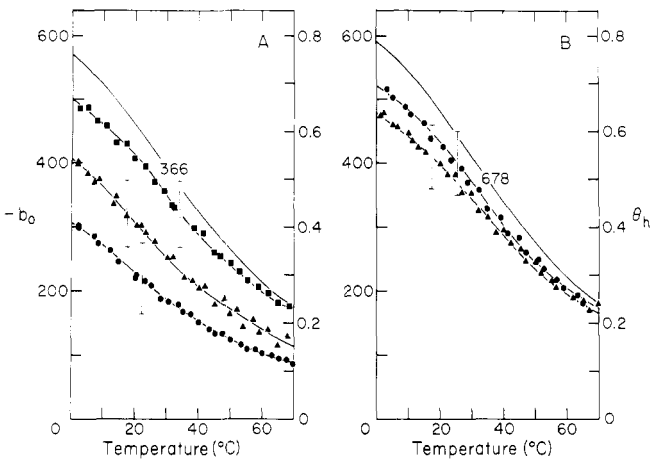

Figure 3. Temperature dependence of b_0 for poly[HBG,L-Thr] copolymers in water. The points are the experimental ones, and the lines are the smoothed experimental curves. The size of the error symbols reflects the experimental error in θ_h arising from errors in the determination of concentration and in the slope of the Moffitt Yang plot: (A) (■) fraction IVG (5.0% Thr, $\overline{DP}_w$ = 345); (▲) fraction IIIB (9.5% Thr, $\overline{DP}_w$ = 366); (●) fraction IIIE (12.4% Thr, $\overline{DP}_w$ = 133); (B) (●) fraction IVB (4.8% Thr, $\overline{DP}_w$ = 678); (▲) fraction IVD (5.1% Thr, $\overline{DP}_w$ = 440). The melting curves of PHBG ($\overline{DP}_w$ = 366 and 678), interpolated from the data of paper 2,[16] are shown as curves without experimental points in A and B respectively.

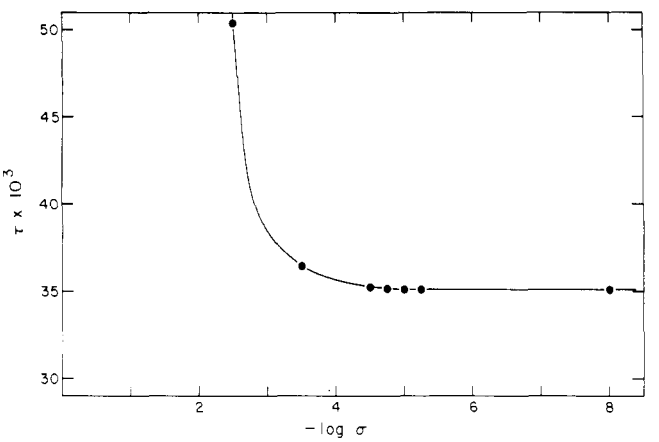

Figure 4. Determination of the best temperature-independent value of σ as the one which corresponds to the lowest value of τ for the threonine copolymers, using the Allegra approximation. See text for details.

Because the value may vary with the nature of the side chain,[26] it was necessary to determine the respective values of b_0 for the copolymers containing threonine. The value of b_0 for copolymer fraction IIIB (9.5% Thr, $\overline{DP}_w$ = 366) was measured in trifluoroethanol at 1 °C. After correcting the data for the dispersion of the refractive index of the solvent using the Sellmeir equation,[27] a b_0 value of −734 was obtained. This result indicates that the value of −750 is a reasonable one for the complete helix in these copolymers. Similarly, the value of −40 was obtained for fraction IIIE (12.4% Thr, $\overline{DP}_w$ = 133) in dichloroacetic acid at 72 °C, indicating that zero is a reasonable value for the complete coil. Thus, the helix content, θ_h, was computed as $-b_0/750$.

C. ORD and CD Data. The ORD and CD spectra for representative fractions of poly(HBG,Thr) in water are shown in Figure 2. These spectra as well as the thermally induced melting curves were obtained as described earlier.[28] Both the ORD and CD spectra indicate the presence of a right-handed α-helical structure[29–31] mixed with random coil. Contributions to these spectra arising from β structure are not evident.

