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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alanine,[6] and is extended here to the guest residue L-serine, with the helix-forming HBG selected as comonomer. Thus, even though poly(L-serine) (PLS) is insoluble in water and tends to form β structures,[8] the host–guest technique permits an evaluation of σ and s for L-serine, provided that the serine content of the polymers is low enough so that the α-helical conformation is maintained. In this way, information is obtainable about the tendency of L-serine either to disrupt or enhance α-helix formation. From the results obtained here, it will be seen that L-serine, like glycine,[5] must be classed as a helix breaker, and the numerical values of σ and s provide a quantitative measure of this helix-breaking tendency. The structural features of L-serine, in particular the side-chain hydroxyl group, which endow it with these properties will be discussed.

Individual serine residues are distributed over both the helical and coil regions in globular proteins; *e.g.*, in lysozyme[9-11] it occurs (1) at either and sometimes at both termini of helical regions (Ser 24 and Ser 36, respectively, in one helix), (2) in the middle of a helical section (Ser 91), and (3) in a nonhelical region (Ser 50). It also appears in β structures in globular and fibrous proteins[11] and in the active sites of certain proteases and esterases.[12] Presumably, its various roles are related to the multiple possibilities for hydrogen bond formation between the side-chain hydroxyl groups and peptide or side-chain groups within the same molecule, or with groups in neighboring chains.

An appreciable number of investigations of synthetic homopolymers and copolymers, containing L-serine and D,L-serine have been carried out.[8,13-25] However, aside from the designation of L-serine as an α-helix breaker,[26-29] all of these have been concerned primarily with the role of L-serine in β structures, rather than its behavior as an α-helix former or α-helix breaker, which is the major interest in this paper.

In the present paper we describe in section I the synthesis of the water-soluble copolymer of N^5-(4-hydroxybutyl)-L-glutamine[30] and L-serine. In section II, we discuss the characterization of these copolymers including the determination of the helix–coil transition curves in water, and in section III an appropriate theory[3] is applied to determine σ and s for L-serine from the transition curves. The conformational role of serine in proteins is then discussed.

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

As in previous polymerizations,[8,13-16,17-19] it was necessary to pay particular attention to the use of an appropriate blocking moiety for the serine hydroxyl group and to avoid racemization during the synthesis of the random copolymers.

The polymers were prepared by copolymerization of the *N*-carboxy-α-amino acid anhydrides (NCA) of *O*-trimethylsilyl-L-serine and γ-benzyl L-glutamate; the resulting copolymers were then converted to the hydroxybutylglutamine derivatives by treatment with hydroxybutylamine (4-amino-1-butanol) and isolated under conditions which simultaneously ensured deblocking of the seryl hydroxyls.

(A) Materials. The solvents used for the synthesis and recrystallization of γ-benzyl L-glutamate NCA and for polymerizations were purified shortly before use. Dioxane, hexane, and benzene were distilled over sodium, 4-amino-1-butanol (Chemical Procurement Laboratories, Inc.) was dried over barium oxide and distilled under reduced pressure, and triethylamine (TEA) was dried and distilled over KOH. Ethyl acetate was dried with calcium sulfate and distilled.

Absolute ethanol (U.S.P., N.F., Industrial Chemicals) and "spectranalyzed grade" methanol (Fisher Scientific Co.) were used without further purification. 2,2,2-Trifluoroethanol (from Matheson Coleman and Bell) was treated with $NaHCO_3$ and distilled from Drierite.

The dichloroacetic acid (Fisher) used for viscosity measurements and the analytical reagent grade anhydrous diethyl ether (Mallinckrodt) used in the fractionation were not purified further.

L-Glutamic acid (Aldrich Chemical Co.) and D,L-serine (Schwarz/Mann) were both used without further purification. The *O*-trimethylsilyl-L-serine NCA was a generous gift from Dr. Ralph Hirschmann[31] of Merck Sharpe and Dohme, Rahway, N. J. Poly-[N^5-(4-hydroxybutyl)-L-glutamine] (PHBG) of $\overline{DP}_w$ = 200 was fraction no. VIB of ref 4.

(B) Synthesis of Copolymers. γ-Benzyl L-glutamate was prepared from L-glutamic acid and treated with phosgene to yield the NCA.[5,32] It was recrystallized repeatedly from ethyl acetate–hexane until the chloride content was less than 0.01%. The product was obtained as white needles (mp 93°).

The two-step synthesis of the water-soluble copolymers of hydroxybutylglutamine and L-serine was carried out according to the following scheme.

$$\gamma\text{-benzyl L-glutamate NCA} + \textit{O}\text{-trimethylsilyl-L-serine NCA} \longrightarrow \text{poly[}\gamma\text{-benzyl L-glutamate-}\textit{co}\text{-trimethylsilyl-L-serine)} \quad (1)$$

$$\text{poly(}\gamma\text{-benzyl L-glutamate-}\textit{co}\text{-trimethylsilyl-L-serine)} + \text{4-amino-1-butanol} \longrightarrow \text{poly[}N^5\text{-(4-hydroxybutyl)-L-glutamine-}\textit{co}\text{-L-serine]} \quad (2)$$

The experimental details are presented below.

Poly(γ-benzyl-L-Glu$^{m'}$-*O*-TMS-L-Ser$^{n'}$), [P(BzG–TMS-Ser)], Copolymers (II–VI) (TMS, trimethylsilyl). Random copolymers of γ-benzyl L-glutamate and *O*-TMS-L-serine were prepared by the polymerization of mixtures of the two corresponding NCA's (of various mole ratios) in dioxane, using triethylamine as the initiator (A/I = 25). The yields, based on the NCA's, varied from 60 to

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TABLE I
COMPOSITIONS AND CHAIN LENGTHS OF THE UNFRACTIONATED HOMO- AND COPOLYMERS

	Polymers containing γ-benzyl L-glutamate			Polymers containing N^5-(4-hydroxybutyl)-L-glutamine		
L-Serine content of reaction mixture, mol %	L-Serine content found, mol %	Av mol wt[a] × 10^{-3}	$\overline{DP}$	L-Serine content found, mol %	Wt av mol wt[b] × 10^{-3}	$\overline{DP}_w$
0	(I)	600	1500			
7	(II)	269	1270	5.4 (VIII)	90.5	466
10	(III)	311	1500	8.9 (IX)	107	562
19	(IV)	259	1290	13.8 (X)	86.3	464
30[c]	22.9 (V)	370	1990	25.1		
50[c]	(VI)	(112)	(640)[d]	(34)[e] (XI)	(9.3)	(59)
100	(VII)		Insoluble[f]			

[a] By viscometry, using the relation of Fujita, *et al.*,[33] for polymers in DCA. It is assumed that DCA deblocks the trimethylsilyl group. [b] By conventional sedimentation equilibrium. [c] These polymers were prepared in our initial survey of the composition range for helix formation but were not investigated further because the serine content was too high to enable us to conclude with confidence that only α-helical and coil conformations were present. [d] Estimated from η_{sp}/c at 0.2% concentration. [e] Uncorrected for possible serine destruction. [f] Prepared with sodium methoxide (A/I = 200) in benzene at 25°.

