Conformational Studies on Copolymers of Hydroxypropyl-L-glutamine and L-Leucine. Circular Dichroism Studies†

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ABSTRACT: Conformational studies have been performed on poly(N^5-(3-hydroxypropyl)-L-glutamine) and random copolymers of hydroxypropyl-L-glutamine with L-leucine in water from 0 to 80° and in various methanol concentrations at 23° by means of circular dichroism. The helical content of the homopolymer and copolymers was found to increase with (1) decreasing temperature, (2) increasing methanol concentration, and (3) increasing mole ratios of leucine in the copolymers. The increase in helicity in the copolymers, P(HPG^{z}Leu^y), with leucine incorporation, as well as methanol addition, is also shown by hypochromicity in the far-ultraviolet absorption region. Utilizing the Zimm–Bragg theory to analyze the helix–coil transition of poly(N^5-(3-hydroxypropyl)-L-glutamine) in H_2O, the helix initiation parameter, σ, was found to be 2×10^{-4}, and this value was also applied to the copolymer data. The thermodynamic parameters at 25° for converting a residue from a coil to a helical state in poly(N^5-(3-hydroxypropyl)-L-glutamine) in water were determined to be: $\Delta G^\circ = +15$ cal/mole, $\Delta H^\circ = -155$ cal/mole, $\Delta S^\circ = -0.57$ eu; and the extrapolated values for a Leu residue in a Leu polymer in water, $\Delta G^\circ = -109$ cal/mole, $\Delta H^\circ = +88$ cal/mole, $\Delta S^\circ = +0.66$ eu. In addition, the helix growth parameter, s, at 25° was found to be 0.98 for poly(N^5-(3-hydroxypropyl)-L-glutamine) and decreased with increasing temperature, while $s = 1.2$ by extrapolation for poly(L-leucine) and increased at higher temperatures. The increased helicity in the latter case is due to stronger hydrophobic interactions with increasing temperatures. The net contributions from hydrophobic interactions of leucyl-leucyl side-chain residues during helix formation were evaluated to be $\Delta G^\circ_{H\varphi} = -560$ cal/mole, $\Delta H^\circ_{H\varphi} = +576$ cal/mole, and $\Delta S^\circ_{H\varphi} = +3.81$ eu, and were in close agreement with theoretical values. When these thermodynamic parameters for poly(L-leucine) are compared to the corresponding literature values for poly(L-alanine), it is seen that the leucine helix is more stable than the alanine helix, due to its larger nonpolar side chains resulting in more favorable hydrophobic stabilization of the helix. A survey of the conformational structure in eleven proteins showed that of all the amino acids in the inner helical regions leucine occurs most frequently. This suggests that leucine may be the strongest helical forming amino acid residue in polypeptides as well as in proteins.

The influence of side chain interactions on the α-helical stability of polypeptides in solution has long been established (Fasman *et al.*, 1962; see review, Fasman, 1967), and their importance in stabilizing the native conformations of proteins has also been shown (Scheraga, 1963). For example, Lotan *et al.* (1966) demonstrated that the α helix is more stable as the side-chain length is increased in the series, poly(N^5-(2-hydroxyethyl)-L-glutamine) (PHEG),[1] poly(N^5-(3-hydroxypropyl)-L-glutamine) (PHPG), and poly(N^5-(4-hydroxybutyl)-L-glutamine) (PHBG). Likewise, Hatano and Yoneyama (1970) and Grourke and Gibbs (1971) showed that the helix stability in the series, poly(L-α,γ-diaminobutyric acid) (PLDBA), poly(L-ornithine) (poly(Orn)), and poly(L-lysine) (poly(Lys)) increases with increasing number of methylene groups on the side chain.

In addition, conformational studies of block as well as random copolymers are helpful in elucidating the contributing factors of amino acid side chains to helical stability. In the case of random copolymers of L-glutamic acid (Glu) and L-leucine (Leu), Fasman *et al.* (1964) as well as Miller and Nylund (1965) found that leucine incorporation into the copolymers, P(Glu^{z}Leu^y), increased the stability of the helix. Sage and Fasman (1966) found that random copolymers of Glu and Phe had a higher helical content than did poly(Glu) under similar conditions. This was confirmed by Auer and Doty (1966) in their block copolymer studies of (DL-Glu)^{50}-(Phe)^{50}(DL-Glu)^{50} in aqueous solutions. On the other hand, random incorporation of L-serine (Kulkarni *et al.*, 1961) as well as L-tyrosine (Doty and Gratzer, 1962) into a glutamic acid helix decreased helical stability. Other studies of block copolymers showed that the poly(L-alanine) (poly(Ala)) helix was extremely stable when incorporated between two blocks of poly(DL-Glu) (Gratzer and Doty, 1963) or poly(DL-Lys) (Ingwall *et al.*, 1968). Similar block copolymers showed poly(L-valine) (poly(Val)) (Epand and Scheraga, 1968b) to be helical in methanol but β forming in water, and the poly(L-leucine) (poly(Leu)) helix (Ostroy *et al.*, 1970) to be more stable than the poly(Ala) helix in water. By means of potentiometric titration of random copolymers of Lys and Ala, Sugiyama and Noda (1970) concluded that the alanine helix is

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[1] Abbreviations used are: DP, degree of polymerization; Hφ, hydrophobic bond; HPG, N^5-(3-hydroxypropyl)-L-glutamine; PBLG, poly(γ-benzyl-L-glutamate), PCHA, poly(L-cyclohexylalanine); PELG, poly(γ-ethyl-L-glutamate); PHBG, poly(N^5-(4-hydroxybutyl)-L-glutamine); PHEG, poly(N^5-(2-hydroxyethyl)-L-glutamine); PHPG, poly(N^5-(3-hydroxypropyl)-L-glutamine; P(HPG^{z}Leu^y), poly[(N^5-(3-hydroxypropyl)-L-glutamine)^{z}Leu^y]; PLDBA, poly(L-α,γ-diaminobutyric acid); poly(Ala), poly(L-alanine); poly(Glu), poly(L-glutamic acid); poly(Gly), poly(glycine); poly(Leu), poly(L-leucine); poly(Lys), poly(L-lysine); poly(Orn), poly(L-ornithine); poly(Phe), poly(L-phenylalanine); poly(Ser), poly(L-serine); poly(Val), poly(L-valine); NCA, *N*-carboxyanhydride.

TABLE I: Reaction Conditions for Polymer Conversion.

Polymer No.	Prepn No.	Polymer	Precursor[a] [A]/[I][b]	Precursor[a] η_{sp}/c[c]	Dioxane (ml)	Aminopropanol (ml)	Duration of Treatment (hr)	Mole Ratio Composition Based On NCA Addition BLG:Leu	Mole Ratio Composition Based On Amino Acid Analy.[d] HPG:Leu
1	GF-15-122-3	PHPG	20	0.55	3.6	7.3	24	100:0	100:0
2	GF-15-146-27	P(HPG^{z}Leu^y)	20	0.49	1.8	3.7	50	90:10	92:8
3	GF-15-311-25	P(HPG^{z}Leu^y)	40	1.10	8.1	14.1	48	70:30	83:17
4	GF-18-439-28	P(HPG^{z}Leu^y)	30	0.72	3.9	5.8	51	80:20	80:20
5	GF-15-290-21	P(HPG^{z}Leu^y)	20	0.81	4.6	12.3	49	75:25	76:24

[a] Poly(γ-benzyl-L-glutamate) or poly(γ-benzyl-L-glutamate-L-leucine) (0.5 g) starting material. [b] Anhydride:initiator ($NaOCH_3$) ratio. [c] Specific viscosity (0.2% in dichloroacetic acid). [d] Determined by analysis on a Beckman Model 120 automatic amino acid analyzer (Spackman *et al.*, 1958).

thermodynamically less stable than the lysine helix. On the other hand, Snell and Fasman (1972) found that the helical stability of random copolymers of Lys and Leu increased with greater leucine content in the copolymers. More recently, Ananthanarayanan *et al.* (1971) showed that glycine (Gly) acts as a strong helix breaker when incorporated in random copolymers of hydroxybutyl-L-glutamine and glycine.

In the present study the helical stability of PHPG as well as its random copolymers with leucine were examined as a function of temperature, solvent, and leucine composition. The helix–coil transition of PHPG was previously investigated by Lotan *et al.* (1965, 1966, 1969) and by Okita *et al.* (1970) using optical rotatory dispersion (ORD). Since circular dichroism (CD) has less overlap of electronic transitions than does ORD (Beychok, 1967), and CD temperature studies of PHPG are not available in the literature, it was decided to use this more sensitive technique in studying the conformational changes of PHPG and its copolymers with leucine. The homopolymer theory of Zimm–Bragg (1959) was applied to obtain the thermodynamic parameters for PHPG, and similar parameters were estimated for leucine from the copolymer data.

Experimental Section

Materials

All chemicals used in the synthesis of the polymers were of reagent grade purity; dioxane was purified by the method of Fieser (1941); benzene was distilled from calcium hydride. 3-Amino-1-propanol (Matheson) was redistilled under high vacuum. Methanol used for CD work was an ACS Spectrograde solvent from Eastman Kodak Co. Methanesulfonic acid was of analytical reagent grade from the same company. Water was doubly distilled from glass.

Synthesis

γ-Benzyl-L-glutamate-N-carboxyanhydride (BLG-NCA). BLG-NCA was prepared by a modification of published procedures (Blout and Karlson, 1956).

