Helix–Coil Stability Constants for the Naturally Occurring Amino Acids in Water. II. Characterization of the Host Polymers and Application of the Host–Guest Technique to Random Poly(hydroxypropylglutamine-*co*-hydroxybutylglutamine)[1]

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ABSTRACT: The melting behavior of the homopolymers poly[N^5-(3-hydroxypropyl)-L-glutamine] and poly[N^5-(4-hydroxybutyl)-L-glutamine], and copolymers of these two amino acids, was determined in water. The homopolymer data were treated by the Zimm–Bragg theory and the copolymer data were analyzed with the lowest order approximation of the theory discussed in the previous paper. Within the experimental error, it was not possible to detect any temperature dependence of the parameter σ or of the parameters ΔH and ΔS for the homopolymers. Using these temperature-independent parameters for one of the homopolymers, it was possible to compute those for the other homopolymer by applying the host-guest technique (and associated theory) to the copolymer data. Good agreement was obtained between the parameters computed directly from homopolymer data and those obtained by the host–guest technique, thus establishing the validity of the latter method for future use in obtaining the helix–coil stability constants for other amino acids.

In paper I[3] of this series, we discussed the melting properties of copolymers, and showed that the low orders of the Lifson–Allegra–Poland–Scheraga (LAPS) hierarchy of approximations were satisfactory for analyzing the melting data obtained from random copolymers for certain ranges of amino acid parameters. With either this approximate theory or an exact theory, it is possible to compute the melting curves for such a copolymer, having any composition of the two constituents, from the parameters which characterize the melting behavior of the two homopolymers. Alternatively, regarding one of the homopolymers as the "host" and introducing the second constituent as the "guest" to form random copolymers having increasing amounts of the guest component, it is possible to calculate the parameters characterizing the helix–coil transition of the homopolymer of the guest units from the melting data for the copolymers and for those of the host homopolymer. This procedure (referred to as the "host–guest" technique) is applicable provided that the copolymers still consist of α-helical and coil regions; it is conceivable that, in some cases, the copolymer may adopt a different conformation if the fraction of guest residues becomes very high; in such a case, the study must be confined to copolymers having a low fraction of guest residues. Thus, with a suitable host homopolymer, it should be possible to determine the helix–coil stability constants in water for all of the naturally occurring (guest) amino acids. The applicability of the theory to various amino acids in this manner is based on the assumption, justified elsewhere,[4–7] that the conformation of a residue depends primarily on nearest-neighbor interactions, *i.e.*, that the statistical weight of a residue is independent of the chemical nature of its neighboring residues, and also on the assumption that the copolymer has been synthesized under conditions leading to a random distribution, at least to the extent required by the theory, as discussed in paper I.[3]

In this paper we consider two nonionic homopolymers, either of which can serve as the host for application of the host–guest technique, *viz.*, poly[N^5-(3-hydroxypropyl)-L-glutamine] (PHPG) and poly[N^5-(4-hydroxybutyl)-L-glutamine] (PHBG). These two polymers had been synthesized, and the helix–coil transition properties of one of them (PHPG) investigated, by Lotan, *et al.*,[8–10] and by Okita, *et al.*,[11] We consider the synthesis and melting behavior of the two homopolymers and of copolymers of varying composition prepared from the two monomers. The host–guest technique for obtaining thermodynamic parameters is demonstrated by using the melting data for PHBG and the copolymers to obtain the parameters for PHPG, which can be compared with the parameters obtained directly from the melting behavior of PHPG. The same procedure is also used to obtain parameters for PHBG from those for PHPG and the melting data for the copolymers. Since similar values of the parameters are obtained by the host–guest technique and by studies on the homopolymers, we may apply the host–guest technique in the future to copolymers for which the guest homopolymer does not satisfy the experimental requirements set forth in the introduction of paper I.[3] The two homopolymers PHBG and PHPG studied here will be used as the hosts for the copolymers to be discussed in future papers in this series.

The application of the host–guest technique will yield two kinds of information about the guest residue. First, and of greater interest, we obtain the σ and s values of the guest *over the range of temperatures studied*, which includes room temperature, for use in assessing the stability of proteins at

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room temperature,[6] as discussed in the introduction of paper I.[3] Second, we can obtain the transition temperature and the thermodynamic parameters ΔH and σ at the transition temperature of the guest homopolymer. Naturally, the more of the guest amino acid which is included in the copolymers (provided the guest confines itself to coil and helical conformations), the less it is necessary to extrapolate and the more reliable will be the parameters at the transition temperature. For some amino acids, the transition temperature of the homopolymer may be 100–200° above or below the range of temperatures studied. In these cases, the inclusion of even moderate amounts of the guest shifts the transition temperature of the copolymer outside the experimentally accessible region, *i.e.*, that in which water is a liquid. In such a case, the *extrapolated* values of the transition temperature and ΔH and σ at the transition temperature may be less accurate.

I. Experimental Section

(A) Materials. 3-Amino-1-propanol (Aldrich Chemical Co.) and 4-amino-1-butanol (Chemical Procurement Laboratories or K and K Chemicals Co.) were dried over barium oxide and distilled either at atmospheric or reduced pressure before use. Dioxane was purified by refluxing for 6 hr over sodium and then distilling at atmospheric pressure. Benzene (thiophene free) was refluxed for 12 hr over calcium hydride and distilled at atmospheric pressure. Triethylamine was dried and distilled over KOH. Dimethylformamide (DMF) was dried over BaO and distilled under reduced pressure before use. *n*-Hexylamine was dried over BaO and distilled at atmospheric pressure. The *N*-carboxy anhydride (NCA) of γ-benzyl L-glutamate, when purchased, was obtained from Pilot Chemical Co. and was recrystallized three times from benzene–dioxane (2/1, v/v) by adding hexane until turbidity first appeared and then chilling the solution to induce further crystallization. All solvents used when synthesizing and subsequently recrystallizing the NCA of γ-benzyl L-glutamate were purified shortly before use. Acetone was spectroanalyzed grade. All other reagents and solvents were anhydrous laboratory reagent grade, and were used without further purification.

(B) Synthesis. Poly(γ-benzyl L-glutamate) (PBLG). γ-Benzyl L-glutamate NCA was synthesized by preparing γ-benzyl L-glutamate from L-glutamic acid and treating it with phosgene as described by Stewart and Ledger.[12]

After a preliminary investigation of the molecular weights of PBLG obtained with various anhydride-to-initiator (A/I) ratios, following the results of Blout and Karlson,[13] the γ-benzyl L-glutamate NCA was polymerized using an initiator, solvent, and A/I ratio to yield PBLG of the desired degree of polymerization (DP). The initiator–solvent systems used were *n*-hexylamine–dimethylformamide, triethylamine–dioxane, sodium hydroxide–dioxane, and sodium methoxide–dioxane. The A/I ratios ranged from 25 to 100 depending on the system. PBLG samples were obtained whose average DP ranged from 100 to 2300 as assessed by viscosity measurements.[14]

Poly[N^5-(3-hydroxypropyl)-L-glutamine] (PHPG) (I–III). The substitution of the hydroxypropyl group for the benzyl group in PBLG was carried out by the procedure described by Lotan, *et al.*,[8] with the following modifications. The amount of dioxane used per gram of polymer varied from 2 to 8 ml/g. The amino alcohol was added in small portions over a period of 3–5 days. Addition of an aliquot was made only after the previous aliquot had reacted to form a completely homogeneous, clear solution with the other reagents in the system. The polymers were isolated by pouring the reaction mixture into 1 *N* acetic acid (300 ml), dialyzing exhaustively against distilled water, filtering the solution through a Millipore filter (0.45-μ pore size, Millipore, Bedford, Mass.), and lyophilizing the filtrate.