90%. A typical polymerization was carried out as follows. Triethylamine (0.040 g, 0.4 mmol) was added to a solution of γ-benzyl L-glutamate NCA (2.45 g, 9.3 mmol) and *O*-trimethylsilyl-L-serine NCA (0.14 g, 0.7 mmol) in dioxane (100 ml). The reaction mixture was protected from atmospheric moisture with a $CaCl_2$ drying tube and kept at room temperature for 3 days with occasional shaking. The resulting viscous, slightly turbid solution was introduced slowly into vigorously stirred absolute ethanol (700 ml), and stirring was continued for an additional 30 min. The white, fibrous precipitate was then collected on a sintered-glass filter, washed with several 50-ml portions of absolute ethanol, and then dried *in vacuo* over P_2O_5. The yield was 1.70 g (80%). The compositions of the copolymers are given in Table I. (Average molecular weights of polymers containing γ-benzyl L-glutamate were determined by viscometry; see ref 33.)

Poly[N^5-(4-hydroxybutyl)-L-Gln^m-L-Ser^n], [P(HBG–Ser)], Copolymers (VIII–XI). The copolymers II–VI were treated with 4-amino-1-butanol to convert them to the corresponding water-soluble copolymers (VIII–XI). A typical reaction was carried out as follows: The P(BzG–TMS-Ser) copolymer (1.5 g) was dissolved with mechanical stirring in freshly distilled dioxane (8 ml) at 60° to give a colorless, viscous solution, and 4-amino-1-butanol (15 ml) was added in 3-ml increments over a period of 4 days. Stirring was continued at 60° in a closed system for a total of 5 days, after which time a sample of the reaction mixture gave no precipitate when diluted with water—the criterion used for completion of the reaction. The viscous, homogeneous mixture was then poured into well-stirred 5% (v/v) acetic acid (250 ml). The resulting clear solution was dialyzed exhaustively against distilled water, filtered through a Millipore filter (0.45-μ pore size), concentrated to about 30 ml *in vacuo*, and then lyophilized. The resulting white product was dried under high vacuum over P_2O_5, at room temperature. The yield here was 1.25 g (90%), and, in general, it ranged from 70 to 90%. The compositions of the copolymers are given in Table I.

(C) Removal of Trimethylsilyl Blocking Group. The $-Si(CH_3)_3$ blocking group was removed at the same time that the benzyl group was replaced by hydroxybutylamine, in the exposure to both 1 *N* acetic acid and water. Since the oxygen–silicon bond is extremely susceptible to cleavage by protic solvents, the trimethylsilyl group is already partially removed in the isolation of P(BzG–TMS-Ser) when the polymer is introduced into ethanol (which may contain traces of water). For this reason, analytical data on silicon content could not be used to compute the serine composition of the copolymers. However, the absence of silicon ($<\pm 0.4\%$) from the water-soluble copolymers served as evidence that the removal of the $-Si(CH_3)_3$ group from the serine side chains was complete.

Analyses for both Si and ash (SiO_2) were performed by Crobaugh Laboratories, Cleveland, Ohio, and Galbraith Laboratories, Inc., Knoxville, Tenn. The errors in these analyses are $\pm 0.4\%$ for Si and $\pm 0.2\%$ for SiO_2, the latter being a more accurate measure of silicon content, especially for samples with low silicon content. The analyses were performed on the various intermediates, as well as on the final water-soluble copolymers. These materials were dried *in vacuo* over P_2O_5 at 60°C for 24 hr before being submitted for analysis.

(D) Spectral Analysis. Proof that the benzyl group was completely replaced by hydroxybutylamine was obtained from the ultraviolet absorption spectra of the aqueous copolymer solutions. Using this technique, only samples having $<1\%$ benzyl absorption at 257 mμ were used in the present study. On this basis, the water-soluble copolymer from sample V was eliminated.

(E) Fractionation. The copolymers VIII–X were fractionated by a procedure similar to that described in paper II,[4] using methanol and ether. The fractions were dissolved in water, lyophilized, and dried *in vacuo* over P_2O_5.

(F) Determination of Composition. The amino acid compositions of the copolymers V, VIII–XI, and subsequently the fractions obtained from these, were determined with a Technicon amino acid analyzer, with a pH gradient from 2.8 to 6.1.

It has been reported[34] that the presence of serine and glutamic acid in a hydrolysate leads to problems in the amino acid analysis because (1) the absorption peaks of the two ninhydrin derivatives are near each other, (2) there is a possibility of ester formation between the γ-carboxyl group of glutamic acid and the hydroxyl group of serine,[35] and (3) there is a possibility that serine may be oxidized to pyruvic acid[8] upon prolonged treatment with concentrated HCl. In order to minimize these difficulties, the following procedure was used. The samples (5–10 mg) were introduced into Pyrex ampoules (0.8 × 13.0 cm) together with 0.1 ml of 0.1 *M* phenol (which inhibits oxidative degradation) and 1.00 ml of constant boiling (6.25 *N*) HCl. The ampoules were sealed, heated in an oven at 105° for 20–24 hr, and cooled to room temperature, and the contents were transferred quantitatively to a 100-ml round-bottomed flask for *immediate* removal of the solvent *in vacuo*. The residue was dried under high vacuum over KOH pellets for several hours and then dissolved in 25.0 ml of 12.5% sucrose solu-

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TABLE II
CHANGE IN RELATIVE AMOUNTS OF L-SERINE AND L-GLUTAMIC ACID UNDER HYDROLYSIS CONDITIONS[a]

Hydrolysis time, hr	Av % serine[b]
Standard solution[c]	50.0 ± 0.0
0[d] (original stock solution)	48.7 ± 0.9
6	50.5 ± 0.1
12[e]	51.4 ± 0.7
12[e]	49.7 ± 1.3
18	50.8 ± 0.5
24	49.4 ± 0.1
36	49.2 ± 0.0
48	49.1 ± 0.4
72	48.4 ± 0.3
96[e]	47.2 ± 1.1
96[e]	46.5 ± 1.0
120	44.3 ± 0.1

[a] 6.25 *N* HCl at 105°, in presence of phenol. [b] From duplicate analyses on each ampoule. [c] The standard solution contained 0.5 μmol each of L-Glu and L-Ser per milliliter of 12.5% sucrose solution. It was not exposed to HCl. [d] Zero-time sample, unheated. The stock solution contains 10 μmol each of L-Glu and L-Ser per milliliter of 6.25 *N* HCl. [e] Two ampoules were analyzed after this particular incubation time.

tion; 0.5-ml portions were subsequently injected into the amino acid analyzer.

In order to obtain accurate, reproducible results and to prove that no degradation of serine had taken place, it was necessary to pay particular attention to several aspects of the procedure outlined above. First, it was necessary to *completely* remove the HCl from the hydrolysis mixture after no longer than about 20–24 hr in order to avoid the formation of a ninhydrin-positive impurity detectable in the amino acid chromatograms. The impurity is presumed to be *O*-(γ-L-glutamyl)-L-serine previously observed in serine analyses;[35] since the formation of the impurity was prevented in the procedure described above, its identity was not established.

Second, with the pH gradient used in the amino acid analyses and with the selection of appropriate concentrations, it was possible to separate the ninhydrin derivatives of serine and glutamic acid satisfactorily.

Third, the stability of serine was established by preparing a stock solution of 1:1 molar ratio mixture of L-serine and L-glutamic acid (both at 1 mmol/100 ml in 6.25 *N* HCl), adding 1-ml portions to separate ampoules, and determining their compositions after various times under the hydrolysis conditions (6.25 *N* HCl at 105°C, in the presence of phenol). From the data of Table II, it appears that the relative amounts of serine and glutamic acid remain constant (and equal to that of the original stock solution) for at least up to 24 hr under hydrolysis conditions. Thus, the hydrolysis time of the copolymers never exceeded 24 hr.[36] Completion of the hydrolysis of the copolymers within this time was assured by the optical clarity of the hydrolysates and by the absence of extraneous oligopeptide peaks in the chromatograms. The average experimental error in the compositions is taken as ±4%.

