Comparison of the Thermal Reaction Rate in Bulk and in Dilute Solution. It is at first sight surprising that the thermal isomerization of side chains of polymers in the rubbery state should proceed as easily as in dilute solution, since changes in the geometry of such bulky groups must require extensive rearrangements in the conformation of neighboring chain molecules. However, the following argument seems to account for the observed behavior. The conversion from a cis to a trans azo group should not be thought of as taking place in a single step, but as a result of many small oscillations of the -N=N—C bond angle.[22] Although the increase of this angle should be slowed down by the requirement that neighboring chains assume a conformation allowing such a change, the reverse process should be impeded to the same extent. Thus, the fundamental postulate of the theory of absolute reaction rates, *i.e.*, that the transition state be in equilibrium with the ground state of the reagent, remains valid, and the restrictions on mobility due to the nature of the medium do not affect the reaction rate. We have here a unimolecular analog of the well-known fact that bimolecular reactions characterized by an appreciable activation energy have rates independent of the viscosity of the medium, since a decreased mobility slows down equally the mutual approach and separation of the reacting species. The above argument appears to hold approximately even for thermal isomerization in the backbone of a polymer chain of polymers in bulk. This rules out, for our case, mechanisms in which it is postulated that changes in the shape of a chain molecule occur by two correlated conformational transitions.[23] Such mechanisms may still apply in cases where conformational transitions involve very low energy barriers, but the spectroscopic method would then, unfortunately, not be applicable for a kinetic investigation.

(22) We are here assuming that the isomerization takes place by the inversion mechanism, in which the C—N=N—C grouping remains coplanar and is linear in the transition state as postulated, *e.g.*, by E. R. Talaty and J. C. Fargo, *Chem. Commun.*, 65 (1967), and by D. Gegiou, K. A. Muszkat, and E. Fischer, *J. Amer. Chem. Soc.*, **90**, 12 (1968).

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

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ABSTRACT: The synthesis and characterization of water-soluble random copolymers containing L-alanine and N^5-(3-hydroxypropyl)-L-glutamine are described, and the thermally induced helix–coil transition of these copolymers in water has been studied. The incorporation of L-alanine was found to increase the helix content of the polymer. The Zimm–Bragg parameters σ and s for the helix–coil transition in poly(L-alanine) in water were deduced from an analysis of the melting curves of the copolymers in the manner described in earlier papers. The values obtained for the enthalpy, entropy, and transition temperature for the conversion of a coil to a helical residue in poly(L-alanine) in water were found to be in good agreement with those obtained earlier using sandwich-type block copolymers of L-alanine and D,L-lysine in the same solvent, thereby supporting the view that the conformational state of an amino acid residue (except possibly proline) in a polypeptide or protein is essentially independent of the chemical nature of its neighbors.

The use of the "host–guest" technique for the evaluation of the helix–coil stability constants of amino acids in water was illustrated in earlier papers of this series.[3–5] This technique involves the study of the perturbation of the helix–coil transition in a homopolymer of a host amino acid residue (with known transition parameters, σ and s, of the Zimm–Bragg theory[6]) by a guest residue (for which parameters are sought) distributed randomly in the copolymer of the two monomers. The validity of this method was established by the determination of the transition parameters of poly[N^5-(3-hydroxypropyl)-L-glutamine] and poly[N^5-(4-hydroxybutyl)-L-glutamine] (PHPG and PHBG, respectively) from both homopolymer and copolymer experimental data,[4] and the values of σ and s for glycine were obtained from an analysis of the melting curves for copolymers of glycine and N^5-(4-hydroxybutyl)-L-glutamine (HBG).[5] In the present paper, this approach is extended to L-alanine, using N^5-(3-hydroxypropyl)-L-glutamine (HPG) as the host residue in the copolymer. The choice of the host was made on the expectation, which was demonstrated by this work, that the melting curve of the homopolymer of the host residue, PHPG, would be progressively shifted toward higher temperatures within the available temperature range in water by the incorporation of L-alanine, since the latter is known to act as a helix former.[7–9]

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(2) (a) NSF Predoctoral Trainee, 1967–1971; NIH Predoctoral Trainee, 1971–1972; (b) NIH Postdoctoral Trainee, 1969–1971.

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TABLE I
COMPOSITIONS AND CHAIN LENGTHS OF THE UNFRACTIONATED COPOLYMERS

	Polymers containing γ-benzyl L-glutamate			Polymers containing N^5-(3-hydroxypropyl)-L-glutamine		
L-Alanine content of reaction mixture, mol %	L-Alanine content found, mol %	Av mol wt[a] $\times 10^{-3}$	$\overline{\mathrm{DP}}$	L-Alanine content found, mol %	Wt av mol wt[b] $\times 10^{-3}$	$\overline{\mathrm{DP}}_w$
10	11.6 (I)	115	560	13.1 (VI)	44	260
20	19.9 (II)	210	1110	19.5 (VII)	89	540
30	28.6 (III)	185	1060	30.2 (VIII)	74	490
40	40.7 (IV)	290	1810	39.9 (IX)	108	760
50	48.1 (V)	300	2070	51.7 (X)	130	980

[a] By viscometry, using the relation of H. Fujita, A. Teramoto, T. Yamashita, K. Okita, and S. Ikeda, *Biopolymers*, **4,** 781 (1966), for polymers in DCA. [b] By conventional sedimentation equilibrium on a solution of concentration 0.3% (w/v); averaged from the values given in Table II.

The values of σ and s for poly(L-alanine) had been determined earlier[7] by a different technique, *viz.*, the use of block copolymers containing L-alanine residues of different chain lengths flanked on either side by D,L-lysine residues which made the copolymers water soluble (presumably without affecting the values of σ and s of the central poly(L-alanine) block). The ease of synthesis and the absence of charged residues in the random copolymers studied here make the host–guest technique a more attractive one. However, if the basic assumptions of both techniques are correct, they should both yield similar parameters for poly(L-alanine) (in water). As will be shown below, the results obtained by both methods are in good agreement.

The synthesis of water-soluble random copolymers of L-alanine and HPG is described in section I, and the experimental characterization of these copolymers and their melting behavior in water are presented in section II. Finally, in section III, the data are analyzed by means of an appropriate form of the theory[3] to determine the helix–coil stability parameters of L-alanine in water. These results are then discussed in terms of a general understanding of the contribution of the stable L-alanine α-helical conformation to polypeptide and protein structures.

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

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

(A) Materials. 3-Amino-1-propanol from Aldrich Chemical Co., Inc., was dried over barium oxide and distilled under reduced pressure. Dioxane and hexane were purified shortly before use by refluxing and distilling over sodium. Triethylamine was dried and distilled over KOH. Ethyl acetate was dried over anhydrous calcium sulfate and distilled. Purified grade dichloroacetic acid (DCA), "spectranalyzed" grade methanol from Fisher Scientific Co., and absolute ethanol, U.S.P., N.F., from Industrial Chemicals were used without further purification. Ether from Malinckrodt Chemical Works was of analytical reagent grade. 2,2,2-Trifluoroethanol obtained from Aldrich Chemical Co., Inc., was stirred over sodium bicarbonate and distilled. L-Alanine and L-glutamic acid were purchased from Aldrich Chemical Company, Inc.

Using the L-leucyl dipeptide method of Manning and Moore,[10] the starting L-amino acids were found to contain no detectable amounts (within ±0.1%) of D residues. PHPG of degrees of polymerization $\overline{\mathrm{DP}}_w = 530$, $\overline{\mathrm{DP}}_w = 360$, and $\overline{\mathrm{DP}}_w = 120$ were fractions IB, IIID, and IIC, respectively, of paper II.[4]

(B) Synthesis. *N*-Carboxyanhydrides. L-Alanine NCA was prepared by the action of phosgene on a suspension of the amino acid in dioxane for 2–3 hr at 50–60°, as described by Kopple and Katz.[11] Several recrystallizations from ether–hexane gave a chlorine-free product (less than 0.01%) with a melting point of 92°. The reaction gave 85–90% yield.