Poly[N^5-(4-hydroxybutyl)-L-glutamine] (PHBG) (IV–VII). The PHBG polymers were obtained by following a procedure similar to that employed for the PHPG polymers,[8] using 4-amino-1-butanol instead of 3-amino-1-propanol. The high molecular weight samples were treated in the same way as samples I–III. In order to avoid losses during dialysis, the low molecular weight samples were isolated by precipitation with chloroform–ethyl acetate (6/4, v/v) and dried over P_2O_5 under vacuum. Samples showing benzyl absorption (of more than 1 benzyl group per 100 amino acid residues[8]) were rejected. Retention of optical configuration was checked by the same procedure used by Lotan, *et al.*;[8] glutamic acid liberated from PHBG had an optical rotation which was 97–101% of the value found for a sample of glutamic acid taken through the same hydrolysis procedure.

Poly{[N^5-(3-hydroxypropyl)-L-glutamine-*co*-(4-hydroxybutyl)-L-glutamine]} [PH(P:B)G] (VIII–XII). These copolymers were prepared from PBLG by one of the following two methods.

In the first procedure, the PBLG polymer was treated with 3-aminopropanol, following Lotan, *et al.*,[8] but stopping the reaction before it was complete; this resulted in the substitution of some but not all of the benzyl groups. The excess aminopropanol was washed out with ethyl acetate, the polymers were dried over P_2O_5 *in vacuo*, and the remaining benzyl groups were substituted with 4-aminobutanol. To obtain copolymers of various P/B ratios, the reaction with 3-aminopropanol was stopped at various times during the propyl substitution. Sample VIII was removed after 5 hr and sample XI after 20 hr, and the separate samples were treated with 4-aminobutanol. After the reaction was complete, the resulting PH(P:B)G copolymer was precipitated and washed free of base with chloroform–ethyl acetate (6/4, v/v); the solid was then dissolved twice in methanol, precipitated with ether to remove any remaining amino alcohol, and dried over P_2O_5 under vacuum.

In the second method, the PBLG polymer was treated with a mixture containing a given ratio of 3-aminopropanol and 4-aminobutanol, the mixture being added in small aliquots over a period of time to avoid completely precipitating the polymer. Sample IX was prepared using 30/70 (v/v) aminopropanol–aminobutanol, and X and XII were prepared using ratios of 45/55 and 80/20, respectively. When the reaction was complete, the copolymer was isolated in the same manner as in the first method.

(C) Fractionation. The homopolymers PHPG and PHBG and the copolymers PH(P:B)G were fractionated by a precipitation procedure in which the polymer was dissolved in pure methanol to give a 2% (w/v) solution (any insoluble material being filtered off) and treated with increasing amounts of ether. The precipitate obtained after each addition was isolated by centrifuging and decanting the supernatant liquid. When necessary, large fractions were refractionated. High molecular weight fractions were dissolved in water and lyophilized, while low molecular weight ones were treated in the same manner as samples IV–VII. All polymers were dried over P_2O_5 under vacuum.

(D) Viscosity. Viscosity measurements were made at 24.8 ± 0.01° with a Cannon–Ubbelohde semimicro dilution viscometer. The outflow times using dichloroacetic acid as the solvent ranged from 120 to 410 sec.

(E) Measurement of Concentration. The concentrations of the homopolymer and copolymer solutions used in all physical measurements on the fractionated material were determined by micro-Kjeldahl nitrogen analysis.[15,16]

Since the optical rotatory dispersion (ORD) and circular dichroism (CD) measurements require the concentration in moles of monomer per unit volume, and since the hydroxypropyl- and hydroxybutylglutamine residues contain two atoms of nitrogen each, the residue concentration can be determined without requiring the knowledge of the propyl/butyl ratio. However, this ratio is required for calculating theoretical melting curves of the copolymers.

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(F) Nuclear Magnetic Resonance Measurements. Nuclear magnetic resonance (nmr) measurements were made on each sample to determine the propyl/butyl ratio, making use of information obtained in an earlier nmr study of these polymers.[17] The spectra were run on a Varian HA-100 spectrometer using a precision-bore Wilmad Glass Co. coaxial insert containing 10% tetramethylsilane (TMS), 5% chloroform, and 85% carbon tetrachloride for the field-frequency lock signal. The frequencies were swept at a rate of 2 cps at ambient temperature (~35°). The polymers were lyophilized from deuterium oxide (D_2O) and then dissolved in D_2O to a concentration of 5% (w/v). Five spectra were run on each sample at each applied radiofrequency field. The areas under the peaks were measured with a Keuffel and Esser planimeter.

Following Joubert, *et al.*,[17] we designate the carbon atoms of the $-NH-CH_2-CH_2-CH_2-OH$ and $-NH-CH_2-CH_2-CH_2-CH_2-OH$ side chains of PHPG and PHBG as 3, 4, 6, and 3, 4, 5, 6, respectively, from left to right. In PHBG, the peaks for protons on C_4 and C_6 are at δ 1.80 and 3.66, respectively;[17] both peaks correspond to two protons, and thus should have the same area. In PHBG, the peaks appear at similar positions (δ 1.62 for C_4 and C_5 protons and 3.65 for C_6 protons); however, the area under the $C_4 + C_5$ peak (δ 1.62) is twice that under the C_6 peak (δ 3.65). In a copolymer, the area under the C_6 peak will be the same as it is in the homopolymers, and the area under the $C_4 + C_5$ peak will be intermediate between the values for pure PHPG and pure PHBG. Thus, the mole per cent of butyl substituent can be determined, since the method makes use of an internal standard (the C_6 peak), by the formula

$$\text{mol \% butyl} = \frac{\text{area}(\delta \sim 1.70) - \text{area}(\delta\ 3.65)}{\text{area}(\delta\ 3.65)} \times 100 \qquad (1)$$

A plot of the applied radiofrequency field against the area under a peak was linear, indicating that no saturation effects were present. As a check on the procedure, several mixtures of known composition of the two homopolymers were prepared and assayed by nmr. The mole per cent of butyl determined by nmr agreed with the known composition within the precision (±4 mol %) of the measurement.

(G) Ultracentrifugal Studies. The molecular weights of the samples were determined from equilibrium runs on aqueous solutions of the polymers in a Spinco Model E ultracentrifuge. Interference optics were used in obtaining both $\bar{M}_w$ and $\bar{M}_z$. The centerpiece for most measurements was a Yphantis type with six channels and parallel walls. The addition of Spinco FC-43 oil produced a visible lower meniscus. In general, solutions of 0.1 to 0.4% (w/v) concentration, having 3–18 interference fringes in synthetic boundary experiments, were used in sedimentation equilibrium runs with 0.17–0.35-cm columns; at equilibrium (achieved in 6–24 hr at 8225–25,980 rpm), 6–18 fringes were present. A Gaertner two-dimensional comparator was used to measure distances on the photographic plates.

