TABLE V
COIL-TO-HELIX PARAMETERS[a] FOR PHPG AND PHBG AT 20°, WITH σ TAKEN AS INDEPENDENT OF TEMPERATURE

	PHPG	PHBG
ΔG, cal/mol	+15.71	−9.73
ΔH, cal/mol	−185	−187
ΔS, eu	−0.685	−0.605
T_{TR}, °C	−3.0	+35.8
σ	2.8×10^{-4}	6.8×10^{-4}

[a] Obtained by successive approximations in the refinement of the homopolymer and copolymer data.

to be correct and a best fit was obtained to the experimental data for *both* PHBG and the copolymers. After several such cycles of refinement, the data converged to the results given in Table V for the homopolymers. It should be emphasized that the data of Table V are the result of curve fitting of data for copolymers and homopolymers rather than for homopolymers alone; this may tend to overweight the copolymer data so that one loses sight of the behavior of the homopolymers. However, we find that the PHBG parameters obtained in this manner differ little from those obtained using only the homopolymer data; hence, the PHBG homopolymer parameters in Table II appear to be good choices when PHBG is used as the host. The PHPG parameters obtained from the refinement are somewhat different from those obtained using only the homopolymer data. This is not unexpected since, as stated above, only the high-temperature ends of the melting curves are obtainable when the solvent is water.

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Helix–Coil Stability Constants for the Naturally Occurring Amino Acids in Water. III. Glycine Parameters from Random Poly(hydroxybutylglutamine-*co*-glycine)[1]

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ABSTRACT: Water-soluble random copolymers, containing glycine and hydroxybutylglutamine, have been synthesized, fractionated, and characterized. From an analysis of their thermally induced helix–coil transition curves, using an approximate theory for random copolymers and the host–guest technique of the previous paper, it was possible to obtain the Zimm–Bragg parameters σ and s which characterize the (hypothetical) helix–coil transition of polyglycine in water. The relatively low values of s in the temperature range of 0–70°, in water, indicate that glycyl residues in polyglycine do not adopt the α-helical conformation under these conditions; when incorporated in a copolymer, they act as strong helix breakers. The implication of these results for the conformational role of glycyl residues in proteins is discussed.

The need for having the helix–coil stability constants for the naturally occurring amino acids in water was discussed in the earlier papers of this series,[3,4] and a method to obtain them was outlined. The method involves the synthesis of random copolymers containing relatively small amounts of the "guest" amino acid whose parameters are sought and a larger proportion of a suitable "host" amino acid whose stability parameters in water are known, and the analysis of the melting behavior of these copolymers using an appropriate form of the theory governing the helix–coil transition in such random copolymers (see paper I[3] for a discussion of the theory). The success of the theoretical treatment in enabling one to obtain the stability constants for the guest amino acid from the experimental data was demonstrated in paper II,[4] using random copolymers of two hydroxyalkylglutamines. We are, therefore, now in a position to apply the technique to obtain the parameters for the naturally occurring amino acids whose homopolymers, unlike those of the hydroxyalkylamines, are either not water soluble or do not form α helices melting in the experimentally accessible temperature range. The first such amino acid, which is the subject of this paper, is glycine, the simplest and yet one of the most interesting of the amino acids. Even though polyglycine has not been observed to form an α helix, nevertheless the parameters to be deduced here apply to the hypothetical transition in which right-handed α-helical polyglycine is converted to the random coil in water.

Water-soluble copolymers containing glycine in amounts less than 20% were synthesized using hydroxybutylglutamine as the "host" amino acid. The homopolymer of the latter (PHBG) was studied in detail in paper II,[4] and its helix–coil transition parameters were determined. PHBG was found to exist as a relatively stable helix in water with a transition temperature (for the "infinite" chain) of about 40°. The expectation that glycine would act as a helix breaker in water and, therefore, that copolymers of hydroxybutylglutamine and glycine, P(HBG:Gly), would have transition curves shifted progressively downward on the temperature scale as the

(1) This work was supported by research grants from the National Institute of Arthritis and Metabolic Diseases, U. S. Public Health Service (No. AM-08465), and from the National Science Foundation (No. GB-7571X).
(2) NIH Postdoctoral Trainee, 1969–1971.
(3) P. H. von Dreele, D. Poland, and H. A. Scheraga, *Macromolecules*, 4, 396 (1971) (hereinafter called paper I).
(4) P. H. von Dreele, N. Lotan, V. S. Ananthanarayanan, R. H. Andreatta, D. Poland, and H. A. Scheraga, *ibid.*, 4, 408 (1971) (hereinafter called paper II).

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

Glycine content of reaction mixture, mol %	Polymers containing benzyl glutamate: Glycine content found, mol %	Av mol wt[a] × 10^{-3}	$\overline{DP}$	Polymers containing hydroxybutylglutamine: Glycine content found, mol %	Wt av mol wt[b] × 10^{-3}	$(\overline{DP})_w$
0	(I)	460	2300	(VIII)[c]	183.2	916
4.0	3.1 (II)	360	1840	3.5 (IX)	37.0	187
8.0	6.2 (III)	390	2040	7.2 (X)	28.0	144
10.0	7.0 (IV)	350	1860	8.1 (XI)	18.4	99
15.0	12.1 (V)	330	1800	12.1 (XII)		
20.0	16.0 (VI)			16.4 (XIII)	12.1	65
25.0	21.5 (VII)	350	2060			

[a] By viscometry, using the relation of Fujita, *et al.*, for polymers in DCA: H. Fujita, A. Teramoto, T. Yamashita, K. Okita, and S. Ikeda, *Biopolymers*, **4**, 781 (1966). [b] By sedimentation equilibrium. [c] This polymer was also used in paper II,[4] where it was designated as sample IV.

glycine content of the copolymer increased was borne out by the results obtained in this investigation.

The synthesis of the water-soluble copolymers is described in section I, and the characterization of these copolymers and their melting behavior in water are presented in section II. The melting data are then analyzed in section III with the aid of an appropriate form of the theory[3] to obtain parameters for the helix–coil transition in polyglycine; these are then discussed in terms of their relevance to the role of glycine in protein conformation and to our understanding of the forces governing the stability of the α helix in polypeptides in general.