γ-Benzyl L-glutamate, which was prepared from L-glutamic acid,[12] was used to synthesize γ-benzyl L-glutamate NCA with phosgene, as described by Blout and Karlson.[13] The product was recrystallized several times from dioxane–hexane and ethyl acetate–hexane until the chlorine content was less than 0.01%. The melting point was 93–94°, and the yield was 93–97%.

Poly(γ-benzyl-L-Glu$^{m'}$-L-Ala$^{n'}$), [P(BzG–Ala)], Copolymers I–V. Random copolymers of L-alanine and γ-benzyl L-glutamate containing from 10 to 50% alanine were synthesized by polymerization of the NCA's in dioxane with triethylamine as an initiator. The two NCA's were dissolved in dioxane (at a concentration of about 10 mmol of total NCA per 100 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 calcium chloride drying tube and allowed to stand at room temperature for 2–4 days. The viscous reaction mixture was then introduced into 700 ml of vigorously stirred absolute ethanol. The white precipitate was collected on a filter funnel, washed thoroughly with ethanol, and dried to constant weight over P_2O_5 *in vacuo.* The yield ranged from 91 to 97%. The compositions and chain-lengths of these copolymers are given in Table I.

Poly[N^5-(3-hydroxypropyl)-L-Gln^m-L-Ala^n], [P(HPG–Ala)], Copolymers VI–X. The γ-benzyl L-glutamate–L-alanine copolymers were treated with 3-amino-1-propanol to yield a series of water-soluble copolymers. The products I–V were dissolved in dioxane at a concentration of 0.14–0.18 g/ml at 60°. 3-Amino-1-propanol was added in five to six equal portions of 3 ml each over a period of 3–5 days, during which the reaction mixture was stirred and maintained at 60°. The reaction was considered to be complete when no precipitate formed upon addition to water. The mixture was poured into about 250 ml of 5% (v/v) acetic acid to give a clear solution, which was dialyzed exhaustively against distilled water. The solution was then filtered through a Millipore filter (0.45-μ size, Millipore, Bedford, Mass.), evaporated to a lesser volume and lyophilized. For samples of higher alanine content (40–50%), the Millipore filtrate was not evaporated to less than about 30 ml because further evaporation would have caused some of the product

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(11) K. D. Kopple and J. J. Katz, *J. Amer. Chem. Soc.*, **78,** 6199 (1956).

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to precipitate. The yield ranged from 89 to 94%. The compositions and chain lengths of the unfractionated water-soluble copolymers are given in Table I.

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

(D) Fractionation. The copolymers VI–X were fractionated with methanol and ether by the procedure described in papers II[4] and III,[5] after which they were dissolved in water, lyophilized, and dried *in vacuo* over P_2O_5.

(E) Determination of Composition. The amino acid compositions of all copolymers were determined on a Technicon amino acid analyzer. The samples I–V were hydrolyzed in 12 *N* HCl at 105° for 48 hr in sealed ampoules, and samples VI–X and fractions thereof were hydrolyzed in 12 *N* HCl at 105° for 72 hr. 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 ±4%.

(F) Determination of Concentration. The concentrations of all polymer solutions were determined by the micro-Kjeldahl nitrogen analysis method described in paper II,[4] using data on the amino acid compositions in the computations. Each concentration determination was performed at least five times. For nonaqueous solutions, the organic solvents were evaporated prior to nitrogen analysis. The average error (one standard deviation) in the concentration determination is estimated to be ±2.3% for water solutions, but ±4% for organic solvent solutions such as methanol, ethanol, and trifluoroethanol.

(G) Viscosity. The viscosities were determined in DCA at 25.0 ± 0.1° in a Cannon–Ubbelohde semimicro dilution viscometer, as described earlier.[4] The relative viscosities were kept in the range of 1.2–1.7.

(H) Optical Purity. The starting materials as well as the final copolymers were checked for racemization by the L-leucyl dipeptide method of Manning and Moore.[10] The L-Leu-L-Ala and L-Leu-D-Ala were separated on a Technicon amino acid analyzer with a sodium citrate elution buffer at pH 3.80. Dipeptide standards were prepared from D,L-alanine.

(I) Determination of Molecular Weights. The molecular weights of polymers I–V shown in Table I were determined by viscometry using DCA as a solvent. These molecular weights are only rough estimates, since the viscosity–molecular weight relation[14] for poly(γ-benzyl L-glutamate) (PBLG) was used without correction for the copolymers. These data were used for semiquantitative comparison purposes only.

On the other hand, the weight-average molecular weights of the fractions of the P(HPG–Ala) copolymers (samples VI–X) were determined with a Spinco Model E ultracentrifuge. Because the conventional sedimentation equilibrium method (as described in paper II[4]) on solutions with concentrations of 0.1–0.5% indicated the presence of significant nonideality [in that the molecular weights increased with decreasing concentration (see section IIA)], it was necessary to determine the molecular weights by the meniscus-depletion method[15] at the relatively low concentrations of 0.010–0.015% (w/v). In order to attain the condition of meniscus depletion from the meniscus to about the middle of the solution, with no material sedimented to the bottom of the cell, the speed ranged from 15,220 to 31,410 rpm. Equilibrium was attained within 14 hr if the equilibrium rotor speed was exceeded for 3–5 hr at the beginning of the run. In order to obtain more interference fringes and hence to improve the precision, the single-channeled cell with a slightly longer column length than is possible with the Yphantis triple-channeled cell was used; three to five fringes were obtained in each run. The value of $\overline{M}_z$ was determined by linear extrapolation of the $\ln c$ *vs.* r^2 points near the bottom of the cell, as described by Yphantis.[15] Although no evidence of convection anomalies was present in the results from the meniscus-depletion method, the molecular weights of several samples were determined in 1.0% sucrose solution to establish definitely the absence of convection. Since the precision of the meniscus-depletion method is lower than that of conventional sedimentation equilibrium, each molecular weight was determined two or three times under slightly different conditions of speed and concentration, and the average value for each is given in Table II. The precision of the conventional sedimentation-equilibrium determinations was ±3%, but that of the meniscus-depletion method was only ±7% (because of the low concentrations used and the resultant small number of fringes available for analysis).

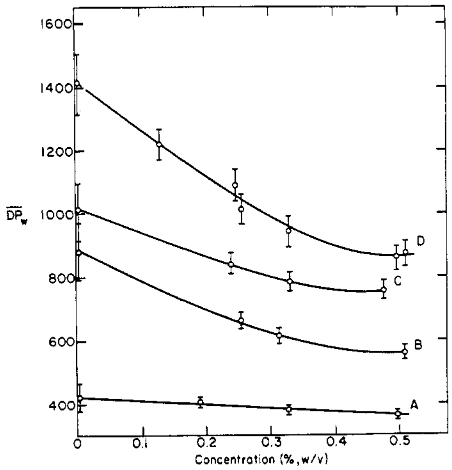

Figure 1. Representative curves illustrating the concentration dependence of molecular weight. All values at concentrations above 0.1% were determined by conventional sedimentation equilibrium and those at lower concentration by the meniscus depletion method: (A) 14.1% Ala, $\overline{DP}_w$ = 422 (VIB); (B) 20.6% Ala, $\overline{DP}_w$ = 880 (VIIB-2); (C) 32.0% Ala, $\overline{DP}_w$ = 1010 (VIIIB); (D) 38.3% Ala, $\overline{DP}_w$ = 1413 (IXB-2). The error symbols represent the experimental error in each measurement.

The partial specific volumes ($\bar{v}$) of the P(HPG–Ala) copolymers necessary for the calculation of molecular weights were determined from the amino acid content as described by Cohn and Edsall.[16] A value of $\bar{v}$ = 0.79[17] for PHPG was used in the computation of $\bar{v}$ for the copolymers.