Leucine-N-carboxyanhydride (Leu-NCA). Leu-NCA was prepared according to the method of Fasman *et al.* (1964).

Poly(γ-benzyl-L-glutamate) (PBLG). BLG-NCA (0.508 g, 1.93×10^{-3} mole) was dissolved in dry benzene (1%) by warming. Sodium methoxide (0.314 ml, 0.308 N, [A]:[I] = 20) was added and the solution was allowed to stand for 24 hr. The polymer was isolated by pouring the polymerization solution into anhydrous ether (200 ml), and the polymer was washed three times with anhydrous ether, lyophilized from dioxane, and dried at 80° for 2 hr *in vacuo* (1 mm Hg): yield 0.32 g (76%), η_{sp}/c = 0.55 (*c* 0.2%, dichloroacetic acid).

Copolymers of γ-Benzyl-L-glutamate and L-Leucine [P(BLG^{z}Leu^y)]. BLG-NCA and Leu-NCA, in the desired ratio, were dissolved in dry benzene (1%) by warming, and initiated with $NaOCH_3$. The solutions were allowed to stand overnight. The polymer was isolated and treated in the same manner as PBLG (above). In Table I are given the mole ratios of the *N*-carboxyanhydrides used in the various polymerization mixtures, the [A]:[I] (anhydride–initiator ratios) and specific viscosities of the polymers obtained.

Poly(N^5-(3-hydroxypropyl)-L-glutamine) (PHPG). PHPG was prepared by the reaction of PBLG with 3-amino-1-propanol as described in Lotan *et al.* (1965).

Copoly(N^5-(3-hydroxypropyl)-L-glutamine):L-Leucine (80:20) [P(HPG^{80}Leu^{20})]. P(BLG^{80}Leu^{20}) (2.59 g, 13.09×10^{-3} mole) was dissolved in freshly distilled dioxane (20 ml) at 60° with mechanical stirring, and 3-amino-1-propanol (30 ml) was added slowly causing precipitation of the polymer in the form of a fine suspension. Stirring was continued at 60° in a closed system for 51 hr until a clear solution was obtained and an aliquot of the reaction mixture gave no precipitate when diluted with H_2O. The reaction mixture was then cooled in an ice bath and poured into a solution of 26 ml of acetic acid and 40 ml of H_2O. The heavy opalescent solution was dialyzed *vs.* H_2O and centrifuged at 10,000 rpm for 2.5 hr to remove water-insoluble material, and the clear supernatant was lyophilized. The polymer was dried under high vacuum for 2 hr at 80°: yield 1.3 g (56%), η_{sp}/c = 0.33 (0.2% w/v in 0.2 M NaCl).

The other conversions of polymers from P(BLG^{z}Leu^y) to P(HPG^{z}Leu^y) were prepared in a similar manner. Table I gives the variation of solvent and reaction conditions, and the amino acid compositions. Table II lists the viscosities and molecular weights.

Viscosity measurements were made at 25 ± 0.1° in 0.2 M NaCl with Ubbelohde viscometers. The polymer concentra-

TABLE II: Characterization of Polymers.

Polymer	Composition of Polymer[a]	Residue Wt	$[\eta]^b$	MW^d	DP	% Helix[c]
1	PHPG	186.2	0.22	47,000	252	14
2	P(HPG^{92}Leu^{8})	180.1	0.22	39,500	220	33
3	P(HPG^{83}Leu^{17})	173.5	0.21	36,000	208	47
4	P(HPG^{80}Leu^{20})	171.6	0.33	50,000	292	57
5	P(HPG^{76}Leu^{24})	168.7	0.22	34,000	202	64

[a] From amino acid analysis. [b] Intrinsic viscosity in 0.2 M NaCl at 25°. [c] Based on $[\theta]_{222}$ of polymers in H_2O at 25° from CD studies. [d] Estimated by taking into account the per cent helicity in the copolymers and extrapolating between the $[\eta]$ *vs.* *MW* plots of Lotan *et al.* (1965) for PHPG in H_2O (14% helix) and in MeOH (90% helix). Okita *et al.* (1970) have shown a linear relationship between % helicity and $[\eta]$, as well as $[\eta]$ and *MW*, for polymer helicity below 70% and $[\eta] < 0.4$.

ıns were 0.2% in all cases. The efflux times for the solvent were between 324 and 344 sec, reproducible to ±0.2 sec.

Preparation of Samples for CD Studies. Stock solutions of PHPG and P(HPG^{x}Leu^y) were prepared by dissolving approximately 10 mg of polymer in 10 ml of H_2O (double glass distilled) with magnetic stirring for 30 min. The resultant solutions appeared clear and were heated to 80° for 2 hr. The heated solutions were cooled to room temperature, and then filtered twice through Millipore filters LSWP 1300 (Millipore Corp., Bedford, Mass.). A slight trace of cloudiness was detected in copolymer solutions with leucine content greater than 17% which remained even after centrifugation. Since these stock solutions (0.1% w/v) were too concentrated for CD studies, they were diluted tenfold. The 0.01% solutions used for CD measurements appeared clear without any trace of turbidity. All solutions were stored at 5° and removed as needed.

Concentration Determination. Concentrations of P(HPG^x-Leu^y) solutions were determined by using the micro-Kjeldahl method (Lang, 1958). Aliquots containing 12–92 μg of nitrogen were reacted with Nessler reagent after digestion. The concentrations of the diluted stock solutions actually used in the CD runs were also measured. Concentrations determined in this manner are reproducible to 2%.

Circular dichroism measurements were made with a Cary Model 60 recording spectropolarimeter equipped with a 6001-CD attachment (slit width programmed to maintain a 15-Å half-bandwidth). Fused-quartz, jacketted cells (1 or 2 mm) (Optical Cell Co., Beltsville, Md.) were used. Temperatures from 0 to 80° were maintained to within ±0.2° (but to within ±0.5° at 0°) with a Tamson-refrigerated circulating bath. Solvent base lines were recorded at the beginning and end for all sample runs. The polymer concentration used was 0.01–0.02% w/v. Most of the runs were conducted using a 0.01% solution in a 1-mm cell, giving an absorbance of less than 0.7 at 190 nm. Measurements were made in the wavelength range of 260–185 nm. CD results are reported as residue molar ellipticity, $[\theta]_\lambda$ ((deg cm^2)/dmole), using the equation $[\theta] = 10 \times \theta_{obsd}/lc$, where θ_{obsd} = observed ellipticity (degrees), l = cell path length (decimeters), and c = residue concentration (moles per liter). The concentration was corrected for changes in the density of H_2O and 90% MeOH with temperature. The CD spectra of each sample were first recorded at 20°, and then the solution was cooled to 10 and 0° to obtain the CD spectra at these temperatures. The sample was then heated to 80° in 10° intervals, allowing 15 min for equilibration at each temperature before the CD spectra were recorded. Finally, the sample was cooled back to 20 and 0° to check for reversibility.

Far-Ultraviolet Absorption Spectra. The ultraviolet (uv) absorption measurements were performed on a Cary 14 spectrophotometer using matched stoppered quartz cells of 1-mm path length (Hellma) at 23°. Prepurified nitrogen was flushed through the instrument at a flow rate of 9 ft^3/min before and during the experiment so that oxygen absorption (below 200 nm) is minimized.

The polymer concentrations varied from 0.01 to 0.1% w/v. Solvent baselines were recorded for all sample runs, and pure solvent (H_2O or 90% MeOH) was used in the reference blank cell in each case. Measurements were made in the wavelength region of 250–185 nm. Uv absorption results are reported as molar extinction coefficient, ϵ (M^{-1} cm^{-1}), using the equation $\epsilon = A/cl$, where A = absorbance, c = residue concentration (moles per liter), and l = cell path length (centimeters).

Sedimentation Velocity. The ultracentrifuge experiments were performed at 25 and 3° in a Spinco Model E analytical ultracentrifuge at 60,000 rpm using 2° single-sector cells. The 0.4% w/v solution of PHPG in 90% MeOH was freshly prepared. Ten photographs were taken in 8-min intervals. The sedimentation constants were measured with a microcomparator (Nikon Profile Projector, Model 6c) using eq 1

$$s_{20,w} = s_{obsd}\left(\frac{\eta_T}{\eta_{20}}\right)\left(\frac{\eta}{\eta_0}\right)_T\left(\frac{1 - \bar{v}\rho_{20,w}}{1 - \bar{v}\rho_T}\right) \quad (1)$$

(Schachman, 1959) where $s_{obsd} = (1/60\omega^2)(\mathrm{d} \ln x)/[\mathrm{d}T]$, x is the distance from the center of rotation, $\mathrm{d}T$ is the time interval, ω is the angular velocity of the rotor, η_T and η_{20} are the viscosities of H_2O at T° and 20°, respectively, η and η_0 are the viscosities of the solvent and H_2O respectively at T°, $\rho_{20,w}$ is the density of water at 20°, ρ_T is the density of the solvent at T° and $\bar{v}$ is the partial specific volume of the solute (taken as 0.79 ml/g for PHPG (Lotan *et al.*, 1965)).

Results and Discussion

Viscosity. Since Lotan *et al.* (1965) found an extremely small dependence of the specific viscosity, η_{sp}/c, of PHPG in H_2O as a function of polymer concentration, we have assumed the specific viscosities determined for the various polymer samples at 0.2% concentration to be identical with

TABLE III: Circular Dichroism Values[a] of PHPG and P(HPG^{z}Leu^y).