The thermally induced melting curves were found to be completely reversible, reproducible, and independent of concentration [in the range from 0.1 to 0.3% (w/v)] for all fractions. Figure 3 presents the melting curves for the five fractions studied, along with curves for the homopolymer, PHBG, of $\overline{DP}_w$ = 366 and $\overline{DP}_w$ = 678. These homopolymer curves were obtained by interpolation of the data in paper 2.[16] The melting curves indicate that threonine is a helix breaker relative to PHBG at all temperatures, with its effect being most noticeable at low temperatures. The error symbols shown in Figure 3 arise from errors in concentration (±3%), from the possible error in the choice of b_0 for the full helix and coil (±3%), and from errors in the slope of the Moffitt–Yang plot ($\pm 300/b_0$%).

III. Discussion

A. Helix–Coil Parameters for Poly(L-threonine). The melting curves described in section II were analyzed according to the LAPS (Lifson–Allegra–Poland–Scheraga) hierarchy of approximations to obtain the Zimm–Bragg parameters σ and s for poly(L-threonine) in water. This procedure has been discussed extensively in earlier papers in this series.[16,24,32] The approximations, which correspond to the theories of Lifson[33] and of Allegra,[34] were used to fit the data and were then checked with the exact method of Lehman and McTague[35] in representative cases.[36]

The results of these two approximations, as well as those of the exact calculations, are compared with some of the original experimental data in Table IV. Both the first-order

Table V
Values of the Zimm–Bragg Parameter s for Poly(L-threonine) in Water as a Function of Temperature [a]

Temp, °C	s	Temp, °C	s
0	0.754	40	0.847
10	0.792	50	0.853
20	0.817	60	0.863
30	0.836	70	0.893

[a] Computed with the Allegra approximation, with $\sigma = 1 \times 10^{-5}$.

Table VI
Thermodynamic Parameters for L-Threonine [a]

$\Delta G°_{20}$, cal/mol	118 ± 27
$\Delta H°_{20}$, cal/mol	451 ± 56
$\Delta S°_{20}$, eu	1.14 ± 0.28
σ	1×10^{-5}

[a] Calculated as described in text.

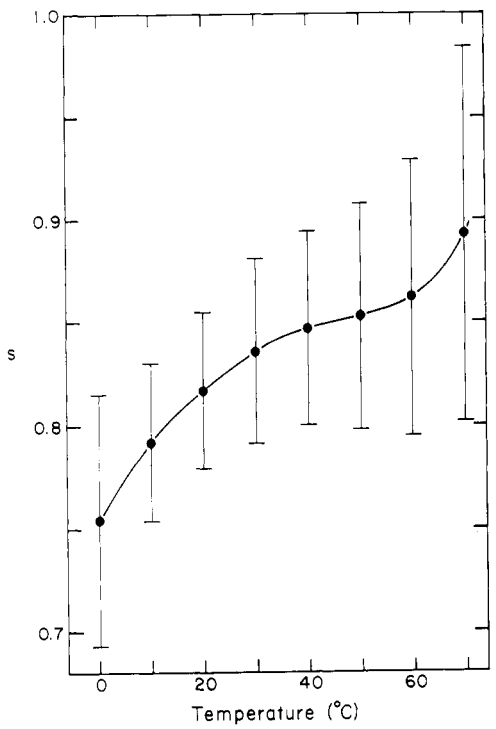

Figure 5. Temperature dependence of s for poly(L-threonine) in water for $\sigma = 1 \times 10^{-5}$. The solid line is drawn to pass through all the points. The error symbols are described in the text.

(Lifson) and second-order (Allegra) approximations yield results in good agreement with those calculated by the exact Lehman–McTague method. The following discussion is based on the results from the Allegra approximation.

The copolymer melting data were analyzed as described in paper 2.[16] σ was assumed to be independent of temperature, and 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 2.[16] The best value of σ is that at which τ is a minimum. From Figure 4 it can be seen that, for values of σ smaller than approximately 1×10^{-5}, τ remains constant. This phenomenon (that τ becomes independent of σ for small values of σ) is discussed in paper 15.[37] Since any value of σ smaller than $\sigma = 1 \times 10^{-5}$ will fit the data equally well, we have chosen this value as the best temperature-independent value of σ.