(J) Optical Rotatory Dispersion and Circular Dichroism Measurements. The optical rotatory dispersion (ORD) and circular dichroism (CD) measurements were made with a Cary Model 60 spectropolarimeter equipped with a model 6001 CD attachment, as described in paper II.[4] The temperature was maintained constant to ±0.4° below 10° and to ±0.1° at higher temperatures with water-jacketed quartz cells. The concentration of the solutions used in these measurements was 0.2–0.5% (w/v). All solutions were filtered through Millipore filters (0.45-μ size) before measurement. The experimental error in b_0 results from (a) the error in the solution concentration (±2.3%), (b) the error in the values of b_0 for the complete helix and complete coil (±2.7%; section IID), and (c) the error in the determination of the slope of the Moffitt–Yang plot (±0.4 to ±6.1%); the effect of an error in the amino acid composition is trivial.

(II) Results

(A) Molecular Weights of the Copolymers. Conventional sedimentation-equilibrium runs in the concentration range of 0.1–0.5% (w/v) indicated that the solutions were nonideal in that the apparent molecular weight increased as the concentration decreased, as shown for some copolymer fractions in Figure 1. It appears that the nonideality increases

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(17) 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, g	L-Alanine content, mol %	Mean residue wt	$\bar{v}$, cm^3/g	$\bar{M}_w \times 10^{-3}$ [b]	$\bar{M}_z/\bar{M}_w$ [b]	$\overline{DP}_w$ [b]	$\bar{M}_w \times 10^{-3}$ [c,d]	$\bar{M}_z/\bar{M}_w$ [c,d]	$\overline{DP}_w$ [c,d]
VIA	0.11	15.0	169							
VIB	0.17	14.1	170	0.788	64.5	1.23	380	71.7	1.18	422
VIC	0.13	10.7	174	0.789	51.2	1.03	294			
VID	0.12	14.7	169	0.788	36.2	0.91	214			
VIE	0.17	11.0	173	0.789	23.0	1.10	132	28.5	1.13	165
VIF	0.06									
VIIA	0.15	19.0	164							
VIIB-1	0.23	18.4	165	0.787	114.2	1.08	693			
VIIB-2	0.22	20.6	162	0.788	95.5	1.19	588	142.9	1.17	880
VIIC	0.13	20.6	162	0.788	84.0	1.02	517			
VIID	0.10	19.7	163	0.787	64.2	1.06	393			
VIIE	0.09	19.5	164	0.787	43.9	0.99	269	52.7	0.99	322
VIIF	0.08									
VIIIA	0.04	32.5	149							
VIIIB	0.15	32.0	149	0.784	117.3	1.05	786	150.6	1.03	1010
VIIIC	0.04	30.6	151							
VIIID	0.20	29.7	152	0.785	85.5	1.09	563			
VIIIE	0.14	30.4	151	0.785	62.9	1.07	417	80.9	0.99	536
VIIIF	0.21	28.0	154	0.785	37.0	0.96	241			
VIIIG	0.09									
IXA	0.13	39.7	140							
IXB-1	0.10	39.0	141	0.782	149.2	1.06	1057			
IXB-2	0.22	38.3	142	0.783	132.0	0.87	929	200.5	1.08	1413
IXC	0.11	39.3	141	0.782	95.6	1.06	679			
IXD	0.12	38.1	142	0.783	42.4	1.02	298	51.9	1.06	365
IXE	0.22									
XA	0.09	54.5	123							
XB	0.06	47.0	132							
XC	0.10	44.1	135	0.781	149.9	1.12	1108	178.8	1.07	1322
XD	0.12	47.3	132	0.780	134.0	1.03	1019			
XE	0.15	49.1	130	0.779	113.2	1.01	874	142.8	0.97	1102
XF	0.32									

[a] Roman numeral corresponds to unfractionated material in Table I and the letter to the fraction obtained in the fractionation procedure. The Arabic numerals indicate those fractions obtained by refractionation. [b] Determined by the method of conventional sedimentation equilibrium with a solution concentration of about 0.3% (w/v). [c] Determined by the method of meniscus depletion with a solution concentration of 0.010 to 0.015% (w/v). [d] These measurements were made only for those fractions whose melting curves were determined.

with increasing alanine content (hence, with increasing helix content) and with increasing degree of polymerization, $\overline{DP}_w$. Although there is roughly a threefold variation in concentration from the meniscus to the bottom of the cell in any single (conventional) equilibrium experiment, there is no evidence of the concentration dependence of the molecular weight in any one given run; *i.e.*, the plot of ln c *vs.* r^2 (where c is the concentration at the distance r from the center of rotation in the cell) is a straight line for each experiment, whereas curvature toward the r^2 axis would be expected from the nonideality present here. However, this curvature arising from nonideality could have been compensated by a curvature in the opposite direction, arising from the small degree of polydispersity of the copolymer fractions, leading fortuitously to a linear ln c *vs.* r^2 plot. Thus, the $\bar{M}_z/\bar{M}_w$ ratios calculated from these experiments at 0.3% concentration cannot be regarded as a true measure of the polydispersity (or nonideality).

Using the meniscus-depletion method, at *very* low concentration where nonideality effects are minimal, we obtain more reliable values of $\overline{DP}_w$ and of $\bar{M}_z/\bar{M}_w$ (see Table II). The latter values indicate that the fractionation procedure yielded relatively homogeneous material.

(B) Synthesis and Characterization of the Copolymers. The average compositions and the average degrees of polymerization of the unfractionated P(BzG–Ala) and P(HPG–Ala) copolymers are shown in Table I. It can be seen that conversion from the parent benzyl glutamate copolymers (I–V) to the daughter hydroxypropylglutamine derivatives (VI–X) resulted in about a halving of the chain length (except in the case of the 40% Ala copolymer, whose chain length decreased even more). Such a decrease in chain length, resulting from the conversion of benzyl to hydroxyalkyl side chains, was also observed by Lotan, *et al.*,[17] in the synthesis of PHPG, by Von Dreele, *et al.*,[4] in the synthesis of PHPG and PHBG and copolymers thereof, and by Ananthanarayanan, *et al.*,[5] in the synthesis of P(HPG–Gly) copolymers. Such an effect is ascribed to a transaminolysis reaction in which the aminopropanol reacts with the peptide backbone as well as with the side-chain ester. As in the case of P(HPG–Gly),[5] it may be that the bulky benzyl side chains could shield such an attack of the backbone, resulting in preferential chain scission at the alanine residues. Hence, if the alanine were distributed in blocks in the copolymers, the reaction with aminopropanol would result in cleavage of the blocks, yielding oligopeptides of alanine which would be lost in dialysis, resulting in a lower alanine content of the P(HPG–Ala) copolymers than the corresponding P(BzG–Ala) copolymers. However, the compositions of the parent and daughter copolymers are essen-

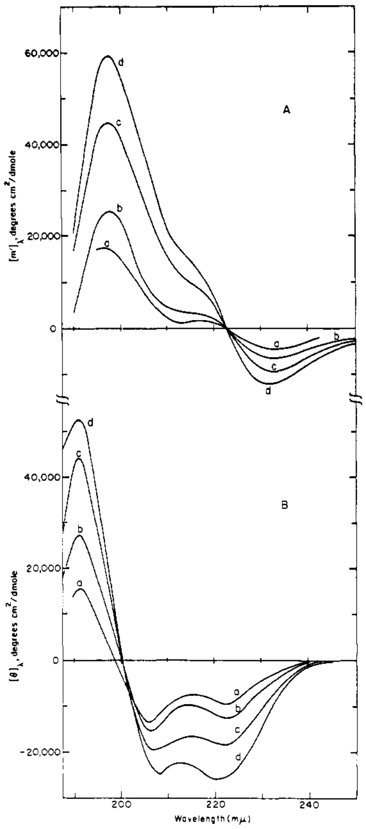

Figure 2. (A) ORD and (B) CD data in water at 26 ± 1° for PHPG and four P(HPG–Ala) copolymers: (a) PHPG, $\overline{DP}_w$ = 530 (fraction IB of paper II[4]); (b) 14.1% Ala, $\overline{DP}_w$ = 422 (fraction VIB); (c) 30.4% Ala, $\overline{DP}_w$ = 536 (fraction VIIIE); (d) 49.1% Ala, $\overline{DP}_w$ = 1102 (fraction XE).

tially the same, as shown in Table I. Also, the compositions of the fractions obtained from a given sample cannot be correlated with chain length (see Table II). This indicates that the alanine residues are probably not accumulated in blocks in the copolymers or, if there are alanine blocks, they are relatively short. Although direct experimental evidence of the degree of randomness of the copolymers is lacking, it is felt that this does not seriously affect the analysis of the melting curves, since it was demonstrated in paper I that the presence of relatively short blocks does not influence the melting behavior of a random copolymer.