Since all of the samples were found to be polydispersed, application of centrifugal force will cause a redistribution of the species throughout the cell depending not only on the distance from the center of rotation but also on the molecular weight of the particles. The value of the weight-average molecular weight, M_{wx}, for the material at any distance x from the center of rotation is given by eq 2,[18] where the symbols are defined as follows: R is the universal

$$M_{wx} = \frac{2RT}{\omega^2(1 - \bar{v}\rho)} \frac{d \ln c}{dx^2} \qquad (2)$$

gas constant, T is the absolute temperature, ω is the angular velocity of the rotor, $\bar{v}$ is the partial specific volume of the solute (taken as 0.79 for PHPG[8] and 0.81 for PHBG—the latter being obtained by adding the partial specific volume for a CH_2 group[19] to the value for PHPG; an average value, depending on the composition, was taken for the copolymers), ρ is the density of the solution (assumed equal to the density of water), and c is the concentration of the solution. For polydispersed systems, M_{wx} takes on different values at different points x in the cell. The weight-average molecular weight of the whole sample in the cell, $\bar{M}_w$, is not simply equal to M_{wx} but must be obtained by averaging M_{wx} over the entire contents of the cell. In computing such an average, if the concentration distribution (c_x at any point x) used is that in a sector-shaped cell, the result as shown by Lansing and Kraemer[20] is the particularly simple expression for $\bar{M}_w$ given in eq 3, where

$$\bar{M}_w = \frac{2RT}{\omega^2(1 - \bar{v}\rho)} \frac{c_b - c_m}{c_0} \frac{1}{x_b^2 - x_m^2} \qquad (3)$$

c_b and c_m are the concentrations at x_b and x_m, respectively, *i.e.*, at the bottom of the cell and at the meniscus, and c_0 is the (uniform) concentration before centrifugation. The *z*-average molecular weight of the whole sample, $\bar{M}_z$ is given by

$$\bar{M}_z = \frac{M_{wb}c_b - M_{wm}c_m}{c_b - c_m} \qquad (4)$$

The multichannel cells have parallel walls rather than sector-shaped walls. We have assumed that the concentration distribution in the parallel-walled cell can be approximated by that in the sector-walled cell, and have therefore used eq 3 and 4 in analyzing our data. We checked this assumption by determining the molecular weight of the same fractionated (but still somewhat polydispersed) samples in both sector-shaped and parallel-walled cells and found that they did not differ by more than 4%, which is within the experimental error of the measurement.

The molecular weights of the homopolymers and copolymers were found to be independent of concentration over the range of 0.07–0.33%.

(H) Optical Rotatory Dipsersion Measurements. Optical rotatory dispersion (ORD) measurements were carried out with a Cary Model 60 spectropolarimeter, equipped with a 450-W Osram xenon arc lamp. Water-jacketed quartz cells, with path lengths of 0.1, 1.0, and 2.5 cm were used. The temperature was kept constant (to ±0.2°) by water flowing through the cell with the use of a Haake Type F circulating bath. A calibrated thermistor probe was used to measure the temperature just beyond the cell. Solvent base lines were recorded for all sample runs at the corresponding temperatures. The data were independent of concentration in the range studied (0.3–1.0%, w/v).

The reduced mean residue rotation, $[m']_\lambda$ ((deg cm^2)/dmol), was calculated from the observed rotation, α_λ, at each wavelength using

$$[m']_\lambda = [3/(n^2 + 2)](100/cl)\alpha_\lambda \qquad (5)$$

where n is the refractive index of the solution, c is the concentration in moles of residues/liter, and l is the cell path length in centimeters. The values of n were corrected for dispersion of n with λ for water (using the Duclaux–Jeantet data)[21,22] and for methanol.[23] The concentration was corrected for changes in the density of water with temperature using the Thiesen equations[21] (this correction varied from 0.2 to 2% as b_0 varied from low to high temperature, respectively).

The parameters a_0 and b_0 of the Moffitt–Yang equation[24] were obtained as suggested by Urnes and Doty.[25] The quantity $[m']_\lambda \cdot (\lambda^2 - \lambda_0^2)/\lambda_0^2$ was plotted against $\lambda_0^2/(\lambda^2 - \lambda_0^2)$, using $\lambda_0 = 212$ mμ; a_0 and b_0 were obtained as the intercept and the slope, respectively, of the resulting straight line. The actual computations and plotting were carried out on a computer, using a least-squares

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fit of the data. The wavelengths used were chosen from 440 to 280 mμ to correspond to equally spaced points on the $\lambda_0^2/(\lambda^2 - \lambda_0^2)$ axis.

(I) Circular Dichroism Measurements. Circular dichroism measurements were made with a CD attachment, Model 6001, to the Cary Model 60 spectropolarimeter, using a quartz cell with 1-mm path length at 24°. Solvent base lines were recorded for all sample runs, and the polymer concentration was 0.015–0.030%, w/v. Measurements were made in the wavelength range of 240–188 mμ.

The observed ellipticity, θ_λ, at each wavelength was converted to molar ellipticity, $[\theta]_\lambda$ ((deg cm^2)/dmol), by means of the equation

$$[\theta]_\lambda = 100\theta_\lambda/cl \qquad (6)$$

where c is the concentration in moles per liter and l is the path length in centimeters.

II. Results

(A) Synthesis and Characterization of Polymers. The compositions and molecular weights of the various polymers are shown in Table I. As observed by Lotan, *et al.*,[8] the conversion from benzyl to hydroxyalkyl side chains was accompanied by a decrease in the degree of polymerization.

The fractionation procedure yields lower molecular weight fractions with increasing amounts of added ether, as seen in Table I for all samples which were fractionated. The degree of polydispersity of the fractions is indicated by the ratio $\bar{M}_z/\bar{M}_w$; a value of 1.00 would indicate a monodispersed polymer. In most cases, the degree of polydispersity is small, by this criterion. An equivalent indication of the polydispersity of these fractions was obtained in the departure from linearity (not shown here) of ln c *vs.* x^2 plots indicating, according to eq 2, that M_{wz} differs throughout the cell. Since $\bar{M}_z/\bar{M}_w$ is so close to unity, we identify $(\overline{\text{DP}})_w$ with $(\overline{\text{DP}})_n$, the latter corresponding to the chain length, N, in the theoretical treatment of the melting data (see section III).