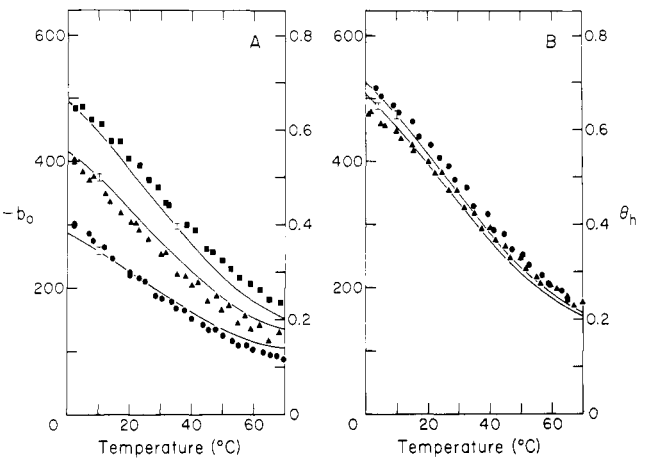

Figure 6. Comparison of the calculated melting curves, obtained with the parameters of the Allegra theory (using $\sigma = 1 \times 10^{-5}$, for poly(L-threonine), with the experimental points: (A) (■) fraction IVG (5.0% Thr, $\overline{DP}_w = 345$); (▲) fraction IIIB (9.5% Thr, $\overline{DP}_w = 366$); (●) fraction IIIE (12.4% Thr, $\overline{DP}_w = 133$); (B) (●) fraction IVB (4.8% Thr, $\overline{DP}_w = 678$); (▲) fraction IVD (5.1% Thr, $\overline{DP}_w = 440$). 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 experimental points).

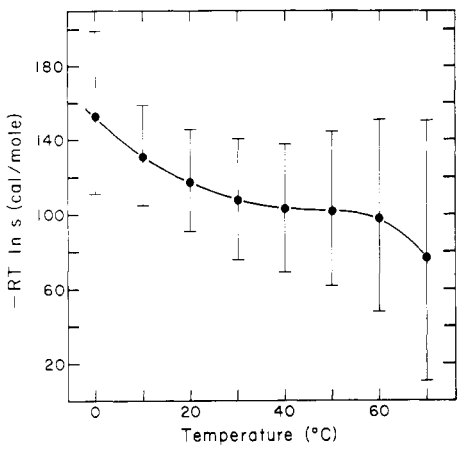

Figure 7. Plot of $-RT \ln s$ (i.e., $\Delta G°$) vs. T for poly(L-threonine) in water. The curve has been drawn through the points obtained from the Allegra approximation using $\sigma = 1 \times 10^{-5}$. The error symbols were calculated from the standard deviation in s.

The values of $s(T)$ obtained from the Allegra theory are listed in Table V. Figure 5 displays the temperature dependence of s, as calculated from the Allegra theory, with the estimated error in s shown at various temperatures. The error symbols represent the standard deviations in s at any given temperature and were calculated as described in earlier papers.[7,10]

The melting curves computed with the best-fit Allegra values for σ and s are shown in Figure 6 along with the experimental points. The agreement is reasonably good. The error symbols on the computed melting curves arise from errors in composition (±1.8%) and molecular weight (±5%).

The thermodynamic quantities $\Delta G°$ (the Gibbs free energy), $\Delta H°$ (the enthalpy), and $\Delta S°$ (the entropy) for the conversion of a residue of L-threonine from the coil state to the 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 $\Delta G°$ ($= -RT \ln s$) vs. temperature. The error symbols were determined from the standard deviations in s. $\Delta H°$ was calculated from the slope of the $\ln s$ vs.

l/T curve using the van't Hoff equation $[-dR \ln s/d(l/T) = \Delta H^\circ]$ and ΔS° was obtained from the relation $\Delta H^\circ - T\Delta S^\circ$. The values of these thermodynamic parameters for L-threonine in water at 20 °C are given in Table VI.