While the starting amino acids were shown to be free of D residues, some racemization could have occurred in the synthesis. Applying the L-leucyl dipeptide method of Manning and Moore[10] to the copolymer hydrolysates, it was found that 0.8 ± 0.2% of the alanine had been racemized and that no detectable racemization (within ±0.1%) of the glutamic acid had occurred. Considering the high alanine content of the copolymers, we may assume that the presence of such a small amount of D-alanine in the copolymers will not affect their melting behavior.

(C) ORD and CD Data. The ORD and CD measurements on three copolymers of P(HPG–Ala) and on fraction IB of PHPG of paper II[4] in water are shown in Figure 2 for the wavelength region (λ) of 187–250 mμ at room temperature. Clearly, all of these copolymers are partially α-helical and show no evidence of β structure. The ORD data show the characteristic trough at 233 mμ, the crossover point at 222.5 mμ, a hump at about 215 mμ, and a peak at 198 mμ, all of which indicate the presence of a right-handed α-helical structure.[18,19] The corresponding CD data also support this conclusion, with two minima at 222 and 208 mμ, a crossover at about 200 mμ, and a maximum at 192 mμ.[20] The helix content is seen to increase as the alanine content of the copolymer increases, supporting our initial presumption that the amino acid L-alanine can be classified as a helix-forming residue.

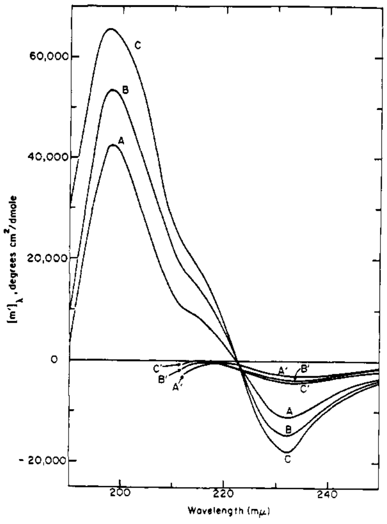

Figure 3. ORD data in water at low and high temperatures. Curves A, B, and C are at 5° and curves A′, B′, and C′ are at 76°: (A and A′) 14.1% Ala, $\overline{DP}_w$ = 422 (fraction VIB); (B and B′) 30.4% Ala, $\overline{DP}_w$ = 536 (fraction VIIIE); (C and C′) 49.1% Ala, $\overline{DP}_w$ = 1102 (fraction XE).

Figure 3 shows the effect of temperature on the conformation of three copolymers. The ORD data indicate that each of these copolymers is more helical at low temperature than it is at room temperature in water (*cf.* Figure 2). At high temperature, the copolymers exhibit ORD curves which are characteristic of the random coil, mixed with little or no α-helical structure. This demonstrates that the copolymers undergo a thermally induced transition from α helix to random coil in water. The large difference in the helix content of the 14.1% Ala, 30.4% Ala, and 49.1% Ala copolymers at low temperature is practically lost at high temperature, where all three contain little α-helical structure.

Figure 4 shows the effect of changing the solvent from water to 90% methanol on the copolymer containing 49.1% Ala. The ORD data demonstrate that this copolymer becomes more helical in methanol. However, this increase in helix content is relatively small compared to that of the P(HBG–Gly) copolymers which are very much more helical in 90% methanol than in water, as shown in paper III.[5]

The above conclusions are verified by the calculation of b_0 as a function of temperature from ORD data in the wavelength range of λ 280–420 mμ. All melting curves were determined at least twice, with solutions whose concentrations ranged from 0.2 to 0.5% (w/v); no concentration dependence of b_0 could be detected. The curves were obtained at 6° intervals from 0 to 30° and at 10° intervals from 30 to 70°. The duplicate curve was determined at 6° intervals from 0 to 24° and at 10° intervals from 24 to 70°. The two curves

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

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

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

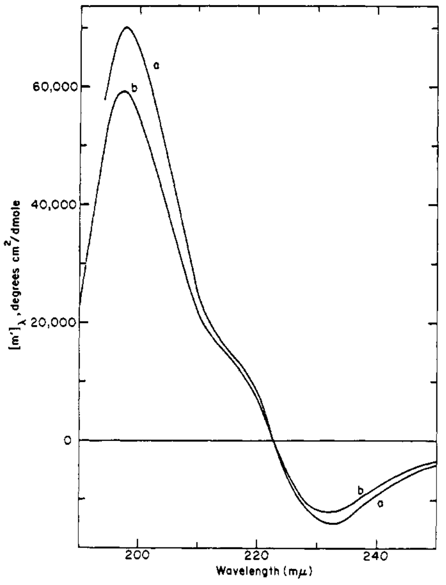

Figure 4. ORD data for copolymer fraction XE, 49.1% Ala, $\overline{DP}_w$ = 1102, at 26° in (a) 90% (v/v) aqueous methanol and (b) water.

superposed very well, indicating good reproducibility. The results are shown in Figure 5, with the vertical error symbols indicating the precision in the experimental determination of b_0. These errors arise from errors in the determination of the concentration, in the values of b_0 for the fully helix and fully coil conformations, and in the slope of the Moffit–Yang plot, and are ±2.3, ±2.7, and ±0.4 to ±6.1%, respectively. The transition was demonstrated to be reversible in all cases, as illustrated by the five typical examples shown in Figure 6.

Several observations can be made from the experimental thermally induced melting curves. Clearly, the melting curves are S shaped, and the sharpness of the transition increases with increasing molecular weight, as predicted by various theories of the helix–coil transition.[21] Furthermore, it can be seen that, for copolymers of a comparable degree of polymerization, the helix content increases as the alanine content increases. However, the order of the melting curves depends not only on composition but also on chain length. Hence, the b_0 *vs.* temperature curve of fraction VIB (14.1% Ala, $\overline{DP}_w$ = 422) lies slightly (almost indistinguishably) above that of fraction VIIE (19.5% Ala, $\overline{DP}_w$ = 322), and the curves of fractions VIIIE and VIIIB (~30% Ala, $\overline{DP}_w$ = 536 and 1010, respectively) are higher than that of fraction IXD (38.1% Ala, $\overline{DP}_w$ = 365). The fact that the curves of fraction VIIIE (30.4% Ala, $\overline{DP}_w$ = 536) and fraction VIIIB (32.0% Ala, $\overline{DP}_w$ = 1010) are experimentally indistinguishable indicates that, for this alanine content, a $\overline{DP}_w$ of 500 is essentially "infinite," in that a further increase in $\overline{DP}_w$ has relatively little effect upon helix content.