In the copolymers prepared by the second procedure in section IB, the P:B ratios are very close to those of the amino alcohols (in excess) in the reaction mixture, despite the fact that it takes 1 and 3 days, respectively, to prepare the homopolymers PHPG and PHBG. This apparent difference in reactivity may be due to a difference in solubility. When the amino alcohol is added, the unreacted PBLG precipitates from solution so that the reaction becomes a heterogeneous one. As the reaction proceeds, *i.e.*, as the benzyl side chains are replaced by alkyl side chains, the polymer becomes more soluble and therefore more accessible to the reagent. The rate at which the polymer dissolves depends on the solubility of each alkyl homopolymer in its respective alkylamino alcohol, which may be different for PHPG and PHBG. The situation is further complicated (at intermediate stages of the substitution reaction) by the existence of cross linking in varying unknown amounts in the partially reacted material. Hence, the different times required for complete reaction in preparing the two homopolymers does not imply the existence of a preferential rate of reaction of aminopropanol over aminobutanol. Further, the high and low molecular weight fractions of a given sample have similar P:B ratios, within experimental error, indicating that there was no selective attack of the short, and possibly more accessible, chains by either reactant. These facts suggest that the synthesized copolymers have an essentially random distribution of P and B units along the chain, although we were not able to check this point experimentally. Fortunately, for the values of σ and s (defined in section IIIA) for these particular copolymers [in contrast to those for polynucleotides and for some other poly(amino acids)], even large deviations of the sequence from a random one will not alter the melting curve significantly. This point was illustrated in paper I,[3] in which similar parameters were used and the melting curves of two amino acid copolymers of the same overall composition (one with a random sequence and the other with alternating regular sequences with blocks 160 residues long) were compared; the two calculated melting curves are essentially collinear. Since such drastic deviations from randomness produce essentially no difference in the melting curves, we need have no concern about possible small deviations from randomness in the copolymers of Table I.

TABLE I
COMPOSITIONS AND MOLECULAR WEIGHTS OF POLYMERS

Sample[a]	P:B[b] ratio	$\bar{M}_w{}^c \times 10^{-3}$	$\bar{M}_z{}^c \times 10^{-3}$	$\bar{M}_z/\bar{M}_w$	Mean residue weight[d]	$(\overline{\text{DP}})_w{}^e$
IB	100:0	98.0	111.0	1.13	186	530
IIB	100:0	40.5	42.5	1.05	186	220
IIC	100:0	22.3	25.5	1.15	186	120
IIIB	100:0	130.3	148.0	1.13	186	700
IIID	100:0	66.2	67.2	1.02	186	360
IVA	0:100	338.0	452.0	1.34	200	1680
V[f]	0:100	144.0			200	720
VIB	0:100	39.8	43.4	1.09	200	200
VIC	0:100	23.9	23.9	1.00	200	120
VID	0:100	14.4	16.1	1.12	200	72
VIIB	0:100	13.3	14.9	1.04	200	66
VIID	0:100	7.45	8.78	1.20	200	37
VIIE	0:100	3.58	6.21	1.73	200	17
VIII[g]A	*i*	190.0				
VIII[g]B	10:90	141.0	161.0	1.14	199	710
VIII[g]C	6:94	33.1	38.8	1.17	200	160
IX[h]B	36:64	77.4	82.4	1.02	195	410
IX[h]C	40:60	32.8	34.9	1.06	195	170
X[h]B	53:47	64.2	64.2	1.00	193	330
X[h]D	55:45	25.5	28.7	1.13	193	130
XI[g]A	*i*	159.0	215.0	1.36		
XI[g]B	65:35	129.0	145.0	1.12	191	670
XI[g]C	62:38	46.3	54.4	1.17	192	240
XII[h]B	83:17	59.1	59.1	1.00	189	310
XII[h]D	82:18	20.9	22.6	1.08	189	110

[a] The Roman numeral refers to the unfractionated material and the letter to the fraction obtained in the fractionation procedure. [b] Determined by nmr. [c] $\bar{M}_w$ and $\bar{M}_z$ were obtained by equilibrium ultracentrifugation. [d] Mean residue weight = mole fraction of butyl side chains × residue weight of PHBG + mole fraction of propyl side chains × residue weight of PHPG. [e] This is $\bar{M}_w$ divided by the mean residue weight. [f] This sample was not fractionated. [g] Synthesized by the first procedure in section IB. [h] Synthesized by the second procedure in section IB. [i] P:B ratio not determined, since this sample was not studied further.

(B) ORD and CD Data. Previous work showed that PHPG is partially α helical in methanol–water mixtures and in water,[8,9] and that in aqueous solution PHBG[9] is more helical than PHPG of similar (high) molecular weight. ORD measurements on both high and low molecular weight fractionated PHBG, in water, are shown in Figure 1. The high molecular weight fraction has the trough at 233 mμ, the crossing point at 223 mμ, the shoulder at 212 mμ, and the peak at 199 mμ, all of which are characteristic of the right-handed α helix,[26,27] while the low molecular weight

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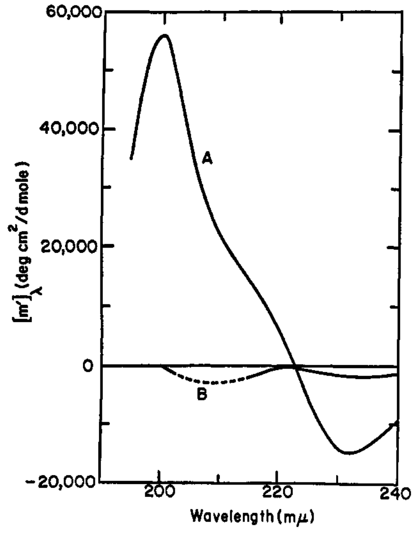

Figure 1. ORD data for PHBG in water at 23°: (A) DP 1680 (fraction IVA); (B) DP 17 (fraction VIIE). The signal-to-noise ratio was very low in the dashed region.

fraction has the shallow trough at 234 mμ and another trough at about 207 mμ.

Figure 2 shows ORD data for two of the PH(P:B)G copolymers. The DP of both of these samples was ~700, and they both have fairly high α-helix content at low temperature. At high temperatures the ORD is similar[27] to that of a mixture of random coil and α helix, indicating that the polymers denature thermally from α helix to random coil. Further, the sample with the higher butyl content has a higher helix content at the low temperature.

Figure 3 shows CD spectra for some of the polymers. The maximum at 192 mμ and the two minima at 208 and 222 mμ correspond closely to the values ascribed by Greenfield and Fasman[28] to α-helix and random-coil mixtures which are high in helix content. Again, the sample with the higher butyl content has a higher helix content at this temperature.

The ORD and CD data for the high molecular weight fractions are similar to those of the ethyl analog[9] (PHEG) in alcohol.[29] The data for the low molecular weight fractions are only qualitatively similar to those of randomly coiled, high molecular weight PHEG in water.[29]

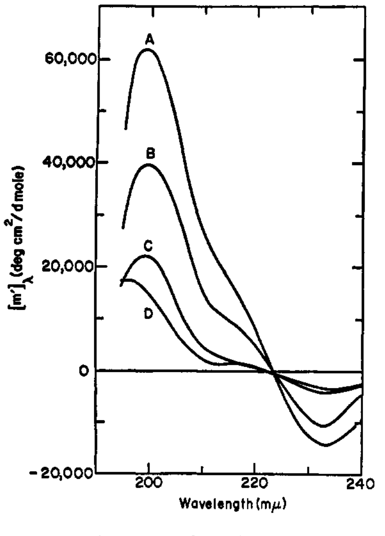

Figure 2. ORD data in water for PH(10:90)G, fraction VIIIB, DP 710, at 4° (A) and at 61° (C); PH(65:35)G, fraction XIB, DP 670, at 5° (B) and at 60° (D).