Polymer	% Concn (w/v)	Solvent	Temp (°C)	$[\theta]_{222}$	% Helix[b]	$[\theta]_{208}$	% Helix[c]	$[\theta]_{193}$	% Helix[d]
P(HPG^{76}Leu^{24})	0.009	H_2O	0	−40,519	77	−35,859	69	+95,219	81
			20	−34,440	67	−32,415	62	+81,037	69
			80	−16,207	32	−18,233	31	+38,500	33
P(HPG^{80}Leu^{20})	0.016	H_2O	0	−37,061	72	−33,048	63	+84,982	72
			20	−30,688	60	−28,327	53	+69,873	60
			80	−13,573	27	−15,934	26	+30,688	26
P(HPG^{83}Leu^{17})	0.02	H_2O	0	−32,546	64	−27,277	51	+75,424	64
			20	−26,244	51	−22,731	41	+57,963	49
			80	−9,092	18	−10,745	15	+21,697	18
P(HPG^{92}Leu^{8})	0.02	H_2O	0	−25,983	51	−24,424	45	+59,761	51
			20	−18,881	37	−19,920	35	+38,975	33
			80	−5,630	11	−8,834	11	+12,125	10
PHPG	0.01	H_2O	0	−18,292	36	−18,292	31	+33,894	31
			20	−8,608	17	−11,944	17	+16,678	14
			80	−2,150	4	−5,165	3	+2,000	2
PHPG	0.01	90% MeOH	0	−50,627	99	−48,197	96	+115,430	98
			20	−48,180	94	−46,877	88	+108,850	92
			40	−43,280	85	−42,224	83	+104,000	88
PHPG	0.015	CH_3SO_3H	0	+3,113	6	0	0	+12,500	11
			20	+1,868	4	0	0	+7,470	6
			80	+996	2	0	0	0	0

[a] % error in $[\theta]_{222}$ = 4.3%, $[\theta]_{208}$ = 6.0%, $[\theta]_{193}$ = 7.3%. [b] Based on $[\theta]_{222}$ = −51,190 for 100% helix; $[\theta]_{222}$ = 0 for 0% helix. [c] Based on $[\theta]_{208}$ = −50,000 for 100% helix; $[\theta]_{208}$ = −4000 for 0% helix. [d] Based on $[\theta]_{193}$ = +117,850 for 100% helix; $[\theta]_{193}$ = 0 for 0% helix.

their intrinsic viscosities. These results are listed in Table II. The molecular weights were estimated from the $[\eta]$ *vs. MW* plots of Lotan *et al.* (1965) for PHPG in H_2O and in methanol, taking into account the variation of $[\eta]$ with the per cent helicity present in the copolymers. The error in DP determined by this method is estimated at roughly 5%.

Circular Dichroism Spectra. From ORD studies, Lotan *et al.* (1965) and Okita *et al.* (1970) have shown that PHPG is essentially in the coil conformation in aqueous solution. From b_0 measurements, it was found that the helicity of PHPG decreases with increasing temperature and with decreasing molecular weight. For PHPG with a DP of 310 (Lotan *et al.*, 1965), it was shown that the helicity decreased from 35% at 0° to 7% at 60°. A similar effect is shown in Figure 1 from CD studies for PHPG (DP = 250) in H_2O as a function of temperature. The molar ellipticities $[\theta]_\lambda$ at 222, 208, and 193 nm are given in Table III. It is seen that PHPG is about 36% helical at 0° and 17% helical at 20°, agreeing well with the earlier ORD studies. When heated to 80°, PHPG is essentially converted completely to the coil form (with 4% helix remaining). Although the shape of the CD curve does not agree with that associated with 100% random coil of poly(Lys) (Greenfield and Fasman, 1969), it is similar to the CD spectra of poly(Glu-Na) films in the unordered conformation as well as various denatured proteins (Fasman *et al.*, 1970). The absolute ratio of the values ($[\theta]_{208}$:$[\theta]_{222}$) increases with increasing temperature, with the minima at 208 and 222 nm shifting to 200 and 225 nm at 80°. The maximum at 193 nm decreases with increasing temperature and shifts to 190 nm at 80°.

The CD spectra of various P(HPG^{z}Leu^y) copolymers in H_2O were obtained as a function of temperature and values of $[\theta]_{222}$, $[\theta]_{208}$, and $[\theta]_{193}$ are listed in Table III. The curves for the temperature melt out of P(HPG^{76}Leu^{24}) are shown in Figure 2. The curves for the intermediate polymers lie between those of Figures 1 and 2. The peak at 193 nm and the minima at 208 and 222 nm for P(HPG^{z}Leu^y) at 0° closely resemble the values given by Greenfield and Fasman (1969) for poly(Lys) with high helical content. It is seen in Figure 2, as well as in Table III, that the absolute value of $[\theta]_\lambda$ at the extrema decreases with increasing temperature, indicating decreasing helicity in the copolymers. CD studies of P(HPG^{76}-Leu^{24}) showed no concentration dependence in the ellipticity values in the range 0.01–0.1% w/v. The CD curves at 0° show that $[\theta]_{208} < [\theta]_{222}$ for all the copolymers, with the absolute value of the ratio $[\theta]_{208}$:$[\theta]_{222}$ increasing as the temperature is raised. Earlier studies have shown that the $[\theta]_{208}$:$[\theta]_{222}$ ratio seems to change in less polar solvents, such as PHEG in MeOH (Adler *et al.*, 1968) and poly (Ala) in trifluoroethanol (Quadrifoglio and Urry, 1968a). In addition, Fasman *et al.* (1970) found $[\theta]_{208}$:$[\theta]_{222}$ of α-helical polypeptide films to decrease with increasing relative humidity.

CD of PHPG in MeOH. From b_0 measurements, Lotan *et al.* (1965) and Okita *et al.* (1970) have shown that PHPG in water–methanol mixtures becomes more helical with increasing methanol concentration. Methanol was demonstrated to be a nearly complete helicogenic solvent from their viscosity studies where PHPG in MeOH was shown to assume a rodlike shape. The effect of varying the concentration of MeOH on the CD spectra of PHPG in H_2O at 23° is shown

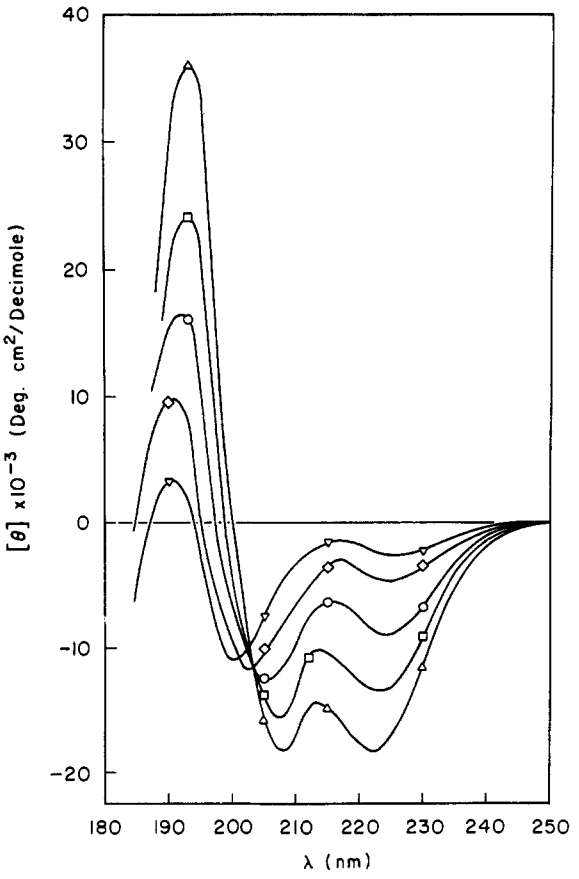

FIGURE 1: Circular dichroism spectra of PHPG in H_2O at various temperatures. 0° (△), 10° (□), 20° (○), 40° (◇), 80° (▽). Polymer concentration = 0.01%; cell = 2 mm.

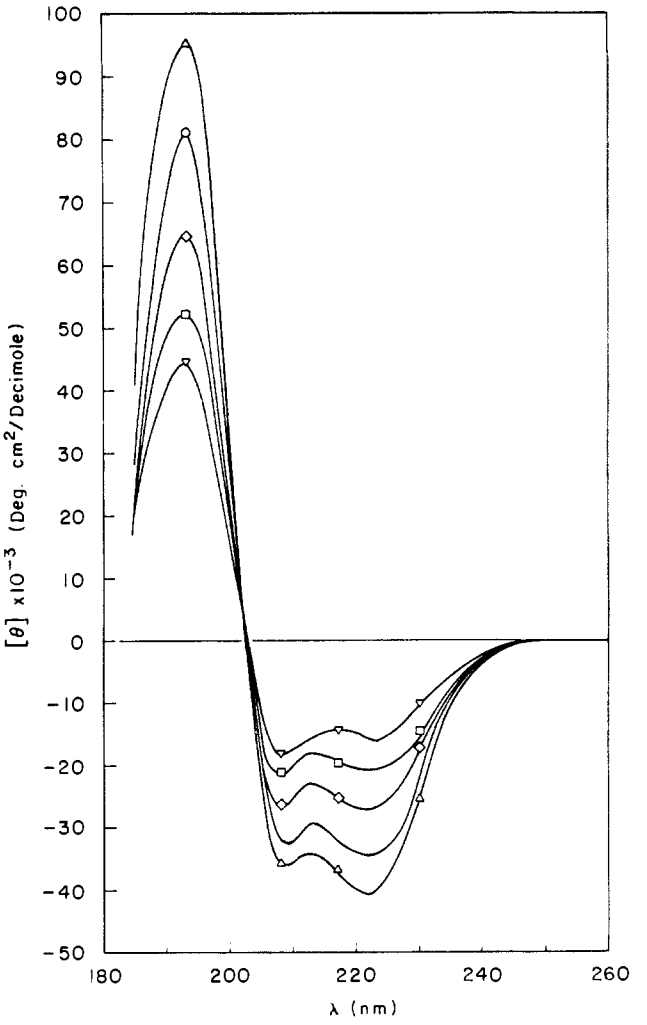

FIGURE 2: Circular dichroism spectra of P(HPG^{76}Leu^{24}) in H_2O at various temperatures. 0° (△), 20° (○), 40° (◇), 60° (□), 80° (▽). Copolymer concentration = 0.009%; cell = 1 mm.