While the procedure described above could be used for the water-soluble copolymers, difficulties were encountered with the water-insoluble blocked copolymers II–VI. Samples containing less than 10% serine were very resistant to hydrolysis with 6 *N* HCl, even after 4 days at 105°. Even the homopolymer poly(γ-benzyl L glutamate) (PBLG) is highly resistant to 6 *N* HCl under these conditions. Presumably, at higher serine content, there is a larger number of labile peptide bonds (Ser–Ser, Ser–Glu, Glu–Ser), and hydrolysis at these sites leads to the formation of more soluble and thus more easily hydrolyzed oligopeptides (peptide bonds involving serine and threonine residues are reported to be among the most readily hydrolyzed[37]). For these reasons, only one water-insoluble copolymer of high serine content (V) was analyzed, the results for duplicate analyses being 22.6 and 23.2%.

(G) Optical Purity of P(HBG–Ser) Copolymers. Although the highly basic conditions which led to partial racemization of seryl residues in previous syntheses[8,15] were avoided, it was nevertheless necessary to check for the presence of D residues in the starting materials and for racemization in the synthetic procedure. For this purpose, the Manning–Moore method,[38] involving the preparation of L-Leu-L-Ser and L-Leu-D-Ser dipeptides from the amino acid hydrolysates and the subsequent chromatographic separation of these dipeptides, using a sodium citrate elution buffer at pH 3.20, was applied. Dipeptide standards were prepared from D,L-serine. In addition, one of the copolymer fractions was hydrolyzed in tritiated HCl[39,40] to determine whether any racemization occurs in the hydrolysis procedure.

(H) Determination of Concentration. Polymer solutions of approximately the desired concentration were prepared just prior to use by adding the weighed amount of sample to the measured amount of distilled, deionized water. Each solution was filtered through a Millipore filter (0.45 μ). Accurate concentrations of each solution were then determined by micro-Kjeldahl nitrogen analysis,[41] together with data on the amino acid composition of the copolymer. For nonaqueous solutions, the organic solvents were evaporated prior to the nitrogen analysis. The overall error in the concentrations of the water-soluble P(HBG–Ser) copolymers determined on the freshly prepared solutions at least in triplicate and usually on two triplicate sets is taken as ±3%. For the water-insoluble and less hygroscopic P(BzG–TMS-Ser) polymers, solutions were prepared on the basis of the dry weight of the sample.

(I) Viscosity. The intrinsic viscosities of the copolymers II–VI were determined with a Cannon–Ubbelohde semimicro dilution viscometer using DCA at 25.0 ± 0.01°. The solvent flow time was 120 sec, and the relative viscosities of the solutions were kept in the range of 1.2–1.7. Since the viscosity data were used only as a rough guide in the synthetic procedure, it did not matter that DCA might have interacted[8] with the seryl hydroxyl group. The solutions were used immediately after preparation.

(J) Determination of Chain Length. Rough estimates of the molecular weight of copolymers II–VI were made from the intrinsic viscosity–molecular weight relationship for PBLG,[33] which was assumed to be (only approximately) applicable to the copolymers, and these are shown in Table I. The data are useful only for semiquantitative comparison.

On the other hand, for the water-soluble P(HBG–Ser) copolymers, accurate weight-average molecular weights, $\bar{M}_w$, were obtained with a Spinco Model E ultracentrifuge and the conventional sedimentation-equilibrium procedure.[4,42] The molecular weights are accurate to within ±4%. The values of $\bar{M}_z$ were also determined, so that $\bar{M}_z/\bar{M}_w$ could be used as a measure of polydispersity. Partial specific volumes ($\bar{v}$) of 0.816[4,43] and 0.63[43] cm³/g were used for HBG and seryl residues, respectively.

(K) 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 and its CD attachment (Model 6001). Conventional procedures were used to obtain the data[4] and to calculate the

(36) It was subsequently found that phenol could be omitted if the hydrolysis time were limited to 20–24 hr. For example, the serine content of copolymer IX of Table I was 9.1 ± 0.1% when the hydrolysis was carried out in the presence of 0.1 *M* phenol and 8.7 ± 0.4 and 8.9 ± 0.3% for two separate hydrolysis experiments without added phenol. Thus, phenol was used only in some analyses, but all hydrolyses were carried out for 20–24 hr.

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TABLE III
CHARACTERIZATION OF THE FRACTIONATED COPOLYMERS

Fraction[a]	Wt of fraction, g	L-Serine content, mol %	Mean residue wt	$\bar{v}$, cm³/gm	$\bar{M}_w \times 10^{-3}$	$\bar{M}_z/\bar{M}_w$[b]	$\overline{DP}_w$
VIIIA	0.12	5.5	194				
VIIIB	0.27	4.8	195	0.81_2	82.7	1.19	424
VIIIC	0.16	5.2	194	0.81_2	87.1		449
VIIID	0.30	5.8	194	0.81_1	49.5	1.00	255
VIIIE	0.14	6.6	193				
IXA	0.18	7.7	192				
IXB	0.39	8.8	190	0.80_9	55.0	1.23	289
IXC-1	0.30	7.5	192	0.81_0	52.5	1.12	274
IXC-2	0.11	11.0	188	0.80_6	18.2	1.16	97
IXD	0.06	c					
XA	0.06	14.0	184				
XB	0.20	12.9	186				
XC	0.12	16.1	182	0.80_2	148	1.73	813
XD	0.36	13.4	185	0.80_4	85.1	1.40	460
XE	0.23	13.6	185	0.80_4	37.7		204

[a] Roman numeral corresponds to unfractionated sample in Table I, and the letter to the fraction obtained in the fractionation procedure (the Arabic numerals indicate those fractions obtained by refractionation). [b] Given only for fractions whose melting properties were analyzed. [c] This last fraction had a very low molecular weight and was not analyzed.

reduced mean residue rotation $[m']_\lambda$, values of the Moffitt–Yang parameters a_0 and b_0, and the molar ellipticity $[\theta]_\lambda$. The range of concentrations covered in the measurements was 0.025–0.45% (w/v). The solutions were filtered through Millipore filters (0.45 μ), and temperature control was maintained to $\pm 0.2°$ by water-jacketed quartz cells. The experimental error in b_0 arises predominantly from (1) the errors in the concentration determinations ($\pm 3\%$) and (2) in the evaluation of the slope of the Moffitt–Yang plot (with a relative error of $\pm 2.5/b_0$). The helix content, θ_h, contains an additional uncertainty arising from the value of b_0 assumed for the complete helix, which is estimated as 2–3%.

(II) Results

(A) Characterization of the Copolymers. Table I summarizes the average compositions and weight-average degrees of polymerization ($\overline{DP}_w$) of the unfractionated P(BzG–TMS-Ser) and P(HBG–Ser) copolymers, and Table III shows the data for the fractionated copolymers. The usual decrease in $\overline{DP}_w$ attributed to transaminolysis, upon conversion to the hydroxybutylglutamine polymers,[4–6,44] was observed for the serine copolymers, with the benzyl derivatives having $\overline{DP}_w$'s in the range of 640–1990 and the converted water-soluble derivatives in the range of 59–562.