(D) b_0 for Complete Helix and Complete Coil. For the homopolymer PHPG studied in paper II,[4] the b_0 value for the complete helix was assigned as −750, and for the complete coil as zero.[4] Because the side chain influences the optical rotatory properties of a polypeptide,[22] it was necessary to determine the respective values of b_0 for the copolymers with L-alanine. Hence, the b_0 of the most helical copolymer, fraction XE (49.1% Ala, $\overline{DP}_w$ = 1102), was determined in several solvents in which it could be expected to be more helical. For these nonaqueous solutions, all values of b_0 were corrected for the dispersion of the refractive index of the solvent using the Sellmeier equation.[23] The results are shown in Table III. The highest helix content of this fraction was attained in 96% trifluoroethanol, for which b_0 = −723. This is only slightly less than the value of b_0 = −731 determined here for PHPG (sample IB of paper II,[4] $\overline{DP}_w$ = 530) in 96% trifluoroethanol. Because it is not certain that the copolymers were truly 100% helical even in this solvent, we feel that it is justifiable to retain the value of −750 for b_0 for the complete helix of P(HPG–Ala) copolymers. The value of b_0 = 0 was assumed for the random coil for both homopolymers and copolymers, since the melting curves tend to approach this value at high temperature. Thus, the helix content of all polymers was calculated as $\theta_h = -b_0/750$. Admittedly, this choice of the limiting values of b_0 is somewhat arbitrary because we cannot attain experimental conditions which unequivocally give fully helical or fully coil conformations. Hence, we include an error in all experimentally determined θ_h's of ±2.7% to account for this uncertainty.

Table III

b_0 at 1.0° of Fraction XE (49.1% Ala, $\overline{DP}_w$ = 1102)

Solvent	$-b_0$
Water	610
90% aqueous methanol	627
90% aqueous ethanol	678
96% aqueous trifluoroethanol	723

(III) Discussion

(A) Helix–Coil Parameters for Poly(L-alanine). After consideration of the theoretical analyses presented in paper I,[3] the procedure employed in fitting the experimental data from the copolymers to obtain the parameters σ and s for poly(L-alanine) was first to use the lowest order of approximation in the LAPS (Lifson–Allegra–Poland–Scheraga) hierarchy and then to proceed to more exact theories sequentially as the results demanded.[24] Hence, the theory corresponding to that of Lifson[25] was used initially. The results were checked in a representative way against the exact ones obtained from the method of Lehman and McTague for finite chain length,[26] and the agreement was demonstrated to be quite good, as shown in Table IV. Also, the second-order approximation, corresponding to the theory of Allegra,[27] was employed; Table IV demonstrates that the Allegra theory provided only a slightly better fit. Although both theories yielded identical results within experimental error, we subsequently used those parameters obtained by the latter higher approximation method (unless specifically stated otherwise).

In paper I[3] it was shown that, if the Zimm–Bragg parameters of the guest residue are close in magnitude to those of the host residue, the approximate theories are valid. This was further verified in paper II,[4] in which copolymers of PHPG

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(21) H. A. Scheraga, "The Proteins," Vol. 1, H. Neurath, Ed., Academic Press, New York, N. Y., 1963, p 477.

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

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

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

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

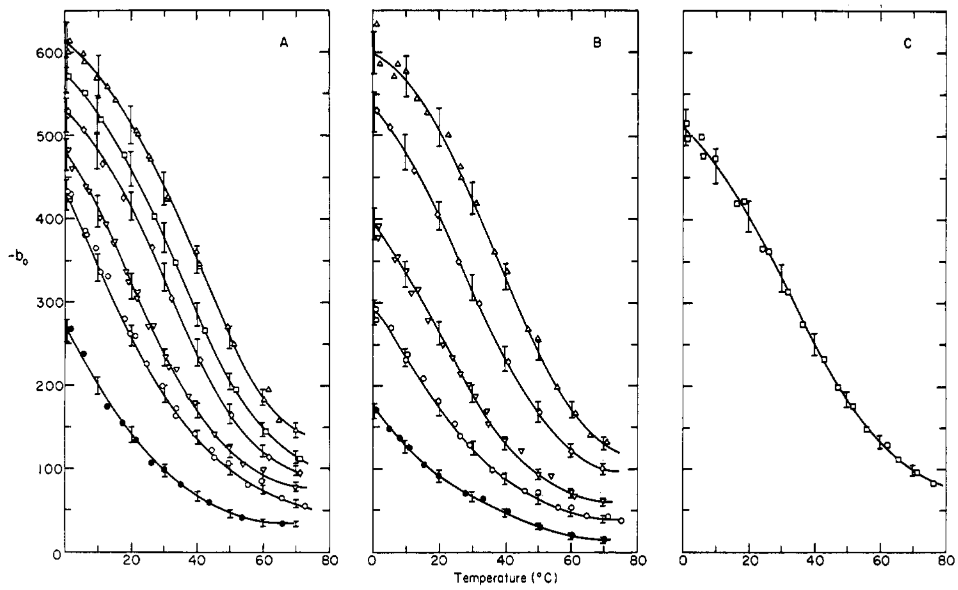

Figure 5. Temperature dependence of b_0 for P(HPG-Ala) copolymers in water: (A) ●, PHPG, $\overline{DP}_w$ = 360 (fraction IIID of paper II[4]); ○, 14.1% Ala, $\overline{DP}_w$ = 422 (fraction VIB); ∇, 20.6% Ala, $\overline{DP}_w$ = 880 (fraction VIIB-2); ◇, 32.0% Ala, $\overline{DP}_w$ = 1010 (fraction VIIIB); □, 38.3% Ala, $\overline{DP}_w$ = 1413 (fraction IXB-2); Δ, 49.1% Ala, $\overline{DP}_w$ = 1102 (fraction XE); (B) ●, PHPG, $\overline{DP}_w$ = 120 (fraction IIC of paper II[4]); ○, 11.0% Ala, $\overline{DP}_w$ = 165 (fraction VIE); ∇, 19.5% Ala, $\overline{DP}_w$ = 322 (fraction VIIE); ◇, 30.4% Ala, $\overline{DP}_w$ = 53.6 (fraction VIIIE); Δ, 44.1% Ala, $\overline{DP}_w$ = 1322 (fraction XC); (C) □, 38.1% Ala, $\overline{DP}_w$ = 365 (fraction IXD). The points are experimental ones and the lines represent the smoothed experimental curves. The experimental errors (arising from errors in the determination of the concentration, in the choice of the values of b_0 for the fully helical and fully coil conformations, and of the slope of the Moffitt-Yang plot) are indicated by the error symbols.

TABLE IV

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

L-Alanine content, mol %	$\overline{DP}$	Temp, °C	$\theta_{h,theor}$			
			Lifson[b]	Lehman-McTague[b]	Allegra[c]	Lehman-McTague[c]
11.0	200	0	0.440	0.436	0.439	0.439
		30	0.175	0.173	0.175	0.175
		60	0.074	0.073	0.073	0.074
30.4	200	0	0.590	0.582	0.589	0.589
		30	0.335	0.327	0.335	0.335
		60	0.133	0.132	0.133	0.133
49.1	200	0	0.682	0.676	0.684	0.684
		30	0.491	0.481	0.493	0.494
		60	0.210	0.208	0.211	0.212

[a] The parameters for the hydroxypropylglutamine were taken from Table II of paper II[4] and are ΔH = 168 cal/mol, T_{tr} = −0.7°, σ = 2.2 × 10^{-4}. [b] The parameters used for L-alanine were obtained by fitting the data by the Lifson theory, as shown in Table V. [c] The parameters used for L-alanine were obtained by fitting the data by the Allegra theory, as shown in Table V.

and PHBG were analyzed. The Lifson method fit the data quite well, and the Allegra method provided no improvement. The difference between the transition temperatures of these two homopolymers was only 38.5°, indicating that the values of σ and s of the guest and host were roughly close in magnitude. In the present paper, L-alanine replaces the PHBG guest of paper II.[4] Since it is found that the transition temperatures of the guest and host differ by 71.9°, the Zimm-Bragg parameters for the guest and host would again be close in magnitude, and we would expect the low order of approximation to be valid. However, the improvement obtained with the Allegra method over that of Lifson is somewhat greater in the case of the L-alanine guest compared to the PHBG guest because there is a greater difference in the values of σ and s for the guest and host in the case of L-alanine.