in Figure 3. At 0% MeOH, PHPG is approximately 17% helical (see Table III) but with an addition of 10 and 20% MeOH, the helicity increases to 40 and 60%, respectively. At 90% MeOH, PHPG is 94% helical with $[\theta]_{193} = +108{,}850$, $[\theta]_{209} = -47{,}800$, and $[\theta]_{222} = -48{,}180$. These values compare favorably to that of PHEG in 95% MeOH at 22° (Adler *et al.*, 1968) with $[\theta]_{193} = +105{,}000$, $[\theta]_{209} = -44{,}845$, and $[\theta]_{221} = -44{,}845$, as well as poly(γ-methyl-L-glutamate) in trifluoroethanol at 25° (Cassim and Yang, 1970) with $[\theta]_{191} = +108{,}000$, $[\theta]_{209} = -52{,}700$ and $[\theta]_{221} = -45{,}900$. It is clear that the above values are much higher than those cited for 100% helix using the poly(Lys) model in H_2O (Greenfield and Fasman, 1969) with $[\theta]_{191} = 76{,}900$, $[\theta]_{208} = -32{,}600$, and $[\theta]_{222} = -35{,}700$. Hence one should exercise caution in selecting limiting $[\theta]_\lambda$ values for different polymers and solvents before calculating per cent helicity.

A plot of $[\theta]_{222}$ for PHPG *vs.* per cent MeOH is seen in Figure 4. The shape of the curve was similarly reproduced when $[\theta]_{193}$ and $[\theta]_{208}$ *vs.* per cent MeOH were plotted. In all three cases, a rapid rise in the absolute value of $[\theta]_\lambda$ characterizes each curve as MeOH was added to the aqueous PHPG solution, with a levelling off in $[\theta]_\lambda$ at 80% MeOH. While this seems to indicate that the maximum values of $[\theta]_\lambda$ have been reached, representing full helicity at 23°, even higher values were obtained by cooling PHPG in 90% MeOH to 0° as shown in Figure 5 and Table III. Similarly, it was observed (Chou and Scheraga, 1971) that values of b_0 and $[m']_{233}$ of poly(Lys) levelled off at high pH at a given temperature but these parameters continued to increase (in absolute value) when the temperature is lowered.

It is debatable whether the higher values of $[\theta]_\lambda$ for PHPG in 90% MeOH at 0° (see Table III) actually represent a higher helical content, and not a solvent effect or an increase in rotational strength, $R_{n\pi^*}$, of the n–π* transition with decrease in temperature (Kauzmann and Eyring, 1941). In a study of poly(Glu) at pH 4.4 and 20° (Cassim and Taylor, 1965), $-b_0$ was found to increase from 650 at 0% MeOH to 755 at 80% MeOH with a corresponding increase in the intrinsic viscosity from $[\eta] = 2.3$–3.0. These data support the interpretation that $-b_0$ increases as the helical content increases with greater MeOH concentration. Further evidence of greater helicity of poly(Glu) in methanol was provided by the electrical birefringence studies of Matsumoto *et al.* (1968) who showed that the length of poly(Glu) in MeOH–H_2O mixtures increased with greater MeOH concentration. Likewise, Teramoto *et al.* (1967) have shown that the intrinsic viscosity of PBLG in dichloroacetic acid–ethylene dichloride mixtures and in dimethylformamide as function of temperature is similar to that of the corresponding curves of $[m']_{578}$ *vs.* temperature, implying a direct correlation of $[\eta]$ with helical content. In addition, Okita *et al.* (1970) found similar changes of $[\eta]$ and $-b_0$ (helical content) for PHPG with temperature in MeOH–H_2O mixtures. Since intrinsic viscosity is a measure of hydrodynamic properties, the increase of $[\eta]$ from

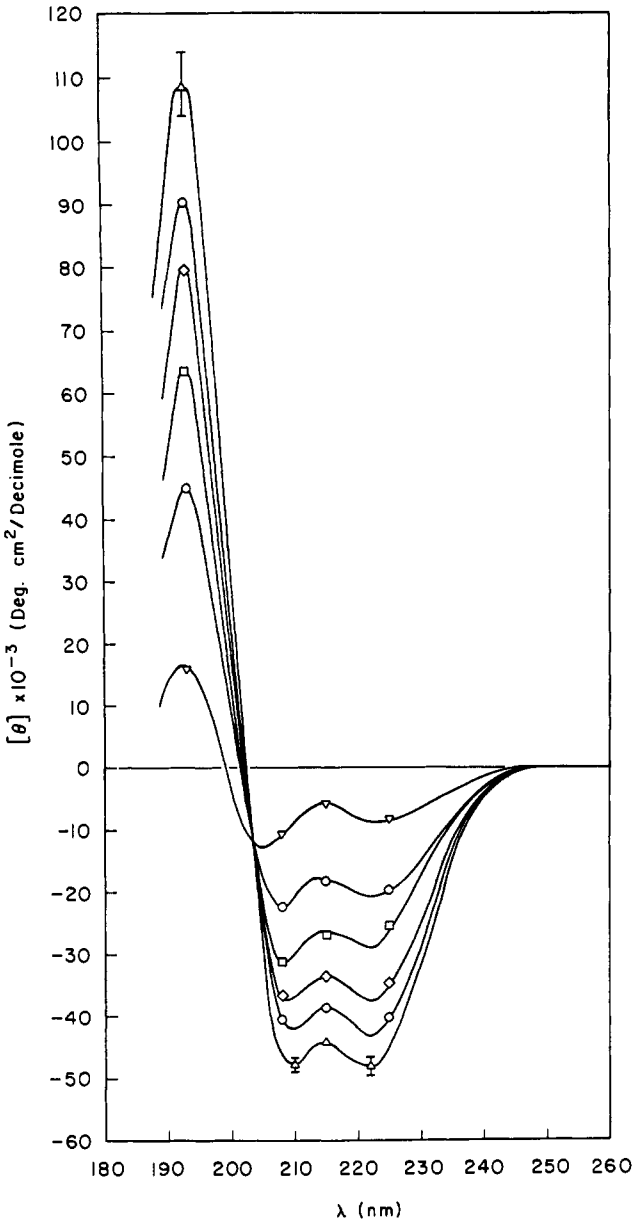

FIGURE 3: Circular dichroism spectra of PHPG as a function of MeOH concentration at 23°. % MeOH: 0 (▽), 10 (○), 20 (□), 30 (◊), 40 (●), 90 (△). Polymer concentration = 0.01%; cell = 1 mm. Error bars represent average deviation of 7 different CD runs.

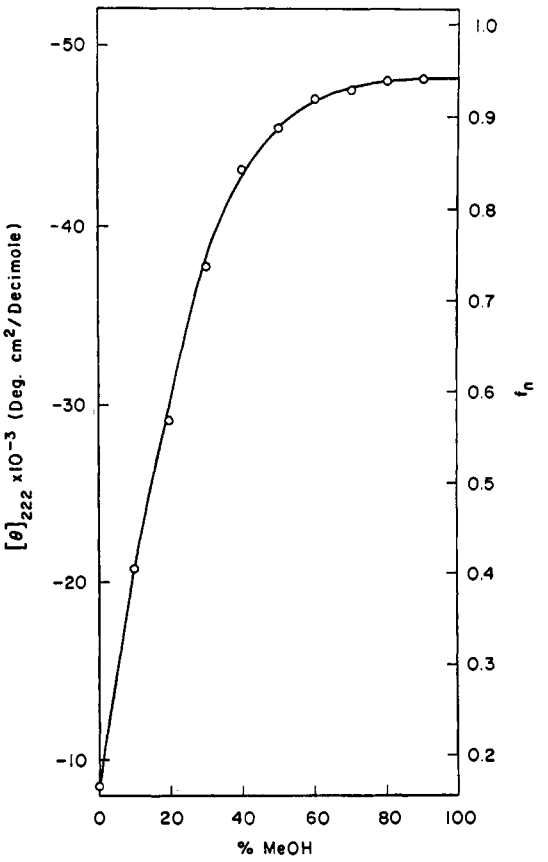

FIGURE 4: Dependence of $[\theta]_{222}$ and fraction helix (f_h) for PHPG as a function of MeOH concentration at 23°.

4.2 at 20° to 4.6 at 0° for PHPG in MeOH (Okita *et al.*, 1970) indicates stiffening of the rodlike molecule. Hence, the use of the higher $[\theta]_\lambda$ values for PHPG in 90% MeOH at 0° as limits for 100% helix is justified.