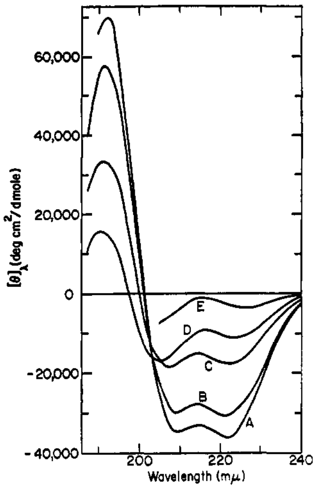

Figure 3. CD data in water at 24°: (A) PH(0:100)G, fraction IVA, DP 1680; (B) PH(10:90)G, fraction VIIIB, DP 710; (C) PH(65:35)G, fraction XIB, DP 670; (D) PH(100:0)G, fraction IB, DP 530; (E) PH(0:100)G, fraction VIIE, DP 17.

The thermally induced helix-coil transition was followed spectropolarimetrically in terms of the temperature dependence of b_0. The melting curves were determined at least twice, generally with b_0 obtained at 10° intervals, first beginning at 0°, and a second time beginning at 5°. The two curves superimpose very well, indicating good reproducibility, especially for large values of b_0. The results for PHPG, PHBG, and for PH(P:B)G copolymers are shown in Figures 4, 5, and 6, respectively. These transitions are fully reversible, as shown for representative samples in Figure 7.

There are some differences between the b_0 *vs.* T data in the interval 0–40° shown in Figure 4 and those of previous workers,[8,11] which may arise from one or more of the following differences between the three sets of experiments. In some cases the polymers were fractionated and the homogeneity of the sample was checked by determining $\bar{M}_z/\bar{M}_w$, while in other cases this was not done. Different instruments for observing ORD and different methods of determining the concentration of the solutions of these hygroscopic solids were used. The number of points measured (to obtain the

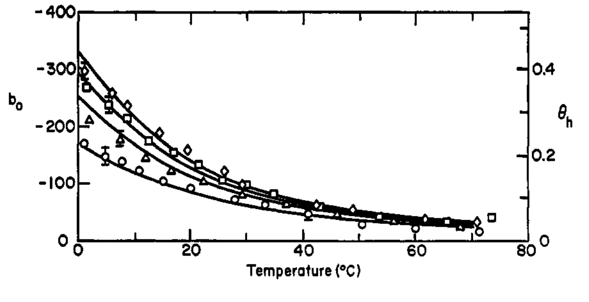

Figure 4. Temperature dependence of b_0 and θ_h for PHPG in water for the following DP's: ◇, 700; □, 360; △, 220; ○, 120. The points are experimental and the lines are theoretical, with the parameters shown in Table II. The several standard deviation symbols indicate the effect of the experimental error from all sources (not just b_0) which affect the quantity θ_h as explained in the text.

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[28] N. Greenfield and G. D. Fasman, *Biochemistry*, 8, 4108 (1969).

[29] A. J. Adler, R. Hoving, J. Potter, M. Wells, and G. D. Fasman, *J. Amer. Chem. Soc.*, 90, 4736 (1968).

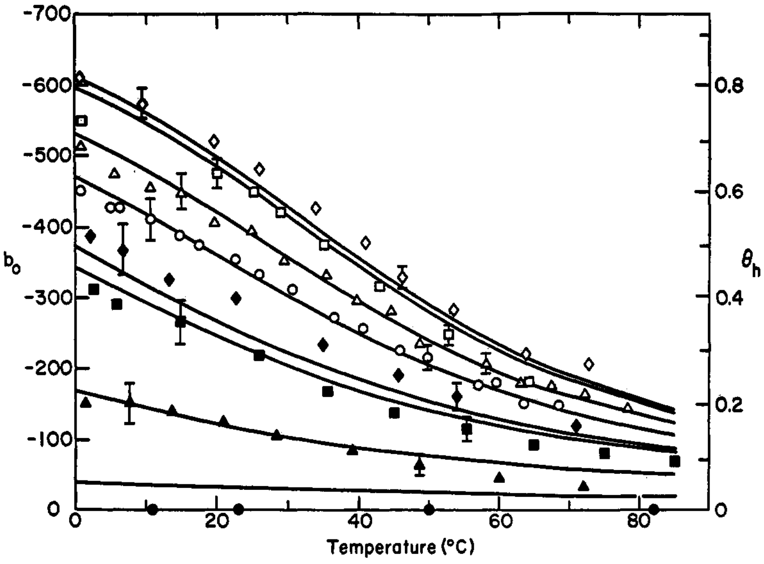

Figure 5. Temperature dependence of b_0 and θ_h for PHBG in water for the following DP's: ◇, 1680; □, 720; △, 200; ○, 120; ◆, 72; ■, 66; ▲, 37; ●, 17. The points are experimental and the lines are theoretical, with the parameters shown in Table II. The several standard deviation symbols are the same as in Figure 4.

b_0 *vs.* T curve) was quite small in some cases and large in others. Such discrepancies in experimental data are not unexpected for samples in which the helix contents, and hence b_0 values, are so low. Despite these and other differences in the techniques used to study the polymers and analyze the the results, the thermodynamic parameters deduced from the three sets of data with the same homopolymer theory agree fairly well, as we will show in section IIIA.

The values of θ_h were computed from the values of b_0 according to the following procedure. The value of zero was assigned to b_0 for the random coil, since this appears to be the limiting value for the data of Figures 4–6 at high temperatures and is also the value obtained for the low molecular weight (DP 17) fraction at all temperatures (Figure 5). Since the b_0 data for PHPG and PHBG both approach a limiting value (as $N \rightarrow \infty$) of -750 for these polymers in methanol (where they both appear to be fully helical), this value was assigned to the fully helical polymer; Okita, *et al.*,[11] also used the value of -750 for fully helical PHPG. Thus, the values of θ_h in Figures 4–6 were computed as $-b_0/750$. The choice of the limiting b_0 values here is admittedly somewhat arbitrary. This is unfortunate, since the choice of the limiting b_0 values affects the thermodynamic parameters obtained from the data.

The source and magnitude of the errors in the experimental melting data are the following. First, the error arising from the Kjeldahl nitrogen determination of the concentration (used in the computation of b_0) was $\pm 3\%$ in b_0 and θ_h. Second, the standard deviation in fitting a straight line to the points on the Moffit–Yang plot was ± 2.5 units in b_0, which leads to errors of ± 0.3 to $\pm 4.0\%$ in θ_h as θ_h decreases from large to small values. Third, the error in the fraction of A

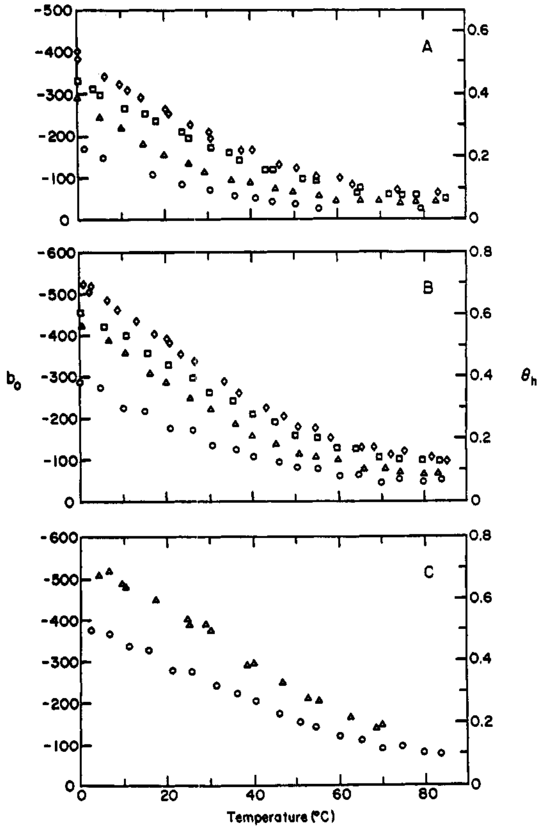

Figure 6. Temperature dependence of b_0 and θ_h for PH(P:B)G copolymers in water for the following compositions and DP's: (A) ◇, PH(65:35)G, 670; □, PH(62:38)G, 240; △, PH(83:17)G, 310; ○, PH(82:18)G, 110; (B) ◇, PH(36:64)G, 410; □, PH(40:60)G, 170; △, PH(53:47)G, 330; ○, PH(55:45)G, 130; (C) △, PH(10:90)G, 710; ○, PH(6:94)G, 160.