The copolymer melting data were analyzed as described in paper II.[4] First, treating σ as temperature independent, several trial values of σ were assumed and s was calculated by fitting the theoretical values of θ_h to the experimental ones for all copolymers at each temperature. A measure of the goodness of fit is the quantity τ defined as in paper II,[4] *viz.*

$$\tau = \sum_T \sum_i [(\theta_{h,exptl})_{i,T} - (\theta_{h,calcd})_{i,T}]^2 \qquad (1)$$

where $(\theta_{h,exptl})_{i,T}$ and $(\theta_{h,calcd})_{i,T}$ are the experimental and calculated values, respectively, of θ_h for a copolymer fraction i at a temperature T. The best fit for the data of all copolymer fractions at all temperatures T is determined by minimizing τ. Figure 7 shows that the minimum in τ is obtained when σ is equal to 0.0008 ± 0.0002 (by both the Lifson and the Allegra theories). The error in σ was determined by repeating the above procedure, *i.e.*, by finding the best fit value of σ for the two sets of data characterized by $\theta_{h,max}$ and $\theta_{h,min}$, respectively, where

$$(\theta_{h,max})_{i,T} = (\theta_{h,exptl})_{i,T} + (\Delta\theta_h)_{i,T} \qquad (2)$$

$$(\theta_{h,min})_{i,T} = (\theta_{h,exptl})_{i,T} - (\Delta\theta_h)_{i,T} \qquad (3)$$

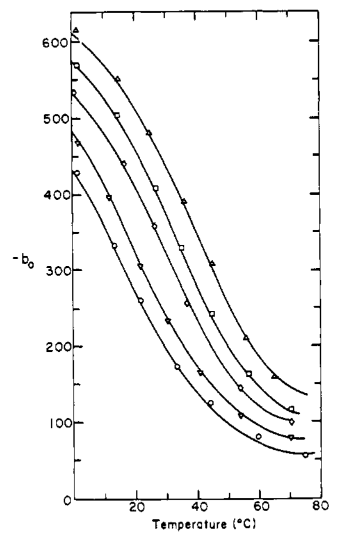

Figure 6. Demonstration of the reversiblity of the helix-coil transition for five representative cases. The curves were obtained during the heating part of the cycle and the points during cooling: O, 14.1% Ala, $\overline{DP}_w$ = 422 (fraction VIB); ∇, 20.6% Ala $\overline{DP}_w$ = 880 (fraction VIIB-2); ◇, 32.0% Ala, $\overline{DP}_w$ = 1010 (fraction VIIIB); □, 38.3% Ala, $\overline{DP}_w$ = 1413 (fraction IXB-2); Δ, 49.1% Ala, $\overline{DP}_w$ = 1102 (fraction XE).

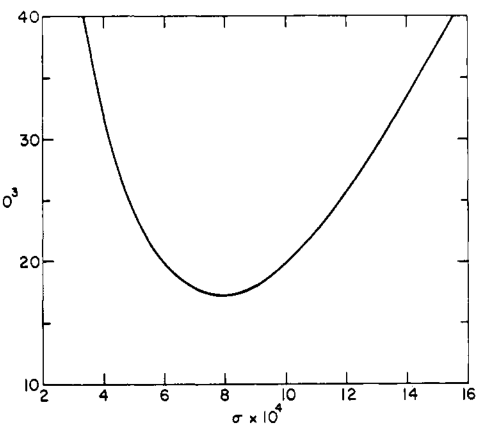

Figure 7. Determination of the best temperature-independent value of σ as the one which corresponds to the lowest value of τ for the P(HPG-Ala) copolymers, using the Lifson theory. The Allegra theory gives the same result.

where $(\Delta\theta_h)_{i,T}$ represents *all* of the experimental errors for each copolymer fraction i at temperature T, *i.e.*, those which affect $(\theta_{h,exptl})_{i,T}$ directly and those which affect only $(\theta_{h,calcd})_{i,T}$. It should be noted that no account was taken of the error in the Zimm-Bragg parameters of the PHPG host.

The values of s, computed from both the Lifson and Allegra theories using the above value of σ, are given in Table V. Figure 8 illustrates the temperature dependence of s. The errors in the values of s (indicated by the vertical error symbols in Figure 8) were determined by holding σ constant at its optimal value and finding the best fit values of s for the two sets of data characterized by $\theta_{h,max}$ and $\theta_{h,min}$, respectively. The standard deviations in s from the best fit value of s at each temperature (indicated by the standard deviation symbols in Figure 8) were calculated by keeping σ at its optimal value, analyzing each of five pairs of alanine copolymers separately for s (each pair having approximately the same alanine content but different DP's), and employing the usual procedure for computing the standard deviation. Obviously, from a comparison of the data of Table V with the error symbols shown in Figure 8, the values of s determined by the Lifson and the Allegra method agree well within the experimental error. Figure 9 compares the melting curves calculated with the Allegra parameters of Table V with the experimental results. The error symbols in Figure 9 correspond to the errors in the calculated values of θ_h arising from errors in the measurement of $\overline{DP}_w$ and in the amino acid analyses. These range from ±0.9 to ±6.8%, totally. It should be noted that the net error in θ_h is different for each $\overline{DP}_w$ and each temperature because of the intricate relationship between these various factors; also, there is an error associated with each of the experimental points of Figure 9 (*cf.* Figure 5). The agreement between the experimental data and the theoretical calculations is excellent. A few experimental curves are somewhat higher or lower than those obtained theoretically. In order to test the fit, from one to four of the experimental curves (in various combinations) were deleted in analyzing the data to determine σ and s. The Zimm-Bragg parameters determined by each set of experimental input curves were the same (far within experimental error). This demonstrates that, though

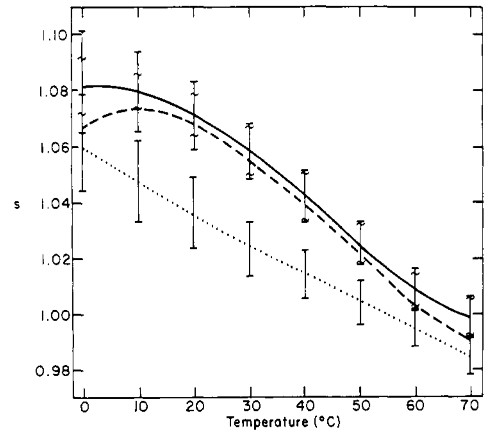

Figure 8. A plot of s *vs.* T for poly(L-alanine) in water. The solid line shows the values obtained from the Allegra analysis with σ = 0.0008, and the dashed line represents the best fit with a temperature-dependent σ. The dotted line shows the results of Ingwall, *et al.*,[7] obtained from block copolymers of the form (D,L-Lys)$_m$-(L-Ala)$_n$-(D,L-Lys)$_m$. The maximum and minimum limits of the fit are given by the vertical error symbols I, and the symbols ⌶ represent the standard deviations; both are computed as described in section IIIA.

TABLE V
VALUES OF THE ZIMM-BRAGG PARAMETER s FOR POLY(L-ALANINE) IN WATER FROM 0 TO 70°[a]

Temp, °C	s Lifson	s Allegra
0	1.07_8	1.08_1
10	1.07_6	1.07_9
20	1.06_8	1.07_1
30	1.05_5	1.05_8
40	1.04_0	1.04_2
50	1.02_4	1.02_5
60	1.00_7	1.00_8
70	0.99_8	0.99_9

[a] The value of σ, obtained from both the Lifson and Allegra theories, is 0.0008 ± 0.0002.