Sample I of Table I is PHBG prepared under similar conditions as those used for the P(BzG–TMS-Ser) copolymers. It can be seen that high, and comparable, $\overline{DP}_w$'s were attained with samples I–V, *i.e.*, even when TMS-serine is incorporated, and the corresponding derivatives (VIII–X) were water soluble. Even for a very high serine content (sample XI), the sample is water soluble if the $\overline{DP}$ is low (59, in this case). The insolubility of sample VII in both water and in 1 *N* HCl (an effective deblocking agent for the TMS group) is consistent with the reported[8,17] water insolubility of *optically pure* poly(L-serine) and supports the assumption that the polymerization of the *O*-TMS-L-Ser NCA proceeds with retention of the L configuration. From the point of view of a suitable composition range for use in evaluating σ and s for serine from partially helical copolymers, preliminary experiments indicated that low serine contents (below 16%) were adequate, and no further effort was expended on the preparation of water-soluble copolymers of higher serine content.

The fractions prepared from a given sample exhibited random variations of serine content with molecular weight, the compositions of the fractions being similar to those of the unfractionated polymer from which they were prepared. Other than this, the only direct evidence we have for the absence of blocks, *i.e.*, departure from randomness in the copolymers, is the absence of indications of β structure from ORD and CD curves (given in section IIC). On the basis of ORD and CD measurements, it has been reported that β structures exist in solutions of serine oligomers of $\overline{DP} \sim 7$[20] and 20,[45] respectively; the latter sample was soluble only after heating to 70°. However, as pointed out earlier,[3] moderate departures from randomness will not affect the melting curves of the polymers.

The values of $\bar{M}_z/\bar{M}_w$ indicate that the VIII and IX series of fractions are fairly homogeneous but that the X series of fractions is less homogeneous. Copolymers with high $\overline{DP}$ and high serine content (*e.g.*, fractions XA and XB, and even IXB) tended to become partially insoluble after storage in the freeze-dried state at room temperature for about 6 months. Thus, fractions XA and XB and also the first and last fractions in each series were not investigated further. If the $\overline{DP}$ is low or moderate, the samples remain water soluble, even if the serine content is high (*e.g.*, fractions IXC-2 and XD). The concentration dependence of the molecular weight of two fractions (VIIID and IXC-1) was checked, and neither exhibited a concentration dependence in the range of 0.2–0.5% (w/v). As a result of the above behavior, the only data used in the computations of σ and s were those obtained from measurements carried out before the samples were aged.

(B) Optical Purity of P(HBG–Ser) Copolymers. Using the Manning–Moore[38] dipeptide procedure, the starting materials, *i.e.*, *O*-TMS-L-Ser and L-glutamic acid, were found to contain no detectable D residues to within $\pm 0.1\%$. Copolymer fraction IXC-1, with 7.5 mol % serine and a $\overline{DP}_w$ of 274 (hence, about 21 of the 274 residues were serine), was

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

(45) F. Quadrifoglio and D. W. Urry, *J. Amer. Chem. Soc.*, **90**, 2760 (1968).

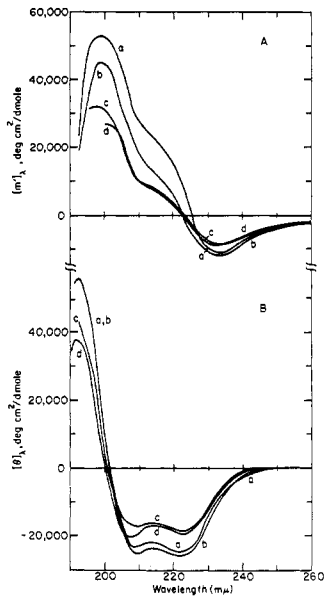

Figure 1. (A) ORD and (B) CD data in water at 25 ± 1° for P(HBG-Ser) copolymers and for PHBG: (a) $\overline{DP}_w$ = 424, 4.8% Ser (fraction VIIIB); (b) PHBG, $\overline{DP}_w$ = 200 (fraction VIB of paper II[4]); (c) $\overline{DP}_w$ = 813, 16.1% Ser (fraction XC); (d) $\overline{DP}_w$ = 289, 8.8% Ser (fraction IXB). The curves for fraction IXC-2, of lowest $\overline{DP}_w$, look qualitatively similar.

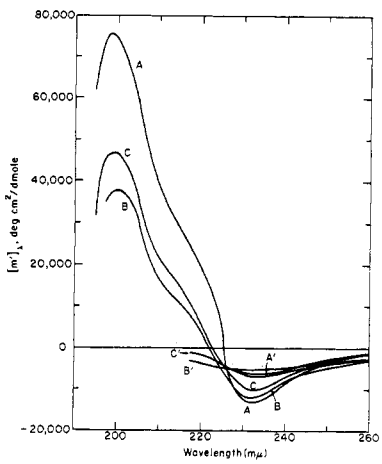

Figure 2. ORD data in water at low and high temperatures: (A and A') $\overline{DP}_w$ = 424, 4.8% Ser (fraction VIIIB) at 2 and 58°; (B and B') $\overline{DP}_w$ = 289, 8.8% Ser (fraction IXB) at 4 and 76°; (C and C') $\overline{DP}_w$ = 813, 16.1% Ser (fraction XC) at 6 and 70°.

hydrolyzed, and L-Leu dipeptides were prepared from the hydrolysate. The analysis of the dipeptides showed the presence of 0.4 mol % of D-serine (*i.e.*, 1.1 residues in 274). From an experiment involving the hydrolysis of this polymer in tritiated HCl,[39,40] it was found that no racemization (within ±0.1%) occurred on hydrolysis of the polymer. Hence, the 1.1 residues of D-serine arose through racemization during the synthesis, probably in the treatment with 4-amino-1-butanol (since the polymerization of NCA's proceeds without significant racemization[46,47]). No racemization of the glutamic

(46) E. Katchalski, M. Sela, H. I. Silman, and A. Berger, in "The Proteins," Vol. II, H. Neurath, Ed., Academic Press, New York, N. Y., 1964, p 405.

(47) R. Hirschmann, R. G. Strachan, H. Schwam, E. F. Schoenewaldt, H. Joshua, B. Barkemeyer, D. F. Veber, W. J. Paleveda, Jr., T. A. Jacob, T. E. Beesley, and R. G. Denkewalter, *J. Org. Chem.*, 32, 3415 (1967).

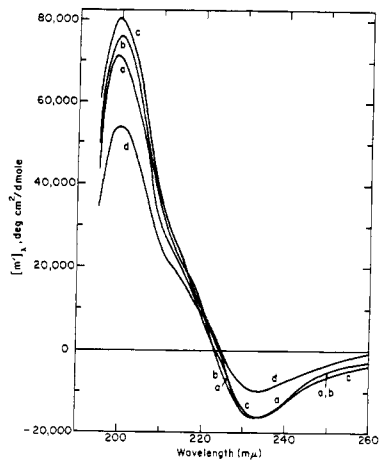

Figure 3. ORD data in 90% (v/v) aqueous methanol at 25 ± 1° for the same fractions (and same notation) of Figure 1.

acid in the polymer (within ±0.1%) was detected by this procedure. Since the sample contained 21 serine residues, only 1.1 of which were the D isomer, we consider this amount of racemization to be too minor to affect the computed values of σ and s for L-serine.