I. Experimental Section. Preparation of the Copolymers

Water-soluble copolymers of hydroxybutylglutamine and glycine, P(HBG:Gly), were prepared by a two-step process

glycine + γ-benzyl L-glutamate ⟶ poly(γ-benzyl L-glutamate-*co*-glycine) (1)

poly(γ-benzyl L-glutamate-*co*-glycine) + hydroxybutylamine ⟶ poly(hydroxybutylglutamine-*co*-glycine) (2)

The experimental details are presented below.

(A) Materials. 4-Amino-1-butanol (Chemical Procurement Laboratories, Inc., College Point, N. Y.) was dried over barium oxide and distilled under reduced pressure. All solvents used in the preparation and recrystallization of *N*-carboxy anhydrides (NCA) and for polymerizations were purified shortly before use, dioxane and hexane by refluxing and distilling over sodium and triethylamine by drying and distilling over KOH. Ethyl acetate was dried over anhydrous calcium sulfate and distilled. Dichloroacetic acid (DCA), used for viscosity measurements, was a purified reagent from Fisher Scientific Co. and was used without any further purification. Methanol was a Spectroanalyzed solvent from the same company. Ether was of analytical reagent grade from Mallinckrodt Chemical Works. Glycine was obtained from M. T. Baker Chemical Co. and L-glutamic acid from Aldrich Chemical Co.

(B) Synthesis. Glycine NCA was synthesized by first preparing *N*-carbobenzoxyglycine and then treating it with phosphorous pentachloride according to Bartlett and Jones,[5] and recrystallizing from ethyl acetate and hexane. γ-Benzyl L-glutamate NCA was synthesized by treatment of γ-benzyl L-glutamate (which in turn was prepared from L-glutamic acid) with phosgene as described by Blout and Karlson;[6] it was recrystallized several times from dioxane and hexane until the chlorine content was less than 0.01%.

Poly(γ-benzyl L-glutamate) (PBLG) (I). PBLG with a degree of polymerization of ~2300 was prepared in 85% yield by the polymerization of γ-benzyl L-glutamate NCA in dioxane with the use of triethylamine as initiator (A/I = 25).[6]

Poly(γ-benzyl-Glu^m'Gly^n') [P(BzG:Gly)] Copolymers (II–VII). Random copolymers containing γ-benzyl L-glutamate and glycine in different ratios were synthesized by the polymerization of the NCA's of the two compounds in dioxane, using triethylamine as the initiator. The yields, based on the NCA's, varied from 76 to 85%. A typical run was carried out as follows. Triethylamine (0.08 g, 0.8 mmol) was added to a solution of γ-benzyl L-glutamate NCA (4.475 g, 17 mmol) and glycine NCA (0.303 g, 3 mmol) in dioxane (150 ml) to give an A/I ratio of 25. The mixture was kept in a desiccator over anhydrous calcium chloride for 3 days. The resulting viscous solution was introduced slowly into vigorously stirred ethanol (700 ml). The white precipitate formed was collected on a filter funnel, washed with several portions of ethanol, and dried *in vacuo* over P_2O_5. The yield was about 85% (3.32 g). The compositions of the copolymers are given in Table I.

Poly[N^5-(4-hydroxybutyl)-Glu^{m}Gly^n] [P(HBG:Gly)] Copolymers (IX–XIII). Copolymers II–VII were treated with 4-amino-1-butanol to yield a series of water-soluble copolymers with glycine content ranging from 3 to 16.4%. (There was some difficulty in obtaining copolymers with higher glycine contents since they did not dissolve readily in water.) The following procedure was used in all cases. The P(BzG:Gly) copolymer (about 3.0 g) was dissolved in dioxane at 60° with mechanical stirring, and 4-amino-1-butanol (16 ml) was added in four equal portions 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 mixture was then poured into 1 *N* acetic acid (300 ml) and the aqueous solution dialyzed exhaustively against distilled water. The solution was then filtered through a Millipore filter (0.45-μ pore size, Millipore, Bedford, Mass.) and lyophilized. The yield ranged from 83 to 85% for IX and X and from 50 to 65% for the copolymers containing higher proportions of glycine (XI, XII, and XIII). The compositions of the copolymers are given in Table I.

(C) Spectral Analysis. The spectra of aqueous solutions of PHBG (VIII) and of the copolymers IX–XIII showed no absorption at 257 mμ arising from benzyl groups, assuring that aminolysis of the benzyl ester groups was essentially complete. Samples showing significant (>1%) benzyl absorption were discarded and are not included in Table I.

(D) Fractionation. The copolymers IX–XIII 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 then dried *in vacuo* over P_2O_5.

(E) Determination of Composition. The amino acid compositions of the copolymers II–VII and IX–XIII, and the fractions obtained from the latter, were determined using a Technicon amino acid analyzer. The samples were hydrolyzed in 12 *N* HCl at 105° for

(5) P. D. Bartlett and R. H. Jones, *J. Amer. Chem. Soc.*, **79**, 2153 (1957).

(6) E. R. Blout and H. R. Karlson, *ibid.*, **78**, 941 (1956).

24 hr in sealed ampoules. No corrections for the destruction of the amino acids during the hydrolysis were necessary, since the recoveries in all cases were 95–100%, even when the hydrolysis time was extended to 72 hr. The analyses were repeated twice on those fractions that were used for obtaining melting curves. The average experimental error in the determination of the compositions is estimated to be ±5%.

(F) Determination of Concentration. The concentrations of all polymer solutions were determined by nitrogen analysis, using the micro-Kjeldahl method (see paper II[4]), together with the data on amino acid compositions. The average error in concentration determination is estimated to be ±3%. The contribution to this error from the error in the amino acid composition is negligible.

(G) Viscosity. The viscosity measurements were made at 25.0 ± 0.01° using a Cannon-Ubbelohde semimicro dilution viscometer, with flow times ranging from 120 to 500 sec in DCA.