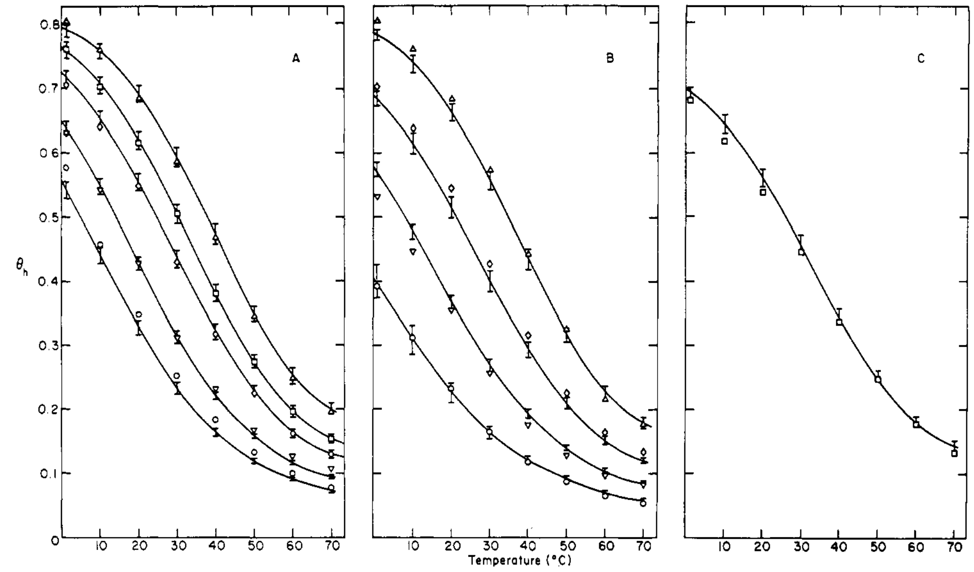

Figure 9. Comparison of calculated melting curves, obtained from the parameters of the Allegra theory for L-alanine given in Table V and those for PHPG of Table II in paper II,[4] with experimental points (obtained from the smoothed experimental data of Figure 5): (A) ○, 14.1% Ala, $\overline{\mathrm{DP}}_w$ = 422 (fraction VIB); ∇, 20.6% Ala, $\overline{\mathrm{DP}}_w$ = 880 (fraction VIIB-2); ◇, 32.0% Ala, $\overline{\mathrm{DP}}_w$ = 1010 (fraction VIIIB); □, 38.3% Ala, $\overline{\mathrm{DP}}_w$ = 1413 (fraction IXB-2); Δ, 49.1% Ala, $\overline{\mathrm{DP}}_w$ = 1102 (fraction XE); (B) ○, 11.0% Ala, $\overline{\mathrm{DP}}_w$ = 165 (fraction VIE); ∇, 19.5% Ala, $\overline{\mathrm{DP}}_w$ = 322 (fraction VIIE); ◇, 30.4% Ala, $\overline{\mathrm{DP}}_w$ = 536 (fraction VIIIE); Δ, 44.1% Ala, $\overline{\mathrm{DP}}_w$ = 1322 (fraction XC); (C) □, 38.1% Ala, $\overline{\mathrm{DP}}_w$ = 365 (fraction IXD). The error symbols indicate the errors in the calculated values of θ_h caused by errors in the measurement of $\overline{\mathrm{DP}}_w$ and in the amino acid analyses. It should be noted that errors (not shown in this Figure ; *cf.* Figure 5) are also associated with the experimental points.

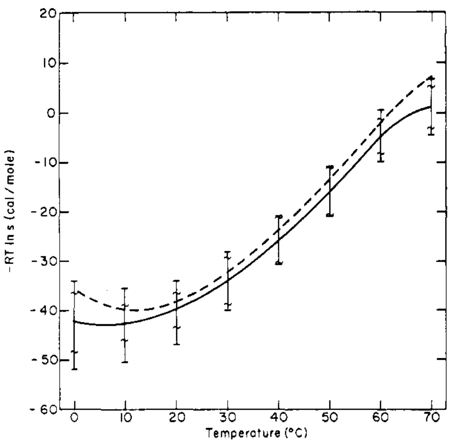

Figure 10. A plot of $-RT \ln s$ *vs.* T for poly(L-alanine) to assess the dependence of ΔG on T. The solid line shows the values obtained from the Allegra analysis with σ = 0.0008, and the dashed line represents the best fit with temperature-dependent values of σ. The maximum and minimum limits of the fit are given by the vertical error symbols ⌶, and the symbols ⌶ represent the standard deviations; both are computed as described in section IIIA.

one or more of the curves may be slightly in error, there is a distinct statistical advantage in analyzing ten independent melting curves.

Because there is some indication that σ should vary with temperature,[28] the experimental data were fit by allowing for a possible temperature dependence of σ. However, over the temperature range studied (0–70°), the departure of this temperature-dependent value of σ for poly(L-alanine) from the temperature-independent value was within the limits of experimental error, except at 0°, where σ = 0.0004. Figure 8 also shows that the values of s corresponding to the temperature-dependent values of σ are the same within experimental error as those determined with a best fit temperature-independent value of σ. Hence, the parameters obtained from the Allegra theory with a temperature-independent value of σ are those used to characterize L-alanine.

The temperature dependence of s can be used to calculate the thermodynamic quantities ΔG (the free energy), ΔH (the enthalpy), and ΔS (the entropy) for the conversion of a coil residue of L-alanine to a helical one at the end of a long helical sequence. Figure 10 shows a plot of ΔG ($= -RT \ln s$) *vs.* temperature. The vertical error symbols and the standard deviation symbols were calculated as described above for Figure 8. It should be noted that the free energy, determined with temperature-dependent values of σ, corresponds very well to that computed with a temperature-independent value of σ. The experimental errors make it somewhat difficult to determine ΔH and ΔS to a high degree of precision. However, if we assume that both are temperature independent, the data yield a least-squares line (weighting those terms with greater experimental error proportionately less by the function[29] $1/\delta_T{}^2$, where δ_T corresponds to the standard deviation in ΔG at each temperature T) whose slope is $-\Delta S$ and whose intercept (at T = 0°K) is ΔH. The transition temperature is then determined as the ratio between ΔH and ΔS. The values of these thermodynamic quantities are listed in Table VI. The errors in ΔH and ΔS were determined by standard procedures.[29] It should be noted that the error in ΔG is *not* directly reflected by the sum of the errors in ΔH and ΔS, because the latter two quantities are not independent. The error in the transition temperature was determined by noting from Figure 10 the greatest displacement, at ΔG = 0 along the temperature axis, for lines drawn by taking the experimental error into account.

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(28) M. Gō, N. Gō, and H. A. Scheraga, *J. Chem. Phys.*, **52**, 2060 (1970); **54**, 4489 (1971).

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

TABLE VI
THERMODYNAMIC PARAMETERS FOR L-ALANINE

$\Delta G_{20°}$, cal/mol	-40.0 ± 6.5
ΔH, cal/mol	-242 ± 21
ΔS, eu	-0.703 ± 0.067
T_{tr}, °C	71.2 ± 4.5
σ[a]	0.0008 ± 0.0002

[a] σ is considered to be independent of temperature.

(B) Implications. The analysis of the experimental data presented above provides a quantitative basis for the qualitative observations concerning the helix-forming capacity of the L-alanine residue in aqueous solution.[7,30-35] Further, the magnitudes of the thermodynamic parameters determined by the host-guest technique correspond quite well to those determined by Ingwall, *et al.*,[7] using block copolymers of the form (D,L-Lys)$_m$-(L-Ala)$_n$-(D,L-Lys)$_m$. In order to make a valid comparison, the Lifson-Roig statistical weights, determined by Ingwall, *et al.*,[7] were converted to Zimm-Bragg statistical weights by the procedure described by Gō, *et al.*[36] The s *vs.* T curve, determined from the experimental data of Ingwall, *et al.*,[7] is shown by the dotted line in Figure 8. The errors were assumed to correspond to those calculated in this study. The agreement between the two curves is almost within experimental error. After conversion of the data of Ingwall, *et al.*,[7] to the Zimm-Bragg formulation,[36] $\Delta H = -195$ cal/mol, $\Delta S = -0.60$ eu, $T_{tr} = 52.0°$. Their Lifson-Roig value of $v = 0.012$ corresponds to a Zimm-Bragg value of $\sigma = 0.00014$.[36] The fact that these two independent methods yield similar quantitative results supports the view that the conformational state of an amino acid residue (except possibly proline) in a polypeptide or protein is essentially independent of the chemical nature of its neighbors. The parameters obtained for a given guest thus seem to be independent of the nature of the host. This assumption can be tested further in the future when more amino acids can be characterized as guests by the technique used here and then be used as hosts with other characterized residue types.