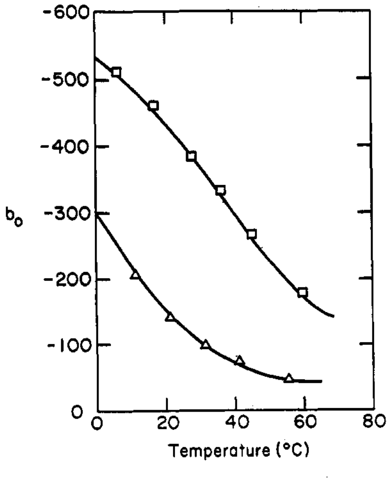

Figure 7. Demonstration of reversibility of transition curves in water: □, PH(10:90)G, DP 710; △, PH(100:0)G, DP 530. The curve was obtained in the heating part of the cycle, and the symbols were obtained during cooling.

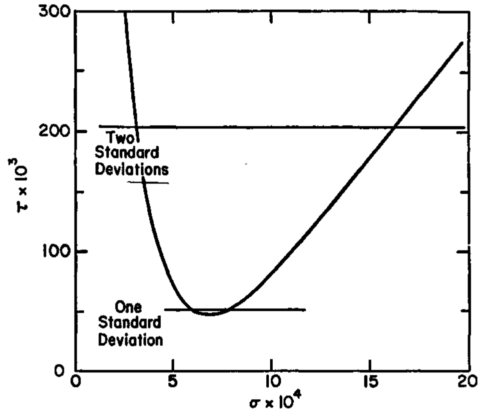

Figure 8. Determination of the best temperature-independent value of σ as the one which corresponds to the lowest value of τ for PHBG. The lines, corresponding to one and two standard deviations in θ_h, are shown.

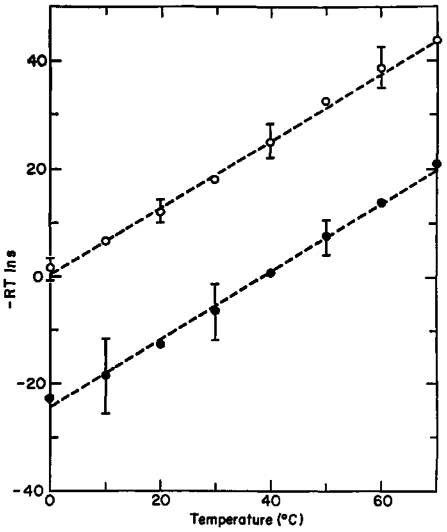

Figure 9. A plot of $-RT \ln s$ *vs.* T to assess the dependence of ΔG on T, using the best-fit temperature-independent values of σ, for PHPG (○) ($\sigma = 2.2 \times 10^{-4}$) and for PHBG (●) ($\sigma = 6.7 \times 10^{-4}$). The error symbols are for the 95% confidence limits. The dashed lines correspond to the parameters in Table II.

units in an A–B copolymer, f_A (±4 mol %), contributes about 6% to the error in θ_h. Fourth, the precision in $\bar{M}_w$ is ±5%. When the molecular weight distribution is Gaussian, $\bar{M}_w/\bar{M}_n \geq \bar{M}_z/\bar{M}_w$,[30] leading to a minimum error of $100(\bar{M}_z/\bar{M}_w - 1.0)\%$ when the value of $(\overline{DP})_w$ is used for the chain length N. The combined errors in chain length produce an error in θ_h ranging from 0.5% for DP 700 to 7% for DP 72. The first two errors affect b_0, but the second two do not. However, the errors in f_A and N affect the value of θ_h computed from the theory. Rather than put error symbols on the theoretical lines in Figures 4 and 5, which might be misinterpreted as errors in the theory, we have combined the errors in f_A and N with those in b_0 to give the error in θ_h shown in Figures 4 and 5.

III. Discussion

(A) Thermodynamic Parameters for Helix–Coil Transition in Host Homopolymers. In order to apply the host–guest technique to obtain the thermodynamic parameters for the guest residues, we must first characterize the host homopolymers, PHPG and PHBG. For this purpose, we have repeated the earlier experiments[8–11] on PHPG (using fractionated samples, and making observations at many temperatures), and extended them also to PHBG. The Zimm–Bragg parameters[31] which characterize the conversion of coil to helix are s and σ, defined as follows

$$s = \exp[-\Delta G/RT] = \exp[-\Delta H/RT + \Delta S/R] \qquad (7)$$

where ΔG, ΔH, and ΔS are the free energy, enthalpy, and entropy, respectively, for converting a random-coil residue to a hydrogen-bonded helical one at the end of a long helical sequence and σ is a cooperativity parameter. The quantities ΔH and ΔS would be expected[7,32] to be temperature dependent over the temperature range (0–80°) considered here. Also, while σ has usually been assumed to be independent of temperature, recent theoretical considerations[7,32] suggest that this may not be the case. We have therefore analyzed the data using both a temperature-dependent and a temperature-independent σ, beginning with the temperature-independent case.

The temperature-independent value of σ and the corresponding set of values of s at each temperature which best fit the experimental data for the homopolymers PHPG and PHBG were obtained as follows.[33] First, a trial temperature-independent value of σ was assumed. Then, *at each temperature*, the value of s which best fits the data for all polymer fractions i was taken as the one which minimized the difference between the experimental $(\theta_{h,\text{exptl}})_i$ and calculated $(\theta_{h,\text{calcd}})_i$ values of θ_h, *i.e.*, for the given σ, the best value of s at each temperature was the one that minimized $\Sigma_i[(\theta_{h,\text{exptl}})_i - (\theta_{h,\text{calcd}})_i]^2$. Thus, a best set of values of s *vs.* T was obtained for a particular selected value of σ. To find the *best* temperature-independent value of σ, this parameter was varied to obtain the value which produced the best fit of the data *over all temperatures*, *i.e.*, the best temperature-independent value of σ was the one that minimized τ, defined in eq 8. The dependence of τ on σ for PHBG is shown in Figure 8.

$$\tau = \sum_T \sum_i [(\theta_{h,\text{exptl}})_i - (\theta_{h,\text{calcd}})_i]^2 \qquad (8)$$

Having determined the best temperature-independent value of σ, the corresponding set of best values of s at each temperature was obtained and plotted as $-RT \ln s$ *vs.* T in Figure 9. When ΔG, or $-RT \ln s$, was fitted with a polynomial in T, it was found that a first-degree polynomial (and hence all higher degree polynomials) described the data within the experimental error. An R^2 test indicated that the linear relationship accounts for 99% of the variance of the data. This implies that a temperature dependence of ΔH and ΔS (for both PHPG and PHBG, and in earlier work[10] on PHPG) beyond the experimental error cannot be detected even though ΔH, ΔS, and σ, in principle, should all vary with temperature.[32] Therefore, our only recourse is to take these parameters as temperature independent. Using the experimental data in the range of 0–70°, and a temperature-independent value of σ, we obtain the thermodynamic parameters of Table II.