Since the CD spectrum of PHPG in H_2O at 80° (Figure 1) does not show peaks at 217 and 197 nm, characteristic of the random coil in polypeptides (Adler *et al.*, 1968), methanesulfonic acid (CH_3SO_3H) was used as a randomizing solvent. This strong acid was utilized in conformational studies of poly(γ-ethyl-L-glutamate) (PELG) (Steigman *et al.*, 1969) as well as poly(L-cyclohexylalanine) (PCHA) and poly(Phe) (Peggion *et al.*, 1970), and these polymer solutions were shown to be stable enough to permit CD measurements.

The CD spectra of PHPG in CH_3SO_3H as a function of temperature (Figure 6) is seen to be typical of polypeptides in the random-coil conformation, with a weak positive band at 217 nm and a negative band around 196 nm. While the molar ellipticity of the positive band is similar to that observed for polypeptides in the coil form at 20° ($[\theta]_{217}$ = +4000), the value of the negative band ($[\theta]_{196}$ = −10,000) is about 1/3 lower (Timasheff and Gorbunoff, 1967). It has been suggested that this behavior is typical of polypeptides dissolved in CH_3SO_3H, and is probably due to solvent effects on the π–π^* peptide transition (Peggion *et al.*, 1970). These investigators observed a value of $[\theta]_{196}$ = −12,500 for PCHA in pure CH_3SO_3H which agrees well with our findings. The crossover points at 208 and 188 nm are similar to that observed for PELG in 99% CH_3SO_3H and 96% H_2SO_4 (Steigman *et al.*, 1969). The small negative band at 238 nm found frequently in random polypeptides (Adler *et al.*, 1968) was not observed.

The effect of the leucine content on the CD spectra of P(HPG^{x}Leu^y) polymers in H_2O at 20° is shown in Figure 7. The spectra of PHPG changes appreciably upon incorporation of 8% Leu and the per cent helix changes from 17 to 37%. The minima at 206 and 223 nm for the homopolymer shift to 208 and 222 nm for the copolymers. The maximum remains at 193 nm for both homopolymer and copolymers, but it is clear that the absolute values of $[\theta]_{193}$, $[\theta]_{208}$, and $[\theta]_{222}$ increased steadily with increasing Leu content. The shape of the CD spectra for P(HPG^{76}Leu^{24}) at 20°, with values of $[\theta]_{193}$ = +81,037 ± 4052, $[\theta]_{208}$ = −32,415 ± 1593, and $[\theta]_{222}$ = −34,440 ± 1100, resembles closely that of fully helical poly(Lys) (pH 11.1) at 22° cited earlier (Greenfield and Fasman, 1969). While it is tempting to infer that the 24% Leu copolymer in H_2O at 20° is fully helical, cooling studies (Figure 2), as well as studies in MeOH (Figure 3), show that the molar ellipticities at the extrema can be increased still fur-

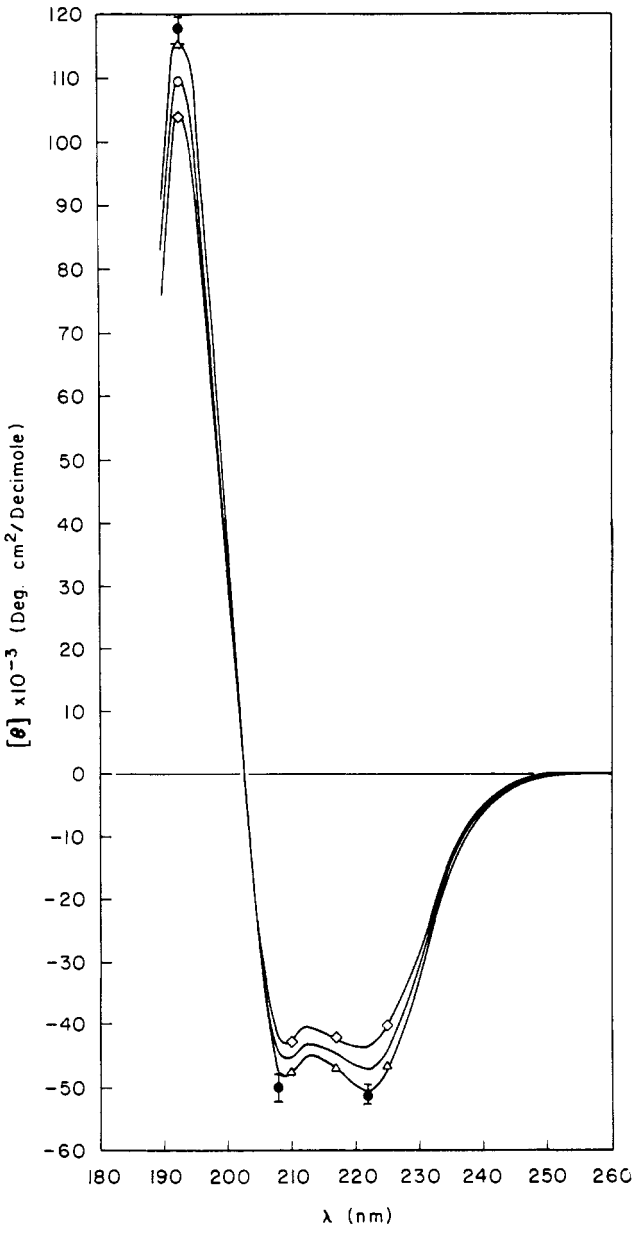

FIGURE 5: Circular dichroism spectra of PHPG in 90% MeOH at various temperatures. 0° (△), 20° (○), 40° (◇). Polymer concentration = 0.01%; cell = 1 mm. Average $[\theta]$ values of PHPG and the four P(HPG^{x}Leu^y) copolymers in 90% MeOH at 0° (●), with average deviation indicated by error bars.

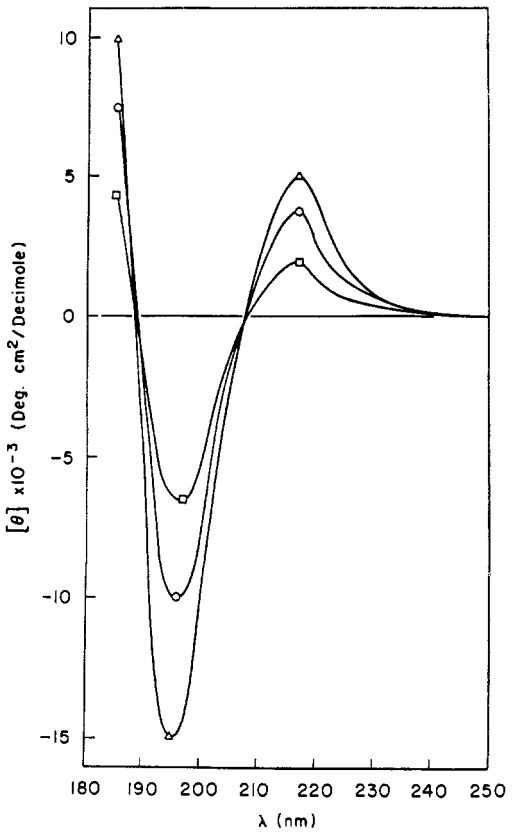

FIGURE 6: Circular dichroism spectra of PHPG in CH_3SO_3H at various temperatures. 0° (△), 20° (○), 80° (□). Polymer concentration = 0.015%; cell = 1 mm.

ther. This may be taken as evidence that P(HPG^{76}Leu^{24}) is not fully helical in H_2O at 20°, even though its CD spectra are almost identical with that of the fully helical poly(Lys). The CD spectra for PHPG in 90% MeOH and CH_3SO_3H at 20° are included in Figure 7 as representative spectra for the helix and random coil in this copolymer system.

The dependence of $[\theta]_{222}$ on temperature for the copolymer series P(HPG^{x}Leu^y) is seen in Figure 8. Similar but not identical curves were obtained for $[\theta]_{208}$ and $[\theta]_{193}$. From this figure, it is seen that with the possible exception of the homopolymer, none of the copolymers appear to melt out completely in H_2O at 80°. P(HPG^{92}Leu^8) and P(HPG^{76}Leu^{24}) have approximately 10 and 30% helix, respectively, at this temperature. While a sign of levelling off in $[\theta]_{222}$ for PHPG in H_2O at 80° is observed in Figure 8, values of $[\theta]_{208}$ and $[\theta]_{193}$ for the homopolymer are still decreasing at 80°. However, increased temperature does cause a diminution of all $[\theta]_\lambda$ values, as seen in Figure 6, for the random coil. It has already been shown (Figure 1) that the CD spectra of PHPG in H_2O at 80° do not resemble those associated with polypeptides in the random conformation, and that the latter spectra can be obtained by dissolving PHPG in CH_3SO_3H (Figure 6). These observations lead to the conclusion that a small residue of helix ($\approx$4%) is still present in PHPG at 80°, so that the $[\theta]_\lambda$ value at 80° does not represent that for a fully random coil. Since the values of $[\theta]_{222}$ and $[\theta]_{193}$ for both homopolymer and copolymers appear to approach the limiting value of zero at high temperatures, and are also the values of PHPG in CH_3SO_3H at 80° (Figure 8), the values of $[\theta]_{222} = [\theta]_{193} = 0$ have been assigned for the random-coil conformation in this system. Similarly, the value of $[\theta]_{208}^{coil} = -4000$ is assigned for the random coil based on the extrapolated value at high temperature in water.