(H) Determination of Chain Length. The molecular weights (shown in Table I) of polymers I–VII were determined by viscometry, using DCA as the solvent. The values obtained for samples II–VII are, however, only rough estimates, since the viscosity–molecular weight relation[7] for PBLG was used, without correction, for the copolymers. The data thus obtained were used for semiquantitative comparison purposes only (see section IIA).

On the other hand, the weight-average molecular weights of PHBG (VIII) and the P(HBG:Gly) copolymers IX–XIII, and the fractions obtained from these samples, were determined by the sedimentation–equilibrium method, using the Beckman Model E ultracentrifuge, as described in paper II.[4] The accuracy of the determination was found to be within ±5%.

(I) 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. The procedures for these measurements, as well as the analysis of the data in terms of $[m']_\lambda$, b_0, and $[\theta]_\lambda$, are described in paper II.[4] The concentrations in these measurements ranged from 0.1 to 0.5% (w/v). The experimental error in b_0 is estimated to be ±4%, and contains the error in the determination of the amino acid composition of the copolymers and of the concentration of the polymer solutions.

II. Results

(A) Characterization of the Copolymers. The average compositions and average degrees of polymerization ($\overline{DP}$) of both the P(BzG:Gly) and P(HBG:Gly) copolymers and the corresponding PBLG and PHBG homopolymers are shown in Table I. It can be seen that the chain lengths of samples I–VII are very high (about 2000). However, conversion to the hydroxybutylglutamine derivatives (VIII–XIII) results in a considerable decrease in chain length, the decrease being larger for the copolymers containing greater amounts of glycine. There was no significant change in glycine content in the conversion of II–VI to IX–XIII.

Considering the fractionated copolymers (see Table II), some variation in glycine content was observed in the several fractions of a particular copolymer. It appears that, as the chain length decreases, the glycine content increases among the fractions from a given copolymer. This behavior may possibly arise from the fact that glycine may act as a better chain terminator than the benzyl glutamate in the synthesis of samples II–VII. The fractions whose melting data were used for the theoretical analysis described in section III had $\overline{M}_z/\overline{M}_w$ ratios close to unity, except for fraction XIC-2 (see Table II), indicating that they were fairly homogeneous in chain length. Since $\overline{M}_w$ did not vary over a threefold change in concentration (determined only for the sample (XIIB) containing the largest amount of glycine), it is concluded that no significant amount of aggregation occurs in the concentration range employed.

TABLE II
CHARACTERIZATION OF THE FRACTIONATED COPOLYMERS[a]

Fraction[b]	Glycine content, mol %	Mean residue weight	$\overline{M}_w \times 10^{-3}$	$\overline{M}_z/\overline{M}_w$[c]	$(\overline{DP})_w$
IXA	3.1	196	100.0		510
IXB	3.0	196	55.3	1.04	282
IXC-1	3.0	196	33.0	1.12	168
IXC-2	3.9	195	20.0	1.05	103
IXC-3	5.0	193	14.7		76
XA	4.1	194	101.2		521
XB	6.5	191	63.7	1.11	334
XC-1	7.9	189	18.9	1.10	100
XC-2	10.8	185			
XIA	6.0	192	40.4		211
XIB	7.1	190	32.8		173
XIC-1			20.2		108
XIC-2	8.9	188	18.6	1.3	99
XIC-3	9.8	186	11.5		62
XIIA	13.4	181	18.5		102
XIIB	18.0	175	16.0	1.05	92

[a] The characterization of the fractionated material from sample VIII is described in paper II[4] (as fraction IVA). [b] 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). [c] Given only for fractions whose melting properties were analyzed.

The decrease in chain length in the conversion of PBLG to the hydroxyalkyl derivative was reported by Lotan, *et al.*,[7] and ascribed to a transaminolysis reaction in which the aminobutanol reacts not only with the side-chain ester group but also with the backbone peptide group. The extent to which the latter reaction, which would cause chain scission, takes place depends on the susceptibility of the backbone to attack by the aminobutanol. Since the chain length of polymers VIII–XIII decreases with increasing glycine content (Table II), it appears that the bulky benzyl side chains may shield the backbone from attack by aminobutanol, and that the replacement of benzyl glutamate by glycine makes it easier for the aminobutanol to reach the backbone; *i.e.*, the chain scission which takes place in the reaction with aminobutanol appears to occur preferentially at glycine residues. (Preliminary observations[8] with copolymers of alanine and hydroxypropylglutamine, whose chain lengths are much larger than those of the corresponding glycine analogs, support this suggestion.) If the glycine residues were distributed in blocks at a few places in the backbone of the copolymers, one would expect the reaction with aminobutanol to result in the separation of these blocks in the form of short glycine oligopeptides which would be very likely to be lost during the subsequent dialysis step in the preparation of the polymers (see section I). As a result, the P(HBG:Gly) copolymers would have a lower glycine content than the corresponding P(BzG:Gly) copolymers. However, as shown in Table I, the compositions of the two sets of copolymers are essentially the same in spite of the low yield (less than 50%) after reaction with aminobutanol. This result indicates that the glycine

(7) N. Lotan, A. Yaron, A. Berger, and M. Sela, *Biopolymers*, 3, 625 (1965).
(8) K. B. Platzer, V. S. Ananthanarayanan, R. H. Andreatta, and H. A. Scheraga, work in progress.