(C) ORD and CD Data for the Copolymers. Representative ORD and CD curves of the copolymer fractions in water at 25 ± 1° for the range 190–260 mμ are shown in Figure 1; data for PHBG, $\overline{DP}_w$ = 200, fraction VIB of paper II,[4] are also shown for comparison. All four ORD curves, even that for 16.1% serine, show a partial α-helix content, with the characteristic[48,49] minimum at 233 mμ, a shoulder at about 215–218 mμ, and a maximum at 198 mμ. The corresponding CD curves at room temperature are given in part B, and again typical, partially α-helical[48] spectra are observed with minima at 222 and 208 mμ and a maximum at 191 mμ.

Allowing of course for the effect of chain length, Figure 1 shows that L-serine decreases the helix content of PHBG. At 25°, the most helical sample studied, fraction VIIIB (of $\overline{DP}_w$ = 424 and 4.8% serine), has 49% helix content, while fraction IXC-2 (of $\overline{DP}_w$ = 97 and 11% serine) is least helical with only 20% helix content.

Figures 2 and 3 show the effect of temperature and solvent (90% (v/v) methanol), respectively, on the same curves. Both low temperature and methanol increased the helix content of all samples studied; a similar effect was found for pure 2,2,2-trifluoroethanol (TFE) and for 90% (v/v) TFE. No evidence for β structure was found in either the ORD or CD curves, although it is difficult to detect small amounts of β structure (in soluble polymers) by comparison of the experimental and computed[49,50] curves for two reasons: (1) the possible presence of β structure is masked by the overwhelming effect from the α helix, and (2) reliable ORD and CD curves for various types of β structures are not yet available. Apparent inversions in the orders of the curves among Figures 1–3 probably represent competing effects of serine content and chain length on helix content, as the solvent or the temperature is changed. The data of Figure 2 show that the P(HBG–Ser) copolymers all undergo a thermally induced helix-to-coil transition in water. The ORD curves at high temperature are

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

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

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

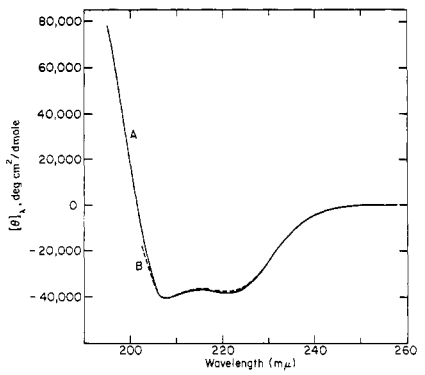

Figure 4. CD data in TFE at 24 ± 1°: (A) $\overline{DP}_w$ = 813, 16.1% Ser (fraction XC); (B) PHBG, $\overline{DP}_w$ = 200.

typical of those for the random-coil conformation admixed with little to none of the helical form.

Despite the aging phenomenon mentioned earlier, no turbidity developed in relatively fresh copolymer solutions over the range of 0–80°, and no changes developed in the ORD spectra, even when the solutions were maintained at the elevated temperature for over an hour (see section IIE for demonstration of reversibility of the transition curves). Presumably, there is no evidence of β structure or insolubility in these solutions because the serine content of the copolymers is low.

(D) b_0 for Complete Helix and Complete Coil. The values of $b_0 = -750$ ● 20 and 0 were used[4] for the complete helix and complete coil, respectively, of PHBG and PHPG, and for various copolymers involving either of these hosts.[4–6] The value of −750 was chosen after examination of the polymers in alcohol solutions at low temperature, in which their helix content was maximal. Thus, the helix content, θ_h, was computed as $-b_0/750$. The use of this same relation to compute θ_h for the serine copolymers is justified by the data of Table IV, from which it can be seen that the b_0 values for the various copolymer fractions and for a moderate-$\overline{DP}$ PHBG fraction are comparable. Also, b_0 tends toward zero for all of the copolymer fractions at high temperature. The values of b_0 were corrected for the dispersion of the refractive index of the solvent with the Sellmeier equation.[51]

Further, it is seen from Table IV (and Figure 2) that serine can be forced to adopt a right-handed α-helical conformation in a favorable environment (*e.g.*, in a copolymer with HBG, and dissolved in TFE (and also, presumably, in water) at low temperature). The absence of any indication of β structure in TFE at room temperature is shown by the CD curves of Figure 4; ORD data at *low* temperature lead to the same conclusion. Thus, our low-serine copolymers behave differently from a (D,L-Ser)$_m$–(L-Ser)$_n$–(D,L-Ser)$_m$ block polymer with $n = m = 30$ and a low-DP poly(L-serine) (mol wt = 650), both of which form β structures at low temperature in water and in alcohol (*i.e.*, 2-propanol).[20]

(E) Helix–Coil Transitions. The thermally induced helix–coil transitions of P(HBG–Ser) copolymers in water were followed by examining the temperature dependence of b_0 (or θ_h) in the temperature range of 0–80° and over the wavelength region of 280–420 mμ. Data were obtained about 5–10° apart (closer at the lower temperature), and by both heating and cooling. Figure 5 shows the melting curves for the five copolymers used to calculate σ and *s*. These include serine contents of 4.8–11.0 mol %, $\overline{DP}_w$'s from 97 to 424, and concentrations ranging from 0.2% to 0.45%. The sigmoidal shape is typical of a polypeptide helix–coil transition in water. No concentration dependence of b_0 was observed in the range considered, and all melting curves were determined at least twice. The values of θ_h decrease with both an increase in serine content and a decrease in chain length in aqueous solution. It is immediately clear that, under the conditions shown, serine, like glycine,[5] is a helix breaker, and values of the Zimm–Bragg parameter *s* less than 1.0 would be expected for this temperature range. The error symbols in Figure 5 correspond to uncertainties in concentration determinations (±3.0%) and in the Moffitt–Yang slope of $100 \cdot (2.5/b_0)$ %, which total to 3.5–7.0%, depending on the value of b_0.

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

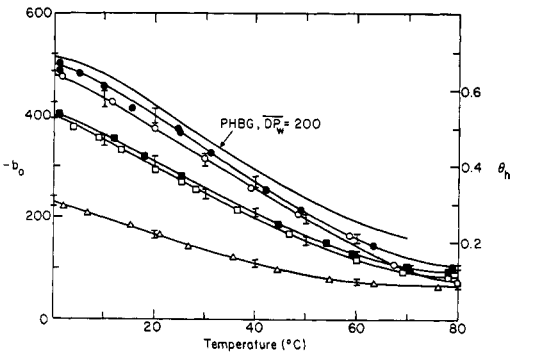

Figure 5. Temperature dependence of b_0 and θ_h for P(HBG–Ser) copolymers and for PHBG (fraction VIB, $\overline{DP}_w$ = 200, of paper II[4]) in water. $\theta_h = -b_0/750$. The $\overline{DP}_w$'s and serine contents of the copolymers are: (●) 424, 4.8% (fraction VIIIB); (○) 255, 5.8% (fraction VIIID); (■) 274, 7.5% (fraction IXC-1); (□) 289, 8.8% (fraction IXB); (Δ) 97, 11.0% (fraction IXC-2). The points are experimental ones and the lines represent the smoothed experimental curves. The error symbols represent the combined error in b_0 for the determination of concentration and the slope of the Moffitt–Yang plot.