No limiting values of $[\theta]_\lambda$ appear at the low-temperature end for the series, P(HPG^{x}Leu^y) in H_2O (Figure 8). The $[\theta]_\lambda$ values at the extrema for these copolymers are still increasing at 0°, suggesting that full helicity has not been reached. When PHPG is dissolved in 90% MeOH, the increase in $[\theta]_\lambda$ with decreasing temperature is diminished (Figure 5) and the $[\theta]_\lambda$ values at the extrema appear to level off near 0°, suggesting full helicity. Furthermore, the CD spectra of all four copolymers, P(HPG^{x}Leu^y), in 90% MeOH at 0° were obtained and found to be almost identical with that of PHPG in this solvent (Figure 5). The $[\theta]_\lambda$ values were averaged to give $[\theta]_{222} = -51{,}190 \pm 1420$, $[\theta]_{208} = -50{,}000 \pm 1950$, and $[\theta]_{193} = +117{,}850 \pm 2140$, representing 100% helicity in this system.

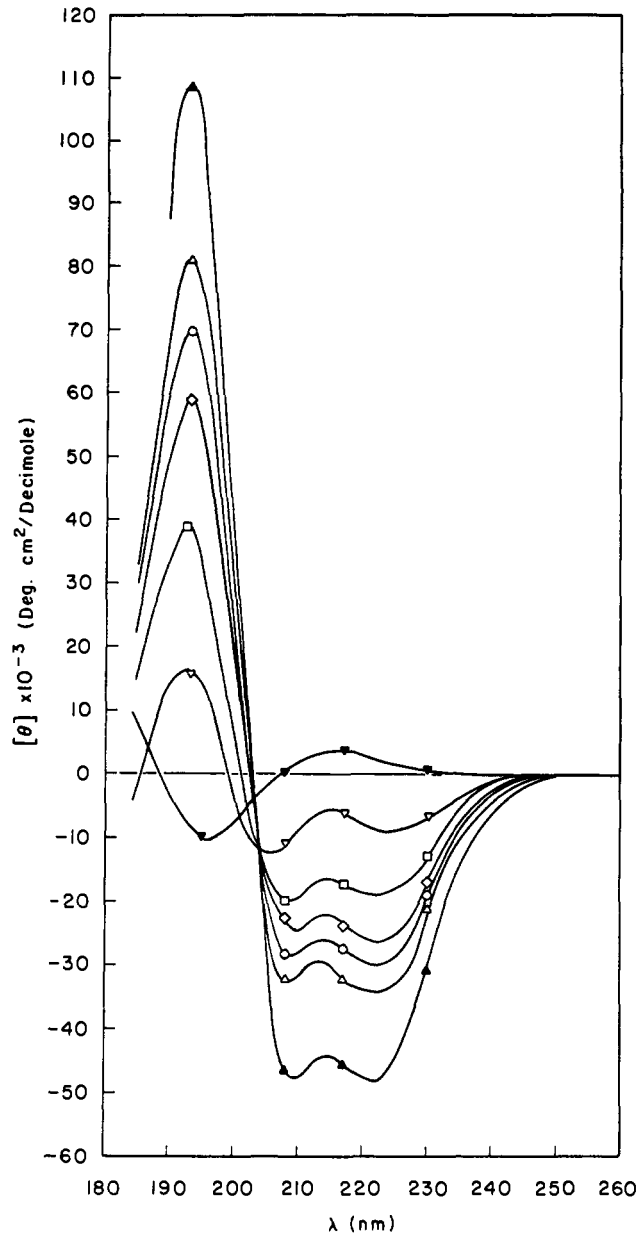

FIGURE 7: Circular dichroism spectra of P(HPG^{x}Leu^{y}) at various ratios in H_2O at 20°. 100:0 (▽), 92:8 (□), 83:17 (◇), 80:20 (○), 76:24 (△). Polymer concentration = 0.01 − 0.02%; cell = 1 or 2 mm. PHPG in 90% MeOH, concentration = 0.01% (▲); PHPG in CH_3SO_3H, concentration = 0.015% (▼).

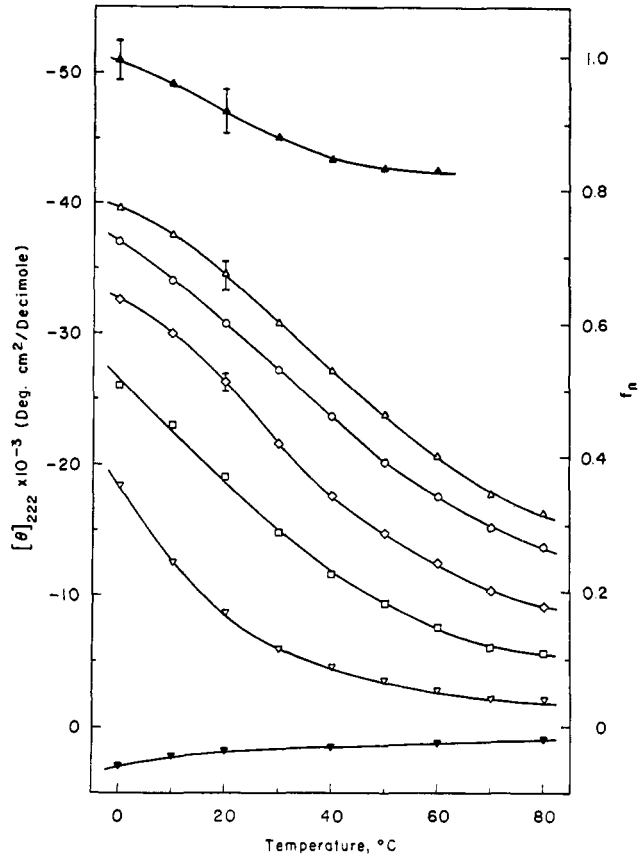

FIGURE 8: $[\theta]_{222}$ and fraction helix (f_n) dependence on temperature for P(HPG^{x}Leu^{y}) series in H_2O. 100:0 (▽), 92:8 (□), 83:17 (◇), 80:20 (○), 76:24 (△). PHPG in 90% MeOH (▲); PHPG in CH_3SO_3H (▼). Average deviation in $[\theta]$ is represented by error bars.

The helix–coil transition of the copolymers was shown to be reversible. The $[\theta]_\lambda$ values at 0° after the heating cycle were about 8% higher than the values before heating, but this discrepancy may be within experimental error. If the copolymer samples were not preheated to 80° prior to CD studies, it was found that the $[\theta]_\lambda$ values at 0° after heating were 25% higher than the values before heating. This suggests that they may be regions in the copolymer where helical formation may be unfavorable, but becomes feasible after heating to 80°, followed by cooling.

Estimation of per cent helix as a function of temperature for the copolymers from $[\theta]_{222}$ and $[\theta]_{193}$ appear to be in excellent agreement (Table III). Estimates from $[\theta]_{208}$ are about 10% lower, and better agreement cannot be obtained by adjusting the limiting $[\theta]_{208}$ values for helix and coil. It was found that the magnitude of the CD minimum at 208 mμ is more sensitive to solvent effects than the CD extrema at 193 and 222 mμ. While the $[\theta]_{193}$ and $[\theta]_{222}$ values of P(HPG^{76}Leu^{24}) at 0° increased by 21 and 25%, respectively, in 90% MeOH relative to H_2O, $[\theta]_{208}$ increased by 34%, with the ratio $([\theta]_{208}:[\theta]_{222})_{H_2O} = 0.89$ increasing to $([\theta]_{208}:[\theta]_{222})_{MeOH} = 0.95$. This is not caused by an increase in helicity, as the $[\theta]:[\theta]_{222}$ ratio decreases with lower temperature when these copolymers become more helical. Comparable changes can also be seen in helical poly(Ala-Gly-Lys) at 7° studied by Quadrifoglio and Urry (1968a) with the ratio $([\theta]_{208}:[\theta]_{222})_{H_2O} = 0.85$ increasing to $([\theta]_{208}:[\theta]_{222})_{F_3CCH_2OH} = 1.08$, in the organic solvent F_3CCH_2OH. Similarly, for helical poly(Lys) at room temperature, the ratio $([\theta]_{208}:[\theta]_{222})_{H_2O} = 0.91$ (Greenfield and Fasman, 1969) increased to $([\theta]_{208}:[\theta]_{222})_{MeOH} = 1.22$ (Epand and Scheraga, 1968a). Since the ratio $([\theta]_{208}:[\theta]_{222})$ for polypeptides in organic solvents is greater than that in H_2O, the per cent helix for polymers in H_2O calculated from $[\theta]_{208}$ will be lower than that calculated from $[\theta]_{222}$. This explains the lower estimates of per cent helix based on $[\theta]_{208}$ in Table III. While values of per cent helix based on $[\theta]_{222}$ and $[\theta]_{193}$ are in close agreement, it was decided not to average these values since the reproducibility in $[\theta]_{222}$ is better than $[\theta]_{193}$, as can be seen from the error bars in Figures 3 and 5. Thus $[\theta]_{222}$ was chosen for calculating the per cent helicity ($f \times 100$) in the copolymers:

$$f_n = \frac{[\theta]_{222} - [\theta]_{222}^{coil}}{[\theta]_{222}^{helix} - [\theta]_{222}^{coil}} = \frac{[\theta]_{222}}{51{,}190} \quad (\text{as } [\theta]_{222}^{coil} = 0) \qquad (2)$$

TABLE IV: Molar Extinction Coefficients per Residue for Oligomeric and Polymeric Peptides in Solution from Ultraviolet Spectra.