The PHPG parameters obtained in the present work can be compared to those of previous authors by analyzing the data over the temperature range 0–40° (since the data of Okita,

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(30) N. Gō, private communication.

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

(32) M. Gō, N. Gō, and H. A. Scheraga, *ibid.*, 52, 2060 (1970).

(33) The computer program is available; footnotes 26 and 27 of paper I[3] should be consulted for the procedure for obtaining this program.

TABLE II

COIL-TO-HELIX PARAMETERS[a] FOR PHPG AND PHBG AT 20° WHEN σ IS INDEPENDENT OF TEMPERATURE

	PHPG	PHBG
ΔG, cal/mol	$+12.6 \pm 2$	-11.3 ± 7
ΔH, cal/mol	-168 ± 30	-195 ± 55
ΔS, eu	-0.616 ± 0.10	-0.627 ± 0.17
T_{TR}, °C	-0.7 ± 3	$+37.8 \pm 6$
σ	2.2×10^{-4}	6.7×10^{-4}

[a] The errors in these quantities correspond to two standard deviations in the error in s when σ (taken as the value shown) is assumed to have no error. Two standard deviations correspond to the following ranges of σ: $1\text{-}5 \times 10^{-4}$ for PHPG and $3\text{-}17 \times 10^{-4}$ for PHBG. We retain the number of significant figures shown here and in later tables in order to maintain self-consistency among the various data.

TABLE III

COIL-TO-HELIX PARAMETERS FOR PHPG AND PHBG AT 20° WHEN σ IS TEMPERATURE DEPENDENT[a]

	PHPG	PHBG
ΔG, cal/mol	$+10.7$	-11.8
ΔH, cal/mol	-117	-187
ΔS, eu	-0.436	-0.598
T_{TR}, °C	-4.8	$+39.5$
σ^b	1.9×10^{-4}	6.9×10^{-4}

[a] In the data fitting to obtain σ and s, these values are coupled, making it difficult to assess the respective errors in σ and s when σ is allowed to be temperature dependent. [b] The values of σ at other temperatures are shown in Figure 11.

et al.,[11] are given only for this range) with the same homopolymer theory (Zimm–Bragg[31]). The PHPG parameters obtained using the data of ref 8 are $\sigma = 1.8 \times 10^{-4}$, $\Delta H = -120$ cal/mol, and $T_{\text{TR}} = 0°$; those using the data of ref 11 are $\sigma = 2.7 \times 10^{-4}$, $\Delta H = -120$ cal/mol, and $T_{\text{TR}} = -3°$; and those of the present work are $\sigma = 2.2 \times 10^{-4}$, $\Delta H = -160$ cal/mol, and $T_{\text{TR}} = -1°$. The results from the various laboratories are not identical, but are very similar.

The homopolymer data were reanalyzed, but this time allowing σ to be temperature dependent. *At each temperature*, the best *pair* of values of σ and s was taken to be that which minimized the quantity $\Sigma_i[(\theta_{\text{h,exptl}})_i - (\theta_{\text{h,calcd}})_i]^2$. The resulting best pairs of values of σ and s are shown in Figures 10 and 11 as s *vs.* T and σ *vs.* T, respectively. The data for PHBG are well behaved, in the sense that s decreases monotonically with increasing temperature as does b_0. However, while the data for s of PHPG decrease monotonically with T between 0 and 30°, they increase above 45° even though b_0 decreases. The increase in s in a region where θ_h decreases is mathematically possible because *both* σ and s are varied to obtain the best fit to θ_h at each temperature. Experimentally, θ_h is rather small above 30°; hence, it is difficult to determine this quantity accurately, and the data can be fit by a wide range of parameters. There are other sets of σ and s that will produce s values which are a monotonically decreasing function of temperature. The dashed lines for PHPG in Figures 10 and 11 represent one such solution, obtained when the s *vs.* T data from 0 to 30° were extrapolated to higher temperatures and the corresponding values of σ found. The data of Figure 10 were replotted as $-RT \ln s$ *vs.* T, which was found to be linear in T between 0 and 70° for PHBG and between 0 and 30° for PHPG; the results are shown in Table III.

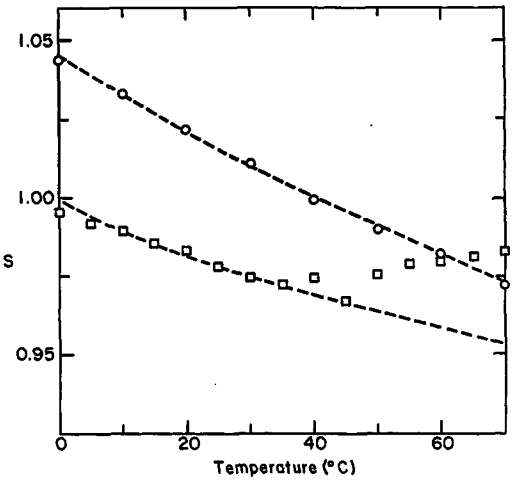

Figure 10. A plot of s *vs.* T for the values of s (and σ) which give the "best fit" of the experimental data at each temperature for ○, PHBG; □, PHPG. For PHPG, the dashed line is an extrapolation of the data between 0 and 30° to higher temperature, as explained in the text; for PHBG, the dashed line corresponds to the parameters in Table III. The precision indicated is that of two standard deviations.

From a consideration of the data of Tables II and III, and of the errors involved, we conclude that it is not possible to detect a temperature dependence of σ or of ΔH and ΔS. Hence, we will base the discussion in the remainder of this paper on the data of Table II, for which σ is assumed to be independent of temperature. The data of Table II were used to calculate the theoretical curves in Figures 4 and 5.

The polymers PHBG, PHPG, and PHEG have T_{TR} values of

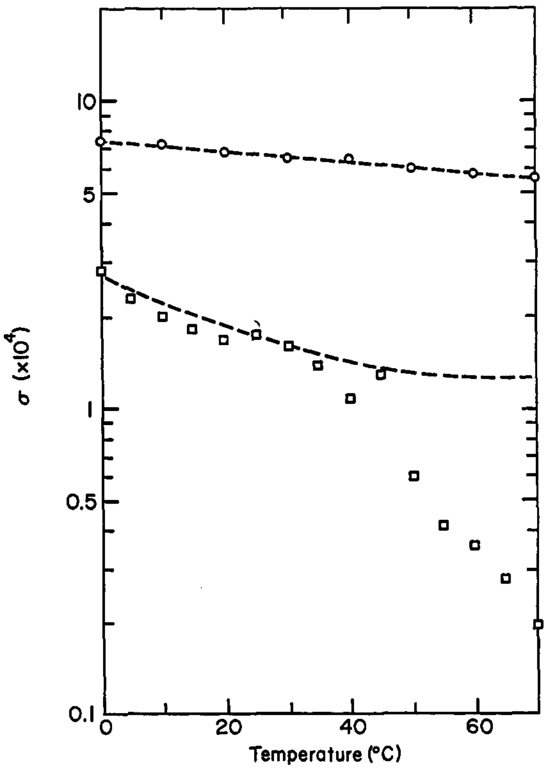

Figure 11. A plot of σ *vs.* T for the values of σ (and s) which give the "best fit" of the experimental data at each temperature for ○, PHBG; □, PHPG. The data of Figures 10 and 11 were obtained in the same set of computations. See legend of Figure 10 for further details.