TABLE IV
VALUES OF b_0 IN ALCOHOLS AND WATER

Fraction	L-Serine content, mol %	Solvent	$\overline{DP}_w$	Temp, °C	$-b_0$
PHBG[a]	0	Water	200	24	389
		Water		0.8	526
		TFE		24	688
		TFE		0.7	732
VIIIB	4.8	Water	424	25	366
		Water		0.8	487
		TFE		24	665
		TFE		0.6	712
IXB	8.8	Water	289	25	291
		Water		1.1	404
		TFE		25	682
		TFE		2.4	721
		90% aq methanol		25	482
		90% aq methanol		1.2	510
XC	16.1	Water	813	24	300
		Water		0.8	398
		TFE		24	722
		TFE		0.4	727

[a] Fraction VIB of paper II.[4]

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

Serine content, mol %	$\overline{DP}$	Temp, °C	$\theta_{h,theor}$ Lifson[b]	Lehman–McTague[b]	Allegra[c]	Lehman–McTague[c]
4.8	424	10	0.599	0.574	0.586	0.594
		30	0.427	0.415	0.419	0.425
		60	0.210	0.205	0.207	0.210
5.8	255	10	0.538	0.510	0.530	0.534
		30	0.380	0.367	0.375	0.378
		60	0.186	0.181	0.185	0.186
7.5	274	10	0.502	0.467	0.501	0.499
		30	0.351	0.336	0.352	0.350
		60	0.168	0.163	0.168	0.168
8.8	289	10	0.472	0.436	0.477	0.472
		30	0.329	0.315	0.333	0.330
		60	0.155	0.151	0.156	0.156
11.0	97	10	0.289	0.270	0.305	0.299
		30	0.208	0.201	0.216	0.213
		60	0.106	0.105	0.108	0.108

[a] The parameters used for hydroxybutylglutamine were taken from Table II of paper II[4] and are $\Delta H = -195$ cal/mol, $T_{tr} = 37.8°$, $\sigma = 6.7 \times 10^{-4}$. [b] The parameters used for L-serine were obtained by fitting the data by the Lifson theory, as shown in Table VI. [c] The parameters used for L-serine were obtained by fitting the data by the Allegra theory, as shown in Table VI.

TABLE VI
VALUES OF THE ZIMM–BRAGG PARAMETER s FOR POLY(L-SERINE) IN WATER FROM 0 TO 80°[a]

Temp, °C	s Lifson	Allegra
0	0.66_7	0.72_6
10	0.72_6	0.76_5
20	0.75_7	0.78_4
30	0.77_4	0.79_3
40	0.77_7	0.79_2
50	0.76_5	0.77_9
60	0.73_1	0.74_4
70	0.70_6	0.71_8
80	0.70_8	0.71_6

[a] The values of σ obtained from the Lifson and Allegra theories are $\sigma = 1 \times 10^{-4}$ and 7.5×10^{-5}, respectively. The error involved in the calculation of s is indicated in Figure 8.

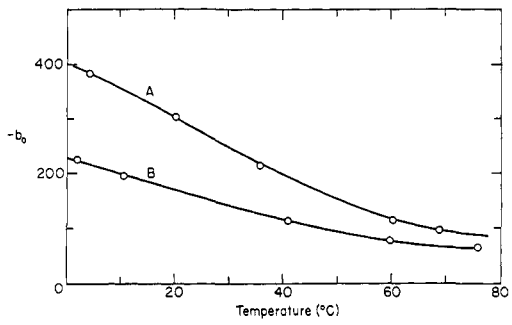

Figure 6. Demonstration of the reversibility of the helix-coil transition for fraction IXB ($\overline{DP}_w = 289$, 8.8% Ser) and fraction IXC-2 ($\overline{DP}_w = 97$, 11.0% Ser) in water. The curve was obtained during the heating cycle and the points were obtained during the cooling cycle.

The reversibility of the helix–coil transition in P(HBG–Ser) is demonstrated in Figure 6 for two fractions; the curves for all other fractions (even fractions XB and XC of high serine content, the former of which became insoluble after storage for 6 months, and the latter of which exhibited an anomalously low decrease in helix content above 50°) were also reversible.

(III) Discussion

(A) Helix–Coil Parameters for Poly(L-serine). As shown in papers I–IV,[3-6] which should be consulted for the details, the lower orders of the Lifson–Allegra–Poland–Scheraga (LAPS) hierarchy of approximations can be used to obtain the helix–coil stability constants for the guest residue in a copolymer provided that the values of σ and s fall within the range of applicability of the approximate theories. In the absence of *a priori* knowledge of the values of these parameters, the low orders of approximation[3] (corresponding to theories of Lifson[52] and Allegra[53]) were used *initially* and checked in a few representative cases against the exact theory of Lehman and McTague for finite chain length.[54,55] The comparison was made in a similar manner to that used in papers III and IV.[5,6]

From the results shown in Table V, it can be seen that both the Lifson and Allegra theories fit the data adequately. However, the Allegra theory (which is a higher order approximation than the Lifson theory) provided a better correspondence to the exact theory. Thus, unless specified otherwise, calculations were carried out with the Allegra theory, with σ assumed to be independent of temperature. The values of s calculated according to both theories are compared in Table VI, and it is seen that the agreement is good. The value of σ was determined as the one which minimized τ (defined in paper IV[6]), as indicated in Figure 7. From Figure 7, it can be seen that τ becomes insensitive to σ at approximately $\sigma = 7.5 \times 10^{-5}$. The value of σ obtained with the Lifson theory was approximately the same (1×10^{-4}). Similarly, the value of σ for glycine could be specified only in the range of 10^{-3}–

(52) S. Lifson, *Biopolymers*, **1**, 25 (1963).
(53) G. Allegra, *J. Polym. Sci., Part C*, **No. 16**, 2815 (1967).
(54) G. W. Lehman and J. P. McTague, *J. Chem. Phys.*, **49**, 3170 (1968).
(55) All computer programs used in this work are available. See footnotes 26 and 27 of paper I[3] for the procedure for obtaining them.

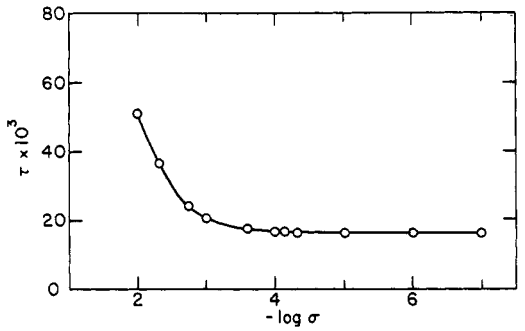

Figure 7. Determination of the best temperature-independent value of σ as that corresponding to the onset of the minimum for τ for P(HBG–Ser) copolymers according to the Allegra theory.

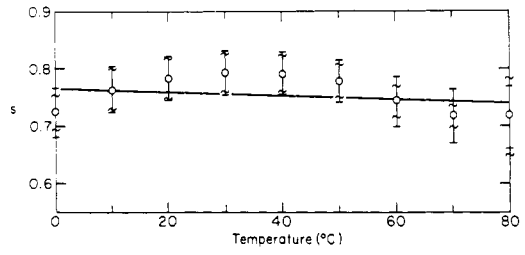

Figure 8. A plot of s *vs.* temperature for poly(L-serine) in water calculated with the Allegra theory with $\sigma = 7.5 \times 10^{-5}$. The line represents a weighted least-squares fit. The precision of the data is indicated by the error symbols which correspond to standard deviations (I) and limiting errors (↕), computed as described in paper IV.[6]

10^{-6}, and a value of 1×10^{-5} was used.[5] In both the glycine and serine copolymers, the composition is low in the guest residue. Hence, the probability of the guest residue initiating a helix is low, and it becomes difficult to determine σ precisely in such cases. We have taken the value of σ for serine as that for which τ *first* reaches its minimum value; both the values of s and the thermodynamic parameters were insensitive to the choice of σ at lower values of σ.