Polypeptide	Solvent	λ_{max} (nm)	ϵ_{coil}	λ_{max} (nm)	ϵ_{helix}	Ref
Poly(Lys)	H_2O, pH 6.0	192	7100			*c*
	pH 10.8			191	4400	
	H_2O, pH 8.8	190	7340			*d*
	pH 10.8			188	5200	
Poly(Glu)	H_2O, pH 10	192	7100			*c*
	pH 3.2			190	4200	
	H_2O, pH 8	190	7000			*d*
	pH 4.9			188	4500	
β-Methyl-L-aspartate						
Trimer	TFE[j]	190	7500			*e*
High polymer	TFE[j]			189	4980	*f*
γ-Methyl-L-glutamate						
Trimer	TFE	190	7700			*e*
High polymer	TFE			188.5	4730	*f*
Oligoglycine	H_2O	188	7700			*g*
Poly(Ala)	TFE–TFA[j] (98:2)			189	4170	*h*
PHPG[a]	H_2O, 23°	190	7250			*i*
	90% MeOH, 23°			191.5 (190)	4850 (4600)	*i*
P(HPG^{76}Leu^{24})[b]	H_2O, 23°			190	5750	*i*

[a] PHPG is 17% helical in H_2O and 94% helical in 90% MeOH at 23° based on CD studies. The corrected values for 0% helix and 100% helix in PHPG are ϵ_{coil} = 7750, ϵ_{helix} = 4250 at 190 nm. [b] P(HPG^{76}Leu^{24}) in H_2O at 23° has 67% helix. [c] Rosenheck and Doty (1961). [d] Tinoco *et al.* (1962). [e] Goodman *et al.* (1963). [f] Goodman and Rosen (1964). [g] McDiarmid (1965). [h] Quadrifoglio and Urry (1968a). [i] This work. [j] TFE = trifluoroethanol; TFA = trifluoroacetic acid.

Far-Ultraviolet Absorption Spectra. It was observed that solutions of P(HPG^{z}Leu^y) at 0.1% concentration were slightly cloudy, but became clear when diluted tenfold. In order to check whether these copolymers were aggregating in this concentration range (0.01–0.1%) ultraviolet absorption measurements were carried out. If aggregation is present at higher concentration, one would expect an increase in absorbance ($A = \epsilon cl$) resulting in higher values of the molar extinction coefficient, ε. The uv spectrum of P(HPG^{76}Leu^{24}) in Figure 9 shows that ε was essentially the same irrespective of concentration in the 0.01–0.1% range. Since the polymer concentration used in the CD work herein varied from 0.01 to 0.02% w/v, it is safe to conclude that there is no aggregation in the copolymer solutions.

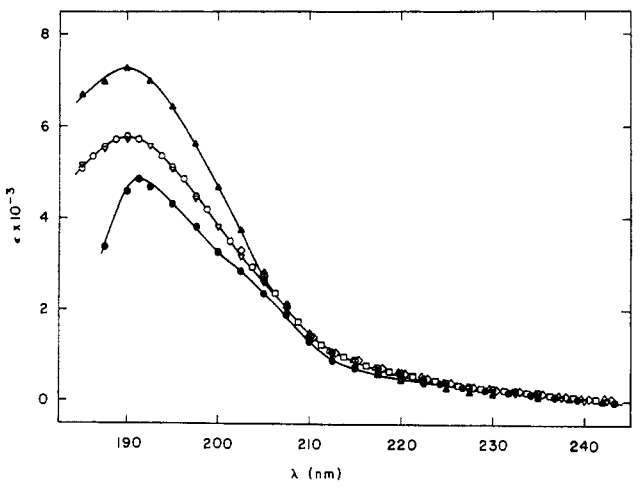

FIGURE 9: Far-uv absorption spectra of P(HPG^{76}Leu^{24}) in H_2O at various concentrations and PHPG in various solvents at 23°. P(HPG^{76}Leu^{24}): 0.009% (▽), 0.021% (○), 0.042% (◇), 0.062% (□), 0.092% (△). PHPG: 0.01% in H_2O (▲); 0.013% in 90% MeOH (●). The spectra were corrected for the extra amide chromophore in the side chain of HPG for both polymer and copolymer.

The uv spectra of PHPG in H_2O and 90% MeOH are also shown in Figure 9, representing the coil and helical conformations respectively. These spectra were corrected for the extra amide chromophore in the side chain of PHPG so that comparison with the copolymer could be made in the following manner. The initial values of ϵ_{190} were 15,000 and 12,300 for PHPG in H_2O and 90% MeOH, respectively. From Table III, it is seen that at room temperature PHPG in H_2O is still 17% helical; hence the uv spectra obtained did not represent the complete coil. Likewise, PHPG in 90% MeOH at 20° was not fully helical (f_n = 0.94). In order to obtain the limiting values of ε in this system, the following equation was used (Tinoco *et al.*, 1962) which had shown that per cent helicity was directly proportional to absorption

$$f_n = \frac{\epsilon_{coil} - \epsilon}{\epsilon_{coil} - \epsilon_{helix}} \quad (3)$$

It is assumed that the magnitude of ϵ_{helix} for PHPG is the same in H_2O as in MeOH. This is supported by literature data in Table IV showing that ϵ_{coil} and ϵ_{helix} values are almost identical in H_2O and organic solvents.

Solving the above equation simultaneously, ϵ'_{coil} = 15,500 was obtained, which represents the absorbance contribution of two amide chromophores in the hydroxypropylglutamine residue. Assuming that these two amides in the coil form contribute to the absorbance equally, ϵ_{190}^{coil} = 7750 was obtained for PHPG in the random-coil conformation. The

ϵ_{coil} values at other wavelengths were calculated so that the uv spectrum due to the extra amide was subtracted from the initial spectra of PHPG. A similar correction was made for the ϵ values of P(HPG^{76}Leu^{24}), taking into account that the extra amide contribution occurs in 76% of the side chains in this copolymer.

The corrected value for the random coil conformation of PHPG is $\epsilon_{coil} = 7750$ at 190 nm which is in excellent agreement with the ϵ_{coil} values for oligomers (Table IV). The ϵ_{coil} values of poly(Lys) are about 7% lower. However, this discrepancy could arise from the fact that at low pH the fully charged polypeptide might assume the form of an extended helix rather than a random coil (Tiffany and Krimm, 1968). The corrected value for 100% helix in PHPG is $\epsilon_{helix} = 4250$ at 190 nm which is in close agreement with $\epsilon_{helix} = 4170$ at 189 nm for poly(Ala). The latter was shown to be fully helical as no change in the CD ellipticities was seen between 7 and 20° (Quadrifoglio and Urry, 1968a). The other ϵ_{helix} values quoted in Table IV are somewhat higher, but it is likely that these polypeptides are not completely helical at room temperature, since they agree more closely with $\epsilon_{helix} = 4850$ at 191.5 nm for PHPG in 90% MeOH which is 94% helical at 23°. The large hypochromic effect associated with the latter can be seen clearly in Figure 9, together with the appearance of a shoulder at 205 nm, characteristic of the helical conformation (Rosenheck and Doty, 1961).

When the solvent environment of PHPG is changed from H_2O to 90% MeOH, the uv absorption peak shows a red shift from 190 to 191.5 nm (Figure 9). Since this red shift is accompanied by a conformational change in PHPG, it is difficult to differentiate between solvent and conformational effects. However, it is interesting to note from Table IV that helical formation of polypeptides in water is accompanied by a slight uv blue shift, which is opposite to the trend observed in Figure 9. Quadrifoglio and Urry (1968b) also found a blue shift (from 190 to 188 nm) in the uv spectra of poly(Ser) when the solvent was changed from H_2O to 80% trifluoroethanol, and attributed this to the lowering of the dielectric constant of the medium, even though no shift in their CD extrema at 197 and 222 nm was observed. On the other hand, Gratzer and Doty (1963) observed a red shift (from 190 to 191.5 nm) in the uv peak of poly (DL-Ala) in H_2O as compared to 33% acetonitrile (also a decrease in solvent dielectric constant). As poly(Ser) is in the β conformation and poly(DL-Ala) is about 30% helical, the red shift may be associated with a helix–solvent interaction. This can be observed by comparing the CD peak of the poly(Glu) helix in H_2O at 191 nm to the PHEG helix in 95% MeOH at 193 nm (Adler *et al.*, 1968). Since the red shift in the uv absorption peak at 190 nm (Figure 9) and the CD minima at 208 nm (Figure 3) for PHPG in 90% MeOH are about the same ($\approx$1.5 nm), it is probable that these shifts are due to the effect of MeOH on the π–π^* transition of helical PHPG. On the other hand, the large changes in magnitudes of the molar extinction coefficient and molar ellipticity upon addition of MeOH to PHPG in H_2O represent conformational changes in the polymer and are not simply due to solvent effects.

It is also interesting to observe in Figure 9 that the uv absorption spectra of P(HPG^{76}Leu^{24}), a copolymer with 67% helix from CD studies, falls in between the spectra of helical and coil PHPG. Calculating f_n on the basis of molar extinction coefficients at 190 nm by using eq 3 we obtain 57% helix for P(HPG^{76}Leu^{24}). Rosenheck and Doty (1961) found that better estimates of helical content from uv absorption spectra (when compared to rotatory dispersion values) could be obtained by using ϵ at 197 nm rather than 190 nm. Likewise, a better correlation is found to the CD studies using ϵ_{197} which gave 65% helix for P(HPG^{76}Leu^{24}) in H_2O at 23°. These results satisfactorily confirm the findings of Applequist and Breslow (1963) "that the changes in extinction coefficient follows rather closely the change in rotation, and that neither of these is a linear function of the degree of ionization." As there is no ionization in our polymer system, the observed ultraviolet hypochromic effect in Figure 9 clearly represents helix formation when leucine is incorporated in PHPG as well as when the homopolymer is in 90% MeOH.