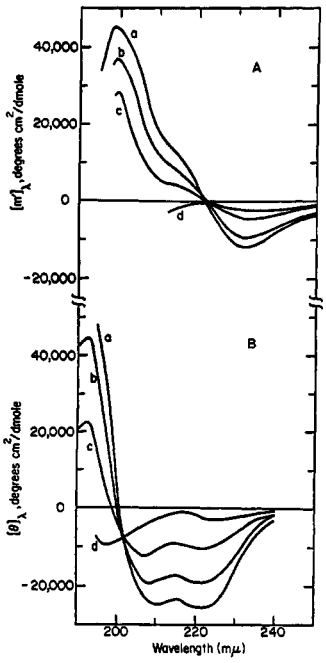

Figure 1. (A) ORD and (B) CD data in water at 24 ± 1° for P(HBG:Gly) copolymers and for PHBG: (a) PHBG, $\overline{DP}$ 200 (fraction VIB of paper II[4]); (b) $\overline{DP}$ 168, 3.0% Gly (fraction IXC-1); (c) $\overline{DP}$ 99, 8.9% Gly (fraction XIC-2); (d) $\overline{DP}$ 92, 18.0% Gly (fraction XIIB).

residues are not accumulated in blocks in the copolymers.[9-11] The distribution of these residues is, however, not completely random, as can be seen by an increased glycine content in the shorter chains (Table II); as mentioned earlier, this could arise if glycine acted as a better chain terminator. In the absence of direct experimental evidence on the randomness of the distribution, we rely on the observations cited above that, if blocks of glycine appear in the copolymer, they are not too long, and also on the conclusions in paper I[3] that such relatively short blocks would lead to the same melting behavior expected from a random copolymer, especially when the fraction of the guest component is relatively small, as it is here.

(B) ORD and CD Data for the Copolymers. The ORD and CD data of some of the copolymer fractions in water in the 190–250-mμ region at room temperature are shown in Figure 1, together with the data for a fraction of PHBG[4] of comparable chain length. The data indicate that fraction XIIB, containing the largest amount (18%) of glycine, is not helical, but that all of the other fractions are, at least partially, α helical. Similar ORD data for the same fractions in 90% aqueous methanol are shown in Figure 2. Comparison of Figures 1 and 2 indicates that the helix contents of all fractions (including fraction XIIB) are higher in methanol. The helix

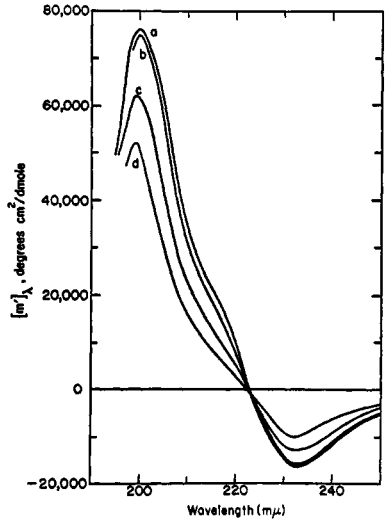

Figure 2. ORD data in 90% (v/v) aqueous methanol at 24 ± 1° for the same fractions (and same notation) of Figure 1.

content in water is also increased by lowering the temperature, as shown in Figure 3.

From Figures 1–3, it can be seen that the introduction of as little as 3 mol % glycine produces a considerable decrease of the helix content of the homopolymer. This decrease is much larger than that which would be expected for PHBG (using the Zimm–Bragg[12] σ and s parameters of paper II[4]) from the reduction in chain length which accompanies the incorporation of glycine. At relatively high glycine content, the ORD and CD spectra at room temperature resemble those of a random coil. For example, the CD spectrum of fraction XIIB ($\overline{DP}$ 92, 18.0% Gly) in water at room temperature (Figure 1B) has minima around 225 and 198 mμ and resembles the calculated CD curve of Greenfield and Fasman[13] for a polypeptide containing mostly random-coil and little or no α-helix or β structure; on the other hand, a sample of PHBG of $\overline{DP}$ 72 (fraction VID of paper II[4]) has ~40% helix content at 24°.[4] At high temperatures, both PHBG and the copolymers exhibit ORD curves which are characteristic of the random coil, admixed with little or no α-helical structure.

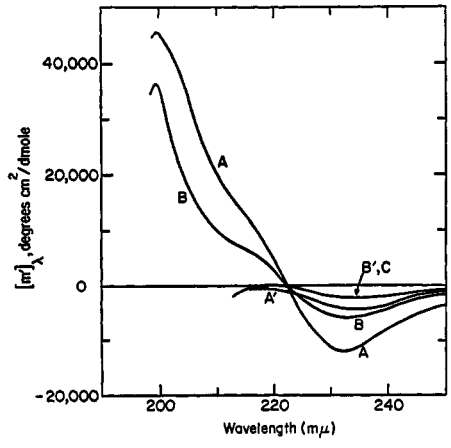

Figure 3. ORD data in water at low and high temperatures: (A and A′) $\overline{DP}$ 168, 3.0% Gly (fraction IXC-1) at 2 and 80°; (B and B′) $\overline{DP}$ 99, 8.9% Gly (fraction XIC-2) at 3 and 75°; (C) $\overline{DP}$ 92, 18.0% Gly (fraction XIIB) at 1° (the data for this fraction at 75° are identical with those at room temperature, shown in Figure 1).

(9) While data are available[10] for the kinetics of copolymerization of the *N*-carboxyanhydrides of glycine and γ-benzyl L-glutamate in dimethylformamide (using diethylamine as initiator) indicating that the rate constant for the polymerization of glycine is four times greater than that for the polymerization of γ-benzyl L-glutamate, these data do not necessarily apply to the dioxane–triethylamine solvent initiator used here. *If they did apply*, the faster intrinsic rate of polymerization of glycine would be partially compensated by the low concentration of glycine used here, leading closer to a random distribution of glycine in the copolymers; the persistence of some departure from randomness in such copolymers (using the solvent-initiator system of Shalitin and Katchalski)[10] was pointed out by Miller, *et al.*[11]

(10) Y. Shalitin and E. Katchalski, *J. Amer. Chem. Soc.* **82**, 1630 (1960).

(11) W. G. Miller, D. A. Brant, and P. J. Flory, *J. Mol. Biol.*, **23**, 67 (1967).

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

(13) N. Greenfield and G. D. Fasman, *Biochemistry*, **8**, 4108 (1969).