B. Comparison with Previous Results. No previous determinations of the Zimm–Bragg parameters have been reported for polymers containing threonine. Based on a statistical analysis of the conformation of threonine in proteins, threonine has generally been considered to be helix indifferent,[38-41] or a mild helix breaker.[42-50] From a theoretical analysis, Finkelstein et al.[51] assigned a value of $s = 0.75$ for L-threonine (which we interpreted, from their context, as pertaining to 20 °C). This is in fairly good agreement with our experimental value of $s = 0.817$ at the same temperature. Of the amino acids studied thus far in this series, Gly,[32] Asp,[3] Ser,[18] and Asn[52] have been found to be helix breakers. Of these, Gly is the strongest breaker, Ser and Asn have values of s very close to that of threonine at 25 °C, while Asp has a value of s between those of Gly and Thr.

IV. Conclusion

Water-soluble random copolymers of L-threonine and N^5-(4-hydroxybutyl)-L-glutamine were synthesized and characterized. From an analysis of the thermally induced helix–coil transition of these copoloymers, the Zimm–Bragg parameters σ and s for poly(L-threonine) were determined. The values of s show that L-threonine is a moderately strong helix breaker in the temperature range of 0–70 °C with the helix destabilizing effect being more pronounced at low temperatures.

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References and Notes

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(2) (a) Hoffman-LaRoche & Co. Ltd., Basel, Switzerland; (b) author to whom requests for reprints should be addressed.
(3) Y. Kobayashi, F. Cardinaux, B. O. Zweifel, and H. A. Scheraga, *Macromolecules*, **10**, 1271 (1977).
(4) B. H. Zimm and J. K. Bragg, *J. Chem. Phys.*, **31**, 526 (1959).
(5) P. H. Von Dreele, D. Poland, and H. A. Scheraga, *Macromolecules*, **4**, 396 (1971).
(6) (a) H. A. Scheraga, *Pure Appl. Chem.*, **36**, 1 (1973); (b) *ibid.*, in press.
(7) F. R. Maxfield, J. E. Alter, G. T. Taylor, and H. A. Scheraga, *Macromolecules*, 8 479 (1975).
(8) 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. Am. Chem. Soc.*, **93**, 2746 (1971).
(9) R. L. Prestidge, D. R. K. Harding, J. E. Battersby, and W. S. Hancock, *J. Org. Chem.*, **40**, 3287 (1975).
(10) R. K. Scheule, F. Cardinaux, G. T. Taylor, and H. A. Scheraga, *Macromolecules*, **9**, 23 (1976).
(11) H. Fujita, A. Teramoto, T. Yamashita, K. Okita, and S. Ikeda, *Biopolymers*, **4**, 781 (1966).
(12) J. E. Alter, R. H. Andreatta, G. T. Taylor, and H. A. Scheraga, *Macromolecules*, **6**, 564 (1973).
(13) K. E. B. Platzer, V. S. Ananthanarayanan, R. H. Andreatta, and H. A. Scheraga, *Macromolecules*, **5**, 177 (1972).
(14) L. J. Hughes, R. H. Andreatta, and H. A. Scheraga, *Macromolecules*, **5**, 187 (1972).
(15) M. F. Ferger, W. C. Jones, Jr., D. F. Dyckes, and V. du Vigneaud, *J. Am. Chem. Soc.*, **94**, 982 (1972).
(16) P. H. Von Dreele, N. Lotan, V. S. Ananthanarayanan, R. H. Andreatta, D. Poland, and H. A. Scheraga, *Macromolecules*, **4**, 408 (1971).
(17) H. D. Spitz, *Anal. Biochem.*, **56**, 66 (1973).