The values of s are plotted against temperature in Figure 8. Although a slight maximum is indicated by the experimental points, a weighted least-squares linear fit is adequate to describe the data when the experimental errors are taken into account. There appears to be a slight decrease in s with increasing temperature. The absence of a pronounced temperature dependence of s probably results from a compensation of various intramolecular energies and solvation.

The error symbols shown in Figure 8 were calculated in two ways, as described in paper IV,[6] taking into account all of the sources of error reported above.

The fact that $s < 1$ over the entire temperature range investigated is a quantitative measure of the classification of serine as a helix breaker. Comparing the values of s for serine and glycine at 20° (0.78 and 0.59, respectively), we see that glycine is the stronger helix breaker in water at this temperature.

Using the Allegra theory with the parameters of Table VI for L-serine and those of paper II,[4] Table II for HBG, theoretical melting curves were computed. These are compared in Figure 9 with the experimental data (smoothed points at 10° intervals taken from Figure 5). The error symbols correspond to errors in θ_h arising from errors in the determination of $\overline{DP}_w$ and of the serine content (which together total to 0.8–4.5%) and of b_0. Except for the lowest DP fraction (IXC-2), the

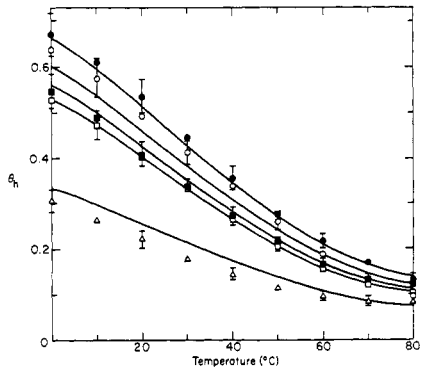

Figure 9. Calculated melting curves (obtained from the Allegra theory with L-serine parameters of Table VI and HBG parameters of Table II, paper II[4]) for P(HBG–Ser) copolymers, together with experimental points (smoothed data from Figure 5 at 10° intervals). The $\overline{DP}_w$'s and serine contents of the copolymers are: (●) 424, 4.8% (fraction VIIIB); (○) 255, 5.8% (fraction VIIID); (■) 274, 7.5% (fraction IXC-1); (□) 289, 8.8% (fraction IXB); (Δ) 97, 11.0% (fraction IXC-2). The error symbols indicate the errors calculated for the individual points and were computed as described in the text.

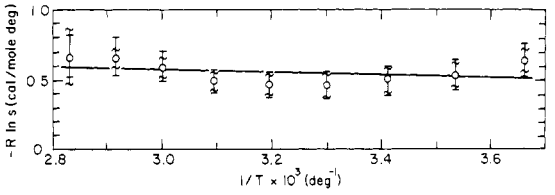

Figure 10. A plot of the data of Figure 8 as $-R \ln s$ *vs.* $1/T$ for poly(L-serine). The precision of the data is given by the error symbols which are defined in Figure 8.

TABLE VII
THERMODYNAMIC PARAMETERS FOR L-SERINE[a]

$\Delta G_{20°}$, cal/mol	158 ± 7
ΔH, cal/mol	−101 ± 95[b]
ΔS, eu	−0.9 ± 0.3[b]
σ[a]	7.5×10^{-5}

[a] σ is assumed to be temperature independent. [b] The errors in ΔH and ΔS were determined in a weighted least-squares analysis of eq I which incorporated the calculated standard deviations in the determinations of the parameter s.

theoretical curves (calculated with the Allegra parameters of Table VI for L-serine and those of Table II of paper II[4] for HBG) agreed with the experimental data within the limits of error.

The temperature dependence of s is given by

$$s = \exp[-\Delta G/RT] = \exp[-\Delta H/RT + \Delta S/R] \quad \text{(I)}$$

where ΔG, ΔH, and ΔS are the free energy, enthalpy, and entropy, respectively, for the conversion of a coil residue to a helical one at the end a long helical sequence. Figure 10 shows a plot of $-R \ln s$ *vs.* $1/T$ for L-serine. Assuming that ΔH and ΔS are temperature independent, the least-squares linear fit yields the thermodynamic parameters shown in Table VII from the slope and intercept, respectively. The limits of error given in Table VII and in Figure 10 were computed as in paper IV[6] and contain no contribution from errors in the parameters for HBG. The values of ln s from the weighted line of Figure 8 agree within 1% with the corresponding values of ln s from the weighted line of Figure 10.

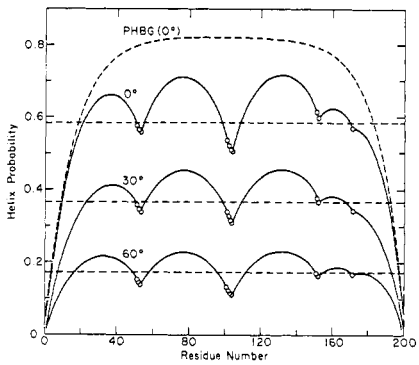

Figure 11. Helix-probability profiles (for the temperatures shown) computed for a P(HBG–Ser) specific-sequence copolymer of chain length 200, containing 5% serine. The positions of the serine residues in the sequence are shown as circles. The dashed curve is for a PHBG homopolymer with DP = 200. The values of σ and s for serine are those of Table VI, while those of HBG are from paper II.[4] The horizontal dashed lines represent the average helix content at each temperature.

As would be expected for a helix breaker, ΔG is positive in the temperature region studied, and at 20° the least-squares value of ΔG is 158 ± 7 cal/mol. It is of interest to compare glycine, with values of 625 ± 100 cal/mol and 1.0 ± 0.3 eu for ΔH and ΔS, respectively, to L-serine, with corresponding values of −101 ± 95 cal/mol and −0.9 ± 0.3 eu. The helix-breaking character (positive value of ΔG at, say, 20°) is dominated by the enthalpy term for glycine but by the entropy term for L-serine. As indicated above for the temperature dependence of s, the relative behavior of these two amino acid residues probably results from (an as yet unaccounted for) combination of various intramolecular energies and solvation. While the transition temperature for the hypothetical helix–coil transition in poly(L-serine), computed from $\Delta H/\Delta S$, is −159°, no significance should be attached to this value, since the temperature-independent values of ΔH and ΔS apply only in the temperature range studied, and not to lower temperatures.