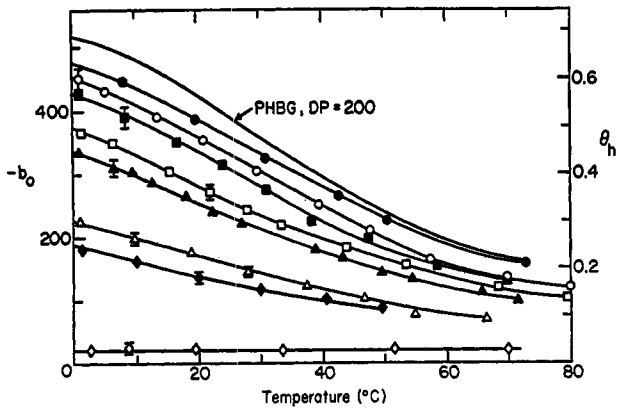

Figure 4. Temperature dependence of b_0 and θ_h for P(HBG:Gly) copolymers and for PHBG (fraction VIB, $\overline{DP}$ 200, of paper II[4]) in water. $\theta_h = -b_0/750$. The $\overline{DP}$'s and glycine contents of the copolymers are: (●) 510, 3.1% (fraction IXA); (○) 282, 3.0% (fraction IXB); (■) 168, 3.0% (fraction IXC-1); (□) 521, 4.1% (fraction XA); (▲) 334, 6.5% (fraction XB); (Δ) 100, 7.9% (fraction XC-1); (◆) 99, 8.9% (fraction XIC-2); (◇) 92, 18.0% (fraction XIIB). The error symbols indicate the average experimental errors in b_0. The curves drawn through the symbols are the *experimental* ones.

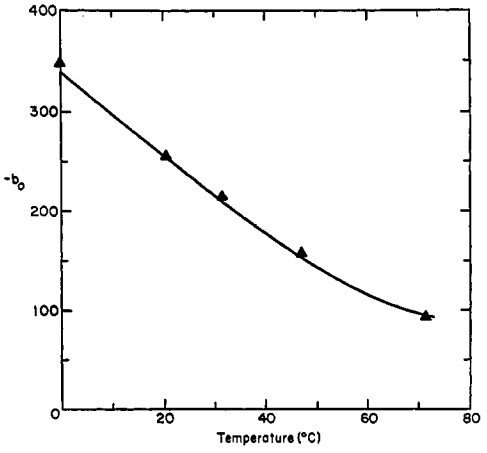

Figure 5. Demonstration of reversibility of the helix–coil transition in fraction XB ($\overline{DP}$ 334, 6.5% Gly) in water. The curve was obtained during the heating part of the cycle, and the points were obtained during the cooling part.

No evidence could be found in any of these spectra for any detectable amount of β structure.

The b_0 data for the various fractions in water, computed from measurements in the wavelength range of 280–450 mμ, support the above conclusions (see Figure 4).

(C) b_0 for Complete Helix and Complete Coil. The values of −750 and 0 were assigned to b_0 for the complete helix and complete coil forms, respectively, of the homopolymer PHBG in water.[4] Since the optical rotatory properties of a polypeptide may depend on the nature of the side chain,[14] it was necessary to establish that these values of b_0 also apply to copolymers containing glycine. For this purpose, the properties of the copolymers in 90% aqueous methanol at ~5° were examined, since they might be expected to attain similar helix contents in this solvent. It was found that, in 90% aqueous methanol at ~5°, b_0 ~ −680 to −700 for all copolymers containing 3–8.9% glycine (with $\overline{DP}$'s ranging from 100 to 300). Since this value is almost the same as that found for PHBG of comparable chain length (fraction VIB, $\overline{DP}$ 200, of paper II[4]), we may use the same value of $b_0 = -750$ for a complete helix in the copolymers. The value of $b_0 = 0$ for the random coil was assumed to be the same for the homopolymer and copolymers, since the high-temperature end of the melting curves of all polymers appear to approach this value. Thus, the helix content of all polymers was taken as $\theta_h = -b_0/750$.

(D) Helix–Coil Transitions. The thermally induced helix–coil transition was followed by examining the temperature dependence of b_0 (or θ_h), and the data are shown in Figure 4. As shown by a typical example in Figure 5, the transition was found to be reversible provided that the temperature was not increased beyond ~80°.[15] This reversibility, as well as the absence of visual turbidity or a concentration dependence of b_0 over the range of 0.1–0.5%, suggests that no aggregation occurs at any stage of the transition, even in the copolymers of highest glycine content.

The errors in b_0 (and in θ_h) were calculated in a manner similar to that discussed in paper II,[4] and are shown for several values of b_0 in Figure 4. It can be seen from Figure 4 that θ_h decreases with increasing glycine content. Also, for a given glycine content, θ_h increases with increasing chain length. Even without a quantitative analysis of the data, we see from Figure 4 that glycine acts as a helix breaker; hence, we would expect the value of the Zimm–Bragg parameter s to be smaller than unity in this temperature range.

III. Discussion

(A) Helix–Coil Parameters for Polyglycine. In paper I,[3,16] it was shown that the lower orders of the LAPS hierarchy of approximations could 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[16] (corresponding to theories of Lifson[17] and Allegra[18]) were used *initially*, and checked in a few representative cases against the exact theory of Lehman and McTague for finite chain length.[19,20] The comparison of the approximate and exact theories was made with glycine parameters deduced below (and the PHBG parameters of paper II[4]) and is shown in Table III; it can be seen that, whereas the Lifson theory is not suitable in this range of parameters (even though it was for the polymers considered in paper II[4]), the Allegra theory (first-order LAPS approximation) is valid for glycine parameters.

The host–guest technique, used to obtain σ and s for glycine from the experimental θ_h *vs.* T values of the copolymers (Figure 4) and from the values of σ and s for PHBG (given in Table II of paper II[4]), was identical with that used previously.[4] In this calculation, σ was assumed to be independent of temperature for both components and s was computed for glycine at *each* temperature in the range of 0–70°. Suffi-

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

(15) The transition is probably reversible above 80° also. However, above this temperature, evaporation of solvent, even in stoppered cells, makes it difficult to carry out experiments.