(18) J. W. van Nispen, D. J. Hill, and H. A. Scheraga, *Biopolymers*, **16**, 1587, (1977).
(19) C. A. Lang, *Anal. Chem.*, **30**, 1692 (1968).
(20) R. J. Noel and L. G. Hambleton, *J. Assoc. Off. Anal. Chem.*, **59**, 134 (1976); *Chem. Abstr.*, **84**, 149347 k (1976).
(21) C. H. W. Hirs, S. Moore, and W. H. Stein, *J. Am. Chem. Soc.*, **76**, 6063 (1954).
(22) J. M. Manning and S. Moore, *J. Biol. Chem.*, **243**, 5591 (1968).
(23) E. J. Cohn and J. T. Edsall, "Proteins, Amino Acids, and Peptides", Reinhold, New York, N.Y., 1943, p 371.
(24) M. K. Dygert, G. T. Taylor, F. Cardinaux, and H. A. Scheraga, *Macromolecules*, **9**, 794 (1976).
(25) D. J. T. Hill, F. Cardinaux, and H. A. Scheraga, *Biopolymers*, **16**, 2469 (1977).
(26) J. N. Vournakis, J. F. Yan, and H. A. Scheraga, *Biopolymers*, **6**, 1531 (1968).
(27) J. R. Partington, "An Advanced Treatise on Physical Chemistry", Vol. IV, Longmans, Green and Co., London, 1960, pp 92 and 99.
(28) H. E. Van Wart, G. T. Taylor, and H. A. Scheraga, *Macromolecules*, **6**, 266 (1973).
(29) E. R. Blout, I. Schmier, and N. S. Simmons, *J. Am. Chem. Soc.*, **84**, 3193 (1962).
(30) N. Greenfield, B. Davidson, and G. D. Fasman, *Biochemistry*, **6**, 1630 (1967).
(31) N. Greenfield and G. D. Fasman, *Biochemistry*, **8**, 4108 (1969).
(32) V. S. Ananthanarayanan, R. H. Andreatta, D. Poland, and H. A. Scheraga, *Macromolecules*, **4**, 417 (1971).
(33) S. Lifson, *Biopolymers*, **1**, 25 (1963).
(34) G. Allegra, *J. Polym. Sci., Part C*, **16**, 2815 (1967).
(35) G. W. Lehman and J. P. McTague, *J. Chem. Phys.*, **49**, 3170 (1968).
(36) All computer programs used in these calculations are available and can be obtained as directed in footnotes 26 and 27 of paper 1.[5]
(37) Y. Konishi, J. W. van Nispen, G. Davenport, and H. A. Scheraga, *Macromolecules*, **10**, 1264 (1977).
(38) P. N. Lewis, N. Gō, M. Gō, D. Kotelchuck, and H. A. Scheraga, *Proc. Natl. Acad. Sci. U.S.A.*, **65**, 810 (1970).
(39) N. Gō, P. N. Lewis, M. Gō, and H. A. Scheraga, *Macromolecules*, **4**, 692 (1971).
(40) P. N. Lewis and H. A. Scheraga, *Arch. Biochem. Biophys.*, **144**, 576 (1971).
(41) P. N. Lewis and E. M. Bradbury, *Biochim. Biophys. Acta*, **336**, 153 (174).
(42) D. Kotelchuck and H. A. Scheraga, *Proc. Natl. Acad. Sci. U.S.A.*, **61**, 1163 (1968).
(43) D. Kotelchuck and H. A. Scheraga, *Proc. Natl. Acad. Sci. U.S.A.*, **62**, 14 (1969).
(44) R. H. Pain and B. Robson, *Nature, (London)*, 227, 62 (1970).
(45) B. Robson and R. H. Pain, *J. Mol. Biol.*, **58**, 237 (1971).
(46) P. Y. Chou and G. D. Fasman, *Biochemistry*, **13**, 211 (1974).
(47) S. Tanaka and H. A. Scheraga, *Macromolecules*, **9**, 142 (1976).
(48) S. Tanaka and H. A. Scheraga, *Macromolecules*, **9**, 159 (1976).
(49) G. D. Fasman, P. Y. Chou, and A. J. Adler, *Biophys. J.*, **16**, 1201 (1976).
(50) S. Tanaka and H. A. Scheraga, *Macromolecules*, **10**, 305 (1977).
(51) A. V. Finkelstein, O. B. Ptitsyn, and S. A. Kozitsyn, *Biopolymers*, **16**, 497 (1977).
(52) R. R. Matheson, R. A. Nemenoff, F. Cardinaux, and H. A. Scheraga, *Biopolymers*, **16**, 1567 (1977).