These thermodynamic parameters are also in agreement with those of Sugiyama and Noda,[32] who found that $\Delta G°_{25°} = -50$ cal/mol (for alanine in 0.06 M NaBr), from potentiometric titration of random copolymers of L-lysine and L-alanine. Correspondingly, $\Delta G_{25°} = -17$ cal/mol by the block copolymer method and -37 cal/mol by the method presented here.

The copolymers considered here had particular experimental advantages. It was possible to synthesize and characterize a series of copolymers containing a wide range of alanine, from 10 to 50%. Although the content of the guest does not affect the validity of the determination of σ and s in the range of temperatures studied, the presence of a high percentage of the guest residue in the copolymer makes it possible to extrapolate the data beyond the temperature range studied with some degree of confidence. Moreover, the transition temperature of poly(L-alanine) was found to be within the range of temperatures studied, further increasing the validity of the applicability of the parameters, determined at this temperature, to the pure homopolymer. The presence of a high percentage of the guest in the copolymers also makes it possible to determine σ with much more precision. The glycine copolymers studied in paper III[5] had a very low guest content; this, together with the fact that glycine is a strong helix breaker, makes the nucleation of helical regions in the glycine portions of the random copolymer an extremely improbable event. However, in copolymers containing up to 50% alanine (a strong helix former) as the guest, such an event is more likely, and the resultant determination of the nucleation parameter is more accurate.

The fact that copolymers of such widely varying content of the guest residue were available makes it further possible to demonstrate that the statistical weights of the host and guest residues are essentially independent of the nature of their neighbors. To support this presumption, the Zimm-Bragg parameters were determined by fitting the ten copolymers in separate pairs, each pair consisting of a low- and a high-$\overline{\mathrm{DP}}_w$ copolymer of a given alanine content, ranging from 10 to 50% alanine in increments of about 10%. Analysis of each of the five different guest/host ratio copolymers yielded the same values of σ and s within experimental error as the average (Allegra) result given above.

While not shown here (in a figure comparable to Figure 9), the Lifson theory was also found to give a very good fit of the experimental data. This was to have been expected,[3] since the magnitudes of σ and s of the host and guest were similar.

A comparison of the parameters determined for L-alanine with those found in paper III[5] for glycine provides insight into the effect of side chains on σ and s. The fact that s is greater than unity for poly(L-alanine) at temperatures up to 71.2° indicates that it is a helix former in this temperature range. In contrast, the value of s for polyglycine is always less than 0.7 for all temperatures studied, proving that it is quite unstable in the helical conformation. The greater stability of the right-handed α-helical form of poly(L-alanine), compared to that of polyglycine, had been attributed earlier[37,38] to a β_1-α_4 type of hydrophobic bond in poly(L-alanine). However, Gō, *et al.*,[28] showed that, whereas such a hydrophobic bond contributes to the stability of the helical form of poly(L-alanine), by far the greater contribution arises from short-range interactions, mainly from the side chain-backbone interactions in poly(L-alanine) which are absent in polyglycine. The short-range interactions affect both s and σ. In the case of s, there is almost no difference between ΔS_s for polyglycine and poly(L-alanine); so, the greater stability of the right-handed α-helical conformation of the latter must be attributed to the difference found for ΔH_s, arising mainly from short-range side chain-backbone nonbonded interactions (primarily between residues separated by two and three residues, respectively, along the chain, but near each other in space because of the α-helical structure).[28] In the case of σ, the difference between polyglycine and poly(L-alanine) is attributed to both ΔH_σ and ΔS_σ, which depend on short-range interactions between a given residue and up to three intervening residues in the helix and coil, respectively. β_1-α_4 hydrophobic bonds in the α helix do not affect the value of σ, and their effect on s is negligibly small in comparison with the effect of the binding of water to the CO and NH groups in

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the coil state;[28] *e.g.*, from Table VIII of the second paper of ref 28, $\Delta H_s - T\Delta S_s$ = 1.07 and −0.02 kcal/mol for polyglycine and poly(L-alanine), respectively, at 300°K, and the contribution of the β_1–α_4 hydrophobic bond to the free energy of the coil-to-helix transformation in poly(L-alanine) is only −0.2 kcal/mol, an amount which affects the transition temperature of poly(L-alanine) but not the fact that the poly(L-alanine) helix is *much* more stable than the polyglycine one, even without the contribution from the hydrophobic bond.

Finally, because of experimental error, we cannot verify the prediction of Gō, *et al.*,[28] that *s* for poly(L-alanine) in water should decrease slightly at low temperature.

(IV) Conclusions

Water-soluble random copolymers containing L-alanine and N^5-(3-hydroxypropyl)-L-glutamine were synthesized and characterized. From an analysis of the thermally induced helix–coil transition of these copolymers, the Zimm–Bragg parameters σ and *s* for poly(L-alanine) in water were deduced. The relatively high magnitude of *s* in the temperature range from 0 to 71° indicates that the L-alanine residue is a helix former. The magnitudes of the thermodynamic parameters determined here by the host–guest technique agree quite well with those determined by Ingwall, *et al.*,[7] who used block copolymers. The fact that the two independent methods yield similar quantitative results supports the assumption that the conformational state of an amino acid residue in a polypeptide or protein is essentially independent of the chemical nature of its neighbors in a given solvent. Also, by separately analyzing each of five pairs of alanine copolymers (each pair having the same alanine content but different DP's), it was demonstrated that the parameters determined for the guest residue are independent of the nature of the neighboring residue. The greater stability of the α-helical form of poly(L-alanine) compared to polyglycine can be attributed primarily to the side chain–backbone nonbonded interactions in the former.[28]

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

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ABSTRACT: Water-soluble random copolymers containing L-serine and N^5-(4-hydroxybutyl)-L-glutamine were prepared by copolymerization of the *N*-carboxy-α-amino acid anhydrides of *O*-trimethylsilyl-L-serine and γ-benzyl L-glutamate, followed by amidolysis with 4-amino-1-butanol. The copolymers were fractionated and characterized, and their thermally induced helix–coil transition curves determined from optical rotatory dispersion measurements. From an analysis of these curves, using an approximate theory for random copolymers and the host–guest technique, the Zimm–Bragg parameters σ and *s* (which characterize the (hypothetical) helix–coil transition of poly-L-serine in water) were obtained. The transition curves and the resulting values of σ and *s* demonstrate quantitatively that L-serine is a helix-breaking residue in water in the temperature range of 0–80°. The conformational behavior of L-serine residues in proteins is discussed.

In this series of papers,[3–6] it has been shown that an experimental evaluation of the Zimm–Bragg[7] helix–coil stability constants, σ and *s*, for the naturally occurring amino acids in water provides information about the conformational behavior of the amino acid residues in proteins in aqueous solution. It is therefore of considerable importance to obtain these parameters in order to understand the factors which influence the folding of a polypeptide chain into the native conformation of a protein.

Since it is not possible to investigate the helix–coil transition for homopolymers of all amino acids in water, for reasons cited earlier, resort is had to the use of random copolymers and the host–guest technique,[3–6] in which the host is a homopolymer of either N^5-(4-hydroxybutyl)-L-glutamine (HBG) or N^5-(3-hydroxypropyl)-L-glutamine (HPG) and the guest residue (which can be any amino acid) is introduced to form a two-component random copolymer. Suitable fractionation provides a series of random copolymers, each of which has a defined average composition and chain length. Such copolymers are soluble in water, partially α-helical, and melt in the temperature range of 0–100°, if the composition of the guest residues is not too high. From an analysis of the helix–coil transition curves for the homopolymers PHBG or PHPG and for the copolymers P(HBG–X) or P(HPG–X), it is possible[3–6] to obtain the values of σ and *s* for the guest residue X.

This technique has already been applied to glycine[5] and L-

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