(16) Paper I[3] should be consulted for a discussion of the theories of the helix–coil transition in random copolymers, and for the use of the Lifson–Allegra–Poland–Scheraga (LAPS) hierarchy of approximations in evaluating σ and s for the "guest" residue in a copolymer.

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

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

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

(20) All computer programs used in this work are available. See footnote 26 of paper I[3] for the procedure for obtaining them.

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

Glycine content, mol fraction	$\overline{DP}$	Temp, °C	$(\theta_h)_{theor}$ Lifson	Allegra	Lehman–McTague
0.030	282	0	0.661	0.613	0.611
		30	0.410	0.392	0.391
		60	0.214	0.209	0.209
0.030	168	0	0.603	0.562	0.558
		30	0.368	0.354	0.352
		60	0.196	0.192	0.192
0.065	334	0	0.534	0.446	0.440
		30	0.305	0.281	0.279
		60	0.158	0.152	0.152
0.079	100	0	0.328	0.284	0.273
		30	0.192	0.182	0.179
		60	0.110	0.107	0.106
0.089	99	0	0.296	0.255	0.249
		30	0.176	0.166	0.166
		60	0.102	0.099	0.099
0.180	92	0	0.099	0.089	0.086

[a] The parameters used for glycine are those of Table IV, while those for hydroxybutylglutamine were taken from Table II of paper II.[4]

cient sensitivity could not be obtained in this analysis to be able to specify the value of σ to better than the range[21] from 10^{-6} to 10^{-3}; therefore, the value of 1×10^{-5} was chosen for σ (although 1×10^{-4} gives the same results),[22,23] and the values of s at each temperature were computed by fitting the theory to the experimental θ_h *vs.* T data.[20] These are shown in Table IV and in Figures 6 and 7, and, as expected, can be seen to be less than unity.

Using the Allegra theory and the parameters of Table IV, theoretical melting curves (shown in Figure 8, together with the experimental data) were computed. Considering the

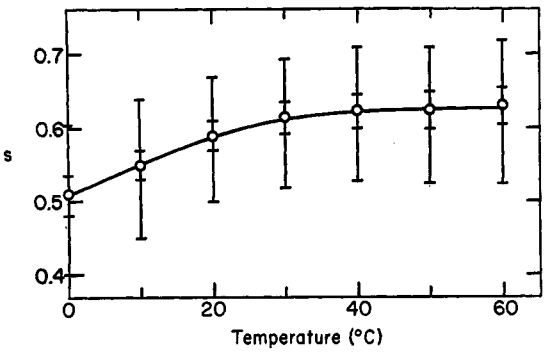

Figure 6. A plot of s *vs.* T (with $\sigma = 1 \times 10^{-5}$) for polyglycine in water. The precision of the data is given by the vertical lines, which correspond to one and two standard deviations, respectively, in s.

(21) This is because of the low glycine content of the polymer, making nucleation of helical regions in a pure-glycine region of the *random* copolymer a very improbable event; even in a homopolymer, the ratio of the number of residues initiating helical sequences to the number in helical sequences at the melting temperature is about $\sigma^{1/2}$, and the probability of a dilute guest component in a copolymer initiating a helical sequence is much smaller.

(22) These values lie in the range of those computed by Gō, *et al.*,[23] for polyglycine.

(23) M. Gō, N. Gō, and H. A. Scheraga, *J. Chem. Phys.*, **54**, 4489 (1971).

TABLE IV
VALUES OF THE ZIMM–BRAGG PARAMETER s FOR POLYGLYCINE IN WATER FROM 0 TO 70°

Temp, °C	s[a]
0	0.510
10	0.550
20	0.591
30	0.615
40	0.625
50	0.625
60	0.631
70	0.610

[a] The value of σ used is 1×10^{-5}. The error involved in the estimation of s is shown in Figure 6.

errors[24] in the values of θ_h (shown by the error symbols in Figure 8), the theoretical curves fit the experimental data very well, with the parameters selected by the curve-fitting procedure.

The slight increase in the value of s with T for glycine is in agreement with that computed by Gō, *et al.*,[23] and attributed by them to decreased binding of water to NH and CO groups in the coil form of the backbone as the temperature is increased. However, the absolute values of s found here are somewhat higher than those computed by Gō, *et al.*[23]

The temperature dependence of s may be expressed as

$$s = \exp[-\Delta G/RT] = \exp[-\Delta H/RT + \Delta S/R] \quad (3)$$

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 of a long helical sequence. A plot of $-R \ln s$ *vs.* $1/T$ for glycine is shown in Figure 7. The experimental error involved (shown as vertical lines in Figure 7 for one and two standard deviations) in the determination of s makes it difficult to obtain a reliable estimate of ΔH and ΔS. Assuming that both of these are temperature independent, the least-squares fit of the data in Figure 7 to a straight line gave the mean values of ΔH and ΔS as 625 ± 100 cal/mol and 1.0 ± 0.3 eu, respectively. These values can be compared with the range predicted by Gō, *et al.*[23,25]

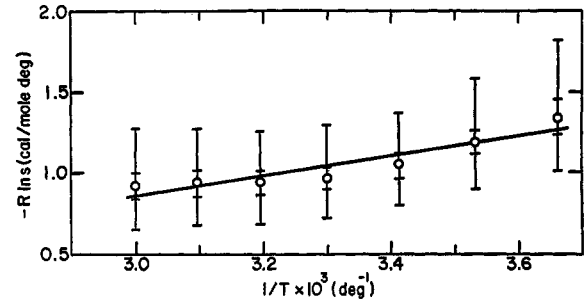

Figure 7. A plot of $-R \ln s$ *vs.* $1/T$ for polyglycine to assess the dependence of ΔG on T (for $\sigma = 1 \times 10^{-5}$). The precision of the data is given by the vertical lines, which correspond to one and two standard deviations, respectively, in s.