Table IV
Comparison of Coil-to-Helix Parameters for PHPG and PHBG at 20°[a]

	PHPG		PHBG	
	Homopolymer	Copolymer	Homopolymer	Copolymer
ΔG, cal/mol	+12.6	+15.8	−11.3	−7.2
ΔH, cal/mol	−168	−145	−195	−164
ΔS, eu	−0.616	−0.549	−0.627	−0.535
T_{TR}, °C	−0.5	−9.0	+37.8	+33.4
σ	2.2×10^{-4}	1.4×10^{-4}	6.7×10^{-4}	5.3×10^{-4}

[a] Obtained from homopolymer melting and from the host–guest technique using a temperature-independent value of σ.

38, −1, and ≪0°, respectively. Since all three polymers are helical in nonaqueous solvents[9] and show the above behavior in water, the increased stability of the helical form in water as the length of the alkyl side chain increases has been attributed to side-chain hydrophobic bonding.[9] The values of ΔG, ΔH, and T_{TR} for the two polymers are consistent with this explanation. The negative values of ΔH and ΔS suggest that the *dominant* interaction in the formation of the helix is hydrogen bonding. The other (attractive) nonbonding interactions, such as hydrophobic bonding, lead to an increment in stability (*e.g.*, to higher values of T_{TR}) as the length of the alkyl side chain increases and to increments in ΔG and ΔH corresponding to the formation of more hydrophobic bonds in the helix than in the coil. While one might, at first sight, expect ΔH and ΔS for PHBG to be more *positive* than the corresponding quantities for PHPG, since the hydrophobic bond is generally characterized as having a positive ΔH of formation and being entropy driven, these characteristics apply to the CH_3 group rather than to the CH_2 group by which PHPG and PHBG differ. However, by examining the data for *n*-alkanes and *n*-alcohols,[34,35] it can be seen that the differences in the parameters for the propyl and butyl polymers are those to be expected for a CH_2 group.

An alternative reason for the higher melting temperature of PHBG over PHPG could be the ability of the extra CH_2 group in PHBG to change the environment of the backbone amide groups in the helix, thus increasing the strength of the peptide hydrogen bonds in water. It is also possible that the additional CH_2 group affects the coil states more than the helical states.

(B) Use of the Host–Guest Technique to Obtain Thermodynamic Parameters for an Amino Acid from Copolymer Data. We turn now to the use of the host–guest technique to obtain σ and s for an amino acid residue from data for the host homopolymer and for copolymers of the host and guest residues. From the data of Figures 2, 3, and 6, we see that the copolymers are partially helical and undergo the helix–coil transition just as the homopolymers do. We will analyze the copolymer data shown in Figure 6 by use of Lifson's copolymer theory[36] (adapted for finite chain length) since we have shown, from a consideration of the LAPS heierarchy in paper I,[3] that Lifson's theory is accurate for the range of parameters pertaining to the polymers of interest here; further, calculations with Lifson's procedure can be performed very rapidly compared to those for the more rigorous theories.[3] However, when the calculations were repeated with Allegra's theory,[37] the same results were obtained.

To illustrate the host–guest technique, we designate PHBG as the host and compute the parameters for PHPG from the melting data for PHBG and for the copolymers. Using a similar procedure as for homopolymers,[33] Lifson's theory,[36] and the PHBG data from Table II, the best fit to the experimental copolymer data *at each temperature* was obtained by varying the (temperature independent) values of σ and the (temperature dependent) values of s for PHPG. A plot of the resulting values of s, as $-RT \ln s$ *vs.* T, was found to be linear in the range of 0–70° within the experimental error, yielding the temperature-independent values for ΔH and ΔS shown in Table IV. By reversing the roles, *i.e.*, by taking PHPG as the host and PHBG as the guest, the temperature-independent parameters for the latter polymer (also shown in Table IV) were obtained. The small differences in the values of the homopolymer parameters obtained by the two techniques (use of homopolymers and host–guest technique, respectively) indicate that the host–guest technique can be used to obtain amino acid parameters.

It was pointed out in paper I[3] that if the values of ΔS for the two homopolymers were not the same then the variation of melting temperature with composition (for very large chain length) would not be linear. This is the reason that, in general, one should not use the melting temperature for the host homopolymer and the melting temperatures of the copolymers to extrapolate to obtain the melting temperature of the guest homopolymer. Further, the extrapolation requires the assumption that σ, ΔH, and ΔS remain independent of temperature through the region of extrapolation; since we want the values of the guest parameters in the range of 0–100°, such extrapolation is avoided, *i.e.*, the parameters are obtained by direct curve fitting in this temperature range.[33]

(C) Further Considerations. When the thermodynamic parameters (Table II) for the helix–coil transition of the two homopolymers PHPG and PHBG are used to compute the theoretical melting curves of PH(P:B)G copolymers, we find that the theoretically expected value of θ_h is generally slightly higher than the experimentally observed value. It is possible that this difference between the theoretical and experimental curves is an undetected experimental error. However, it could also arise from a failure of any of the assumptions of the theory. For example, by taking into account a possible near-neighbor interaction (using a 4×4 matrix instead of the 2×2 matrix of the Lifson theory[36]), a better fit is obtained between the calculated and experimental curves. However, in light of the discussion of the experimental errors in section IIIB, it would not be possible to tell whether the effect of near-neighbor interactions were real or not. Nevertheless, it is intended to carry out further experiments to test whether the parameters deduced for the guest residues depend on the nature of the host.

We believe that the small discrepancies shown in Table IV between the values computed for the homopolymer and those computed using the host–guest technique may in part be due to the lower accuracy in the PHPG data, because only the high-temperature ends of the melting curves are obtainable (see Figure 4). Therefore, we have used a procedure of successive approximations, in which the parameters for PHBG were initially assumed to be correct, and a best fit was obtained to the experimental data for *both* PHPG and the copolymers. Then the resulting PHPG values were assumed

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(34) G. Nemethy and H. A. Scheraga, *J. Chem. Phys.*, **36**, 3382 (1962), and references therein.

(35) N. Laiken and G. Nemethy, *J. Phys. Chem.*, **74**, 3501 (1970), and references therein.

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

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

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.

Acknowledgments. The authors wish to thank Mr. Hua Tjan for carrying out the micro-Kjeldahl analyses, Mr. James MacNeil for his assistance in obtaining the nmr spectra, Mrs. Mary Dygert, Mr. John Alter, and Mrs. Lou Hughes for their help in characterizing three of the PHBG fractions, and Dr. Nobuhiro Gō for helpful discussions.

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

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[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).