Sedimentation Velocity. It has been demonstrated from literature evidence that the higher absolute values of $[\theta]_\lambda$ for PHPG in 90% MeOH at 0° as compared to 20° (Figure 5) indicate an increase in helical content, and we have therefore based the limits for complete helicity at the lower temperature. Since the values of $[\theta]_{222} = -50{,}627$ and $[\theta]_{193} = +115{,}430$ for PHPG in 90% MeOH at 0° are higher than all existing literature values for helical polymers, one may suspect that these higher values may be due to aggregation of helices. It has been shown by Jennings *et al.* (1968) that aggregation of poly(Glu) helices at low pH occurs below 20° and that this phenomenon is paralleled by an increase in negative optical rotation in the visible and ultraviolet spectral range. The same group (Schuster, 1965; Schuster *et al.*, 1969) have shown from ultracentrifuge schlieren patterns that while poly(Glu) at pH 4.3 and 20° exhibited a single sedimenting boundary, the same sample cooled for 2 hr at 5° revealed a second faster moving boundary, which is associated with helical aggregates.

In order to see whether PHPG helices in 90% MeOH were aggregating at low temperatures, an ultracentrifugal experiment was performed. Stock solutions of PHPG in 90% MeOH stored at 5° were not used since these samples may be in the aggregate form. A fresh 0.4% w/v solution of PHPG in 90% MeOH was prepared at room temperature and the sedimentation experiments were carried out immediately (Lotan *et al.* (1965) found that solutions of PHPG in anhydrous MeOH gradually precipitated on storage for more than 1 day at room temperature).

The ultracentrifugal schlieren pattern of PHPG in 90% MeOH at 25° and 3° showed that there is no appearance of a second boundary in the sedimentation patterns upon cooling, and the sedimentation coefficients for 0.4% PHPG in 90% MeOH at 25 and 3° were $s_{20,w} = 0.97 \times 10^{-13}$ and 0.95×10^{-13} sec, respectively. It is concluded that there is no aggregation for PHPG helices at low temperatures, and thus there is justification for using the higher $[\theta]_\lambda$ values of PHPG in 90% MeOH at 0° as limits for 100% helicity.

Thermodynamic Parameters. The thermally induced helix–coil transition for polypeptides in solution is defined by two parameters in the Zimm–Bragg theory (1959): the cooperativity parameter, σ, measures the difficulty of initiating a helical region, while the growth parameter, s, represents the equilibrium constant for helix formation:

$$s = \exp[-\Delta G/RT] = \exp[-\Delta H/RT + \Delta S/R] \qquad (4)$$

where ΔG, ΔH, and ΔS are the changes in free energy, enthalpy, and entropy respectively, when a random-coil residue is converted into a helical residue at the end of a helical section.

The fraction of helical residues, f, in a very long polypeptide chain is given by the equation (Zimm and Bragg, 1959; Applequist, 1963)

$$f = \frac{1}{2} + \frac{s - 1}{2[(s - 1)^2 + 4\sigma s]^{1/2}} \qquad (5)$$

TABLE V: Thermodynamic Parameters for Coil to Helix Conversion in PHPG and P(HPG^{z}Leu^y) Copolymers in H_2O at 20°.

Thermodynamic Parameters	P(HPG^{z}Leu^y)								
	76:24	80:20	83:17	92:8	100:0	PHPG[a]	PHPG[b]	PHPG[c]	PHBG[c]
ΔG (cal/mole)	−18.8	−11.5	−9.2	−1.5	+10.9	+10.0	+9.8	+12.6	−11.3
ΔH (cal/mole)	−121	−105	−112	−116	−155	−119	−107	−168	−195
ΔS (eu)	−0.35	−0.32	−0.35	−0.39	−0.57	−0.44	−0.40	−0.63	−0.63
T_c (°C)	+73	+56	+46	+24	−1	0	−5.0	−0.7	+38
$\sigma \times 10^4$	2	2	2	2	2	2	2.8	2.2	6.7

[a] Data from Lotan *et al.* (1969). [b] Data from Okita *et al.* (1970). [c] Data from von Dreele *et al.* (1971).

As the high molecular weight polymer samples of P(HPG^{z}Leu^y) were insoluble (all the polymers and copolymers used are in the intermediate range with DP ≈ 250), it was not possible to obtain σ by the Zimm–Bragg method. However, Poland and Scheraga (1970) have derived an equation whereby σ can be evaluated for finite chain lengths, N

$$\sigma^{1/2} = \frac{1}{2N\left[\frac{1}{2} - (f_n)_{s=1}\right]} \quad (6)$$

for $(f_n)_{s=1} > 0.25$

where $(f_n)_{s=1}$ is the fraction helix of the finite chain at $s = 1$. Lotan *et al.* (1969) and von Dreele *et al.* (1971) obtained $T_c \approx 0°$ for PHPG in H_2O with large values of N where $(f_n)_{s=1} = 1/2$. At this transition temperature in the present studies PHPG with $N = 250$ in H_2O has a helical content of 36% (see Figure 8). Substituting these values in eq 6 gives $\sigma = 2.04 \times 10^{-4}$. This is in excellent agreement with the value $\sigma = 2 \times 10^{-4}$ obtained by Lotan *et al.* (1969) for PHPG in H_2O, and is similar to those found by other investigators (Okita *et al.*, 1970; von Dreele *et al.*, 1971). This value is also identical with the σ for PBLG found by Zimm *et al.* (1959). Since the value of $\sigma = 2 \times 10^{-4}$ falls in the range for that of poly(Leu) ($\sigma = 1.2 \times 10^{-4}$ to 2.5×10^{-3}) found by Ostroy *et al.* (1970), it was decided to use this value for the other copolymers as well. To test the validity of this assumption, eq 6 was used to evaluate the σ values for the copolymers. Since a high DP sample of P(HPG^{z}Leu^y) was not available to determine T_c, the value of $T_c = 54°$ was obtained from the data of Lotan *et al.* (1965) for the PHPG (DP = 750) in 30% MeOH for the infinite chain. Furthermore, the b_0 *vs.* T plot of PHPG (DP = 1360) in 30% MeOH (Okita *et al.*, 1970) extrapolated to $T_c \approx 60°$ at 50% helicity. Since the behavior of the copolymers in 90% MeOH was similar to PHPG in the same solvent (Figure 5), the values of $T_c = 60°$ and 54° were tested as the transition temperatures for P(HPG^{z}Leu^y). In this way the $(f_n)_{s=1}$ values for the copolymers at $T_c = 60$ or 54° were used in eq 6 and an average value of $\sigma = 2.2 \times 10^{-4}$ was obtained using $T_c = 60°$, and $\sigma = 4.7 \times 10^{-4}$ using $T_c = 54°$. Since these values are of the same magnitude as $\sigma = 2 \times 10^{-4}$ for PHPG, it may be assumed that $\sigma_{Leu} \approx \sigma_{HPG}$, so that in *this particular case*, the σ values for homopolymer PHPG and copolymers P(HPG^{z}Leu^y) are the same.[2]

For the polymer samples in the DP range of 250 the following equations derived by Okita *et al.* (1970) were found to be satisfactory

$$f_n = f - \frac{2f[f(1-f)]^{1/2}}{(N-2)\sigma^{1/2}} \quad (7)$$

$$f = \frac{1}{2} + \frac{\ln s}{[16\sigma + 4(\ln s)^2]^{1/2}} \quad (8)$$

where f_n is the fraction helix for a finite chain, f is the fraction helix for an infinite chain, and N is the degree of polymerization.

The relation between f_n and ln s is plotted in Figure 10 for different degrees of polymerization using $\sigma = 2 \times 10^{-4}$. Teramoto *et al.* (1970) found eq 7 to be a good approximation for the exact equation over the entire range of the helix–coil transition. In addition, values of f_n at $s = 1$ (where it is most sensitive) from eq 7 were checked against an exact equation for $(f_n)_{s=1}$ valid at all DP's (Poland and Scheraga, 1970), and were found to be in excellent agreement (within 1%). Thus the theoretical curves in Figure 10 can be used with confidence for homopolymers with $\sigma = 2 \times 10^{-4}$, as is the case with the thermodynamic calculations for PHPG herein. Since the theoretical curves of f_n *vs.* ln s for copolymers are unavailable from the literature, and the computer methods for obtaining them are quite complex (Poland and Scheraga, 1969), it was decided to extend the homopolymer theory to the copolymers for qualitative comparisons. Hence the thermodynamic parameters obtained herein confirm qualitatively that leucine is a helix stabilizer. These parameters may also have quantitative validity since their use gave s_{Leu} values in close agreement with literature values, as will be discussed below.

From the f_n *vs.* T curves for P(HPG^{z}Leu^y) in Figure 8 and the f_n *vs.* ln s curves in Figure 10, the ΔG dependence on temperature was obtained for P(HPG^{z}Leu^y) shown in Figure 11. As can be seen, straight lines can be drawn through the experimental data, whose slopes are equal to $-\Delta S$ and whose intercepts are equal to ΔH (when the abscissa is plotted in T°K). Alternatively, ΔH may be obtained as the negative slope of a R ln s *vs.* $1/T$ plot. The results found by both methods are identical, and are tabulated in Table V. The experimental ΔG's at both the low- and high-temperature ends in