(24) The errors in θ_h shown in Figure 8 were computed by the procedure described in paper II.[4] This involves the calculation of the error in θ_h arising from errors in the determination of b_0 (shown in Figure 4), molecular weight, and composition. The error in θ_h arising from molecular weight measurement was computed to be from 0.5 to 1.6% over the $\overline{DP}$ range of 334–92, while the error in θ_h originating from compositional analysis was estimated to vary from 1.3 to 7.5% over the composition range of 3–18% glycine.

(25) The values of Gō, *et al.*,[23] were computed for temperature-*dependent* ΔH and ΔS, whereas this temperature dependence is not detectable in the data of Figure 7 because of the experimental error. The ranges of values of Gō, *et al.*,[23] were 1380–470 cal/mol for ΔH and 0.4 to −2.5 eu for ΔS in the temperature range of 7–87°.

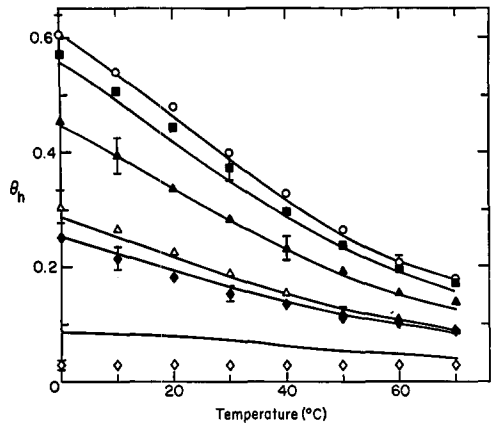

Figure 8. Calculated melting curves (Allegra theory with glycine parameters of Table IV and HBG parameters of paper II[4]) for P(HBG:Gly) copolymers, together with experimental data (smoothed points from Figure 4 at 10° intervals). The DP's and glycine contents of the copolymers are: (○) 282, 3.0% (fraction IXB); (■) 168, 3.0% (fraction IXC-1); (▲) 334, 6.5% (fraction XB); (△) 100, 7.9% (fraction XC-1); (◆) 99, 8.9% (fraction XIC-2); (◇) 92, 18.0% (fraction XIIB). The error symbols indicate the average errors in θ_h, computed as described in the text.

The values of s found here for glycine are compared with those for several other polypeptides in water in Figure 9. The data for alanine[26] and leucine[27] were obtained with block copolymers, while those for HPG and HBG are from paper II.[4] It can be seen from Figure 9 how the relative helix-forming abilities (*i.e.*, variation in s at a given temperature) compare for the various amino acids. While the dehydration of the coil form dominates for glycine, it is outweighed by other intramolecular interactions[28] for the other polymers for which s decreases with temperature; in the case of leucine, hydrophobic interactions[27] account for the increase in s with temperature.

It should be emphasized that the parameters σ and s for glycine given in Table IV apply to the temperature range given there, and cannot be extrapolated (without additional assumptions) to higher or lower temperatures. Also, these parameters apply to a glycine (in water) in a PHBG environment. While we do not yet know how sensitive these parameters are to the nature of the host, we would not expect their values to vary much from host to host, on the basis of the discussion of the importance of near-neighbor interactions (between a side chain and the backbone atoms of the same residue) referred to in the introduction of paper I,[3] and on the basis of the experimental results reported in paper II.[4] Hence, these parameters for glycine may be used, as discussed in the next section, to assess its role in influencing the conformation of a protein.

(B) Conformational Role of Glycine in Proteins. The low values of s for glycine, in the temperature range of 0–70°, provide a quantitative basis for classifying this residue as a helix breaker in a protein, even though it is sterically capable of fitting into a helical region; the energetic considerations (primarily the binding of water to the random-coil form and the absence of side-chain-to-backbone interactions) which prevent polyglycine from adopting an α-helical conformation were discussed elsewhere.[23] It was shown previously[28] that

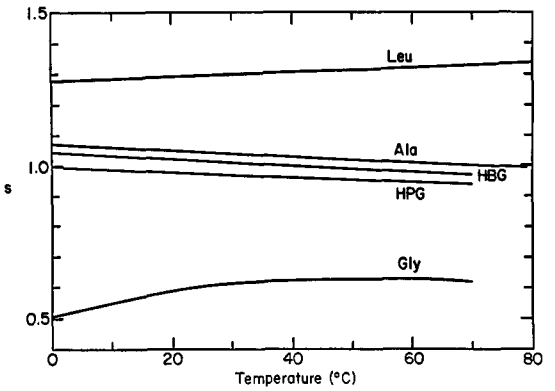

Figure 9. Temperature dependence of s for various amino acid residues. The sources of the data are given in the text.

a helical region in a protein can tolerate one helix-breaking residue without a serious disruption, but that two or more helix-breaking residues terminate a helical sequence. These conclusions can now be presented more quantitatively, by means of helix-probability profiles.[29] Using the parameters which are in Table IV for glycine, and those for HBG of Table II, paper II,[4] we have computed the helix-probability profiles of a specific (arbitrary) sequence of these two residues in a chain of $\overline{DP}$ 200, 5% glycine, using the procedure of Lewis, *et al.*;[29] these are shown in Figure 10 at several temperatures. First of all, the helix-breaking character of glycine is evident at all temperatures. However, the strong helix-forming property of the host (HBG) residue tends to force an isolated glycine residue (*e.g.*, no. 17 in Figure 10) into the helical region, through the near-neighbor interaction (characteristic of the one-dimensional Ising model) existing in the copolymer. Secondly, the profound effect of the proximity of two (and three) glycyl residues can be seen.[28] The relatively smaller effect of the glycyl residues as the temperature increases arises from the fact that s increases for glycine, while it decreases for HBG.