(B) Conformational Role of Serine in Synthetic Polypeptides and in Proteins. The helix-breaking property of L-serine (though less pronounced than observed for glycine[5]) is indicated by the probability profiles of Figure 11; these were computed by the procedure of Lewis, *et al.*,[28] with the parameters of Table VI for serine and those of Table II, paper II,[4] for HBG, applied to a specific sequence of these two residues in a chain of DP = 200 containing 5% serine. This specific sequence was selected to demonstrate the effect of a single serine residue and of blocks of two, three, and four consecutive serine residues. The helix-breaking character of serine is evident at all temperatures, but the strong helix-forming propensity of the host (HBG) residues can force an *isolated* serine residue (*e.g.*, no. 171 in Figure 11) into the helical region, through the near-neighbor interaction (characteristic of the one-dimensional Ising model) present in the copolymer. The specific sequence in Figure 11 also demonstrates the pronounced effect of contiguous serines, the helix breaking being greater as the number of serines increases from one to four. The serine residues of globular proteins occur in *short* sequences, and blocks as large as four do occur, *e.g.*, Ser 168–171 in tosyl elastase;[56] a three-serine block, Ser 21–23, exists in bovine ribonuclease.[11] The various curves of Figure 11 reflect the decrease of helix content with increasing temperature. The smaller effect of the seryl residues at the higher temperatures results from the fact that the values of s for HBG decrease, while those for serine change very little with increasing temperature.

It is observed in Figure 11 that the minima all lie on the right side of the contiguous seryl blocks. The origin of this skewness of the helix-disrupting effect of the serines is in the unequal and lower value of the nucleation parameter, σ, for serine relative to that for the host residue HBG. The explanation for this effect is as follows: in the nearest-neighbor Ising model, the factor σ is arbitrarily assigned to the first residue of a helical sequence. Hence, if a guest residue G is in the middle of a helical sequence where a break might occur and has a lower value of σ than the host H, it would prefer to be associated with the end of one helical sequence (with a statistical weight of s_G) rather than with the beginning of the next helical sequence (where its statistical weight would be $\sigma_G s_G$). If $\sigma_G = \sigma_H$, there is no preference and the guest residues are distributed symmetrically around the minima; if $\sigma_G > \sigma_H$, the guest residues would accumulate on the right side of the minima. Since σ for Ser is less than that for HBG, the guest residues accumulate on the left side of the minima in the curves of Figure 11.

Besides being a helix breaker, as demonstrated here quantitatively, L-serine has also been shown to exist to a large extent in β turns,[57] *e.g.*, Ser-Tyr-Ser-Thr, residues 75–78 in bovine ribonuclease,[57] and Ser-Ser-Gly-Orn in the cyclic hexapeptide ferrichrome A.[58] While several conformational energy calculations have been carried out for L-serine, none of them took the solvent (water) into account, as Gō, *et al.*,[59] did for glycine and L-alanine. Therefore, it is not possible at this time to provide an explanation (in terms of intramolecular potentials and solvation) as to why serine appears to be a β-turn former and a helix breaker in water.

The position of the hydroxyl group has been shown to be related to the helix-breaking character of serine, since polymers of the two higher homologs with side groups of $-(CH_2)_2-OH$ and $-(CH_2)_3-OH$, respectively, *i.e.*, poly(2-amino-4-hydroxybutyric acid) and poly(δ-hydroxy-L-α-aminovaleric acid), can both form helical conformations, as shown by ORD measurements. The former[19] is slightly helical in water and has a higher helix content in dimethyl sulfoxide, whereas the latter[60] is fully helical in both dimethylformamide and TFE. In the Fasman–Blout classification, the threonine residue is regarded as a helix breaker,[15] and poly(*O*-acetyl-L-threonine) has been reported to be nonhelical.[26] All three of these polymers have additional (nonpolar) methylene or methyl groups, compared to serine, which may contribute side chain–backbone interactions to influence the stability of the helical form. At the present time, we can only repeat the conjectures of Blout, *et al.*,[26,61] and of Fasman and Tooney[20] that the proximity of the seryl hydroxyl group to the backbone, with possible side chain–backbone hydrogen bonding, reduces the stability of helical conformations in synthetic polypeptides and in proteins.

An examination of the known protein structures reveals

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that serine serves diverse functions as (1) helix breaker, (2) participant in β turns and in β regions, (3) occasional helical residue, and (4) active-site residue. These diverse functions probably arise from the following characteristics of the $-CH_2-OH$ side group: (1) small size (even the α-helical conformation being sterically allowed), (2) polar but nonionic structure, (3) capability of forming one or multiple hydrogen bonds, and (4) closeness to backbone which allows hydroxyl–peptide bond interactions.

(IV) Conclusion

Water-soluble copolymers of L-serine and N^5-(4-hydroxybutyl)-L-glutamine were synthesized and characterized. The *O*-trimethylsilyl group was found to be a suitable blocking group (with preservation of stereochemical configuration) for copolymerizations involving seryl residues. The thermally induced helix–coil transitions in water were examined over the temperature range of 0–80°, and the Zimm–Bragg parameters σ and s were determined by application of the host–guest technique[3-6] and the Allegra theory. The value of 0.78 found for s at 20° is a quantitative measure of the helix-breaking character of serine in water, and shows that serine is a less effective helix destabilizer than glycine. The computed values of s were insensitive to the value selected for the nucleation parameter σ below 7.5×10^{-5}. Assuming that σ, ΔH, and ΔS are independent of temperature in the range of 0–80°, the values of $\Delta G_{20°}$, ΔH, and ΔS were found to be 158 cal/mol, −101 cal/mol, and −0.9 eu, respectively. The various roles of serine in proteins are attributed to the small size, polar but nonionic structure, and ability to form one or more hydrogen bonds.

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Conformational Properties of Structurally Rigid Polyamides. Conformation of Model Diamides

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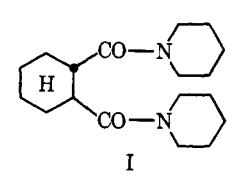
ABSTRACT: The conformational equilibria of some model diamides which represent the building blocks of a series of polyamides derived from *trans*-1,2-cyclohexanedicarboxylic acid and different aliphatic amines have been studied. We have used nmr and dipole moment measurements, coupled with some *a priori* conformational energy estimates, to ascertain the conformational preferences of diamides I, II, and III, which seem appropriate model compounds of polyamide IV (see text). The results indicate that diamide I exists preferentially in an extended conformation and that in diamides II and III the conformational equilibrium is strongly biased toward conformations containing axial methyl groups (nearly equipopulated mixture of the four diaxial rotamers possible). Nmr solvent differential shifts are also reported for some diamides, and the results are interpreted in terms of the collision complex concept.

The optical properties of a series of asymmetric polyamides derived from cyclic 1,2- and 1,3-dicarboxylic acids, with varying degrees of rigidity imposed on the polymer chain, have been reported.[1] The results indicate that several of these polyamides may exist in preferred conformations in solution but cast no light on the identity of the specific conformations which may be present.[1]

To further investigate this problem, we have studied the conformational equilibria of some model diamides which represent the building blocks of our polymers. In the present approach we have used nmr and dipole moment measurements, coupled with some *a priori* conformational energy estimates, to ascertain the conformational preferences of diamides I, II, and III, which seem appropriate model compounds for polyamide IV.



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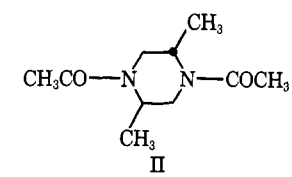

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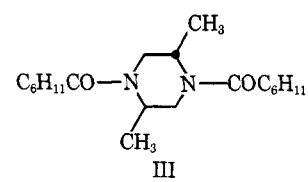
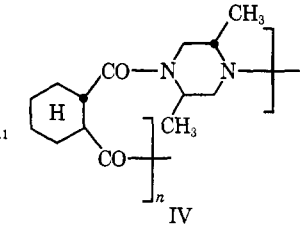


III



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