(C) Comparison with Other Results. Glycine acts as a helix breaker, not only in water, but also in organic solvents (see the next section). This behavior in organic solvents had

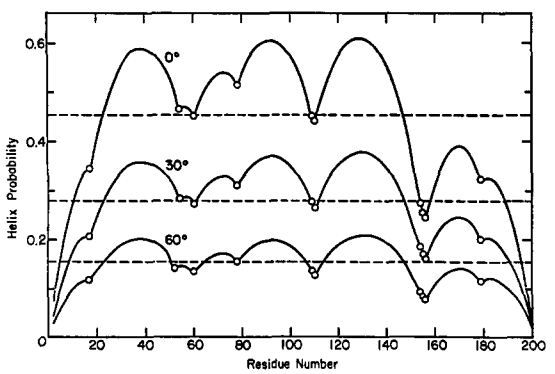

Figure 10. Helix-probability profiles (for the temperatures shown, computed for a copolymer, P(HBG:Gly), of chain length 200) containing 5% glycine. The positions of the glycine residues in the sequence are shown as circles. The values of σ and s for glycine are those of Table IV, while those for HBG are from paper II.[4] The horizontal dashed lines represent the average helix content at each temperature.

(26) R. T. Ingwall, H. A. Scheraga, N. Lotan, A. Berger, and E. Katchalski, *Biopolymers*, 6, 331 (1968).
(27) S. E. Ostroy, N. Lotan, R. T. Ingwall, and H. A. Scheraga, *ibid.*, 9, 749 (1970).
(28) D. Kotelchuck and H. A. Scheraga, *Proc. Nat. Acad. Sci. U. S.*, 62, 14 (1969).
(29) P. N. Lewis, N. Gō, M. Gō, D. Kotelchuck, and H. A. Scheraga, *ibid.*, 65, 810 (1970).

been observed previously by others.[30,31] Goodman and Rosen[30] found that cooligomers of γ-ethyl L-glutamate (ELG) and glycine exhibited no secondary structure ($b_0 = 0$) in trifluoroethanol, whereas the oligomers of ELG of comparable size showed considerable helix content ($b_0 = -165$ for a $\overline{DP}$ of 8). They attributed the effect of glycine to the ease of solvation of amide groups. Fraser, *et al.*,[31] noticed that the incorporation of glycine in a regular-sequence copolymer with ELG reduced the stability of the ELG helix, as detected by its effect on the solvent-induced helix–coil transition in a mixture of ethylene dichloride (EDC) and dichloroacetic acid (DCA). Block and Kay,[32] who studied block copolymers of glycine and γ-benzyl L-glutamate in the same solvent mixture, found that glycine residues at the ends of the block copolymer did not adopt the helical structure formed by the glutamate residues in the middle of the copolymer. This is in agreement with the low values of s found here for glycine. They[32] also studied random copolymers of these two monomers and observed a much higher helix content than they expected for a truly random copolymer; from this they concluded that their copolymers were not random, since they expected every glycine residue (including isolated ones) to be nonhelical. However, as shown here, in a *random* copolymer, isolated glycine residues (imbedded among strong helix-forming ones) can be forced to adopt a helical conformation (see Figure 10).

(D) Helix–Coil Transition in Organic Solvents. The random copolymers P(BzG:Gly) (samples II–VII) are soluble in mixtures of DCA and EDC. Hence, their thermal transitions in a 70/30 (w/w) DCA–EDC mixture (which are inverted ones[33] in this case) were examined, and will be reported in greater detail in a subsequent paper.[34] It was observed[34] that the thermal transition curves of these random copolymers are shifted to higher temperature with increasing glycine content, indicating that glycine is a helix breaker in this mixed solvent. An analysis for the σ and s values of polyglycine in this organic solvent,[34] and a comparison with the values of Table IV for water, should provide information about the influence of solvent on σ and s.

IV. Conclusion

In summary, we have synthesized water-soluble random copolymers containing glycine and hydroxybutylglutamine and studied their melting behavior in water. By applying the "host–guest technique"[3,4] for obtaining parameters for the "guest" amino acid (glycine), we have determined the Zimm–Bragg parameters s and σ for the coil-to-helix transition for polyglycine in water. The relatively low values of s and its temperature dependence have been found to agree with those predicted by Gō, *et al.*,[28] on the basis of conformational energy calculations. They also provide an explanation for the nonoccurrence of the α-helical form of polyglycine in water at normal temperatures. The implications of the finding that glycine acts as a strong helix breaker in water are discussed in connection with the analysis of protein conformation.

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(30) M. Goodman and I. G. Rosen, *Biopolymers*, **2**, 537 (1964).
(31) R. D. B. Fraser, B. S. Harrap, T. P. Macrae, F. H. C. Stewart, and F. Suzuki, *ibid.*, **5**, 251 (1967).
(32) H. Block and J. A. Kay, *ibid.*, **5**, 243 (1967).
(33) P. Doty and J. T. Yang, *J. Amer. Chem. Soc.*, **78**, 498 (1956).
(34) V. S. Ananthanarayanan, E. Leroy, R. H. Andreatta, and H. A. Scheraga, manuscript in preparation.

A Theory of the Rate of Conformational Change of Isotactic Poly(α-olefin) Molecules in Solution

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ABSTRACT: A theory of the effect of hydrodynamic interaction on the uncoiling of isotactic poly(α-olefins) in solution is given. The quantity calculated is the optical rotation in a temperature-jump experiment. The model uses Kramers' theory for the escape of particles over potential barriers and neglects cooperative interactions. As an example, the optical activity change of poly((*S*)-4-methyl-1-hexene) resulting from a temperature jump is predicted.

The rate of conformational change in molecules is a subject of considerable interest. For small molecules these rates depend on properties of neighboring atomic groups and bonds and on solute–solvent interactions. In macromolecules, they may depend, in addition, on the degree of polymerization.

A case of particular interest is the rate of unwinding of spiraled macromolecules. For example, a number of authors have considered, theoretically, the rate of unwinding of the double helix of DNA. In this paper we wish to consider a somewhat simpler case, that of isotactic poly(α-olefins). These molecules exist in solution primarily as helices because of steric factors. They were chosen for study because they are, at least in first approximation, free of complicating long-range cooperative interactions, and because their conformational changes are amenable to convenient experimental study, in many cases, through monitoring their optical activity.

The paper is organized as follows. Section I discusses the model and solves the problem formally. Section II concerns the rotatory friction coefficients which enter the solution. Section III presents the final results and Section IV is a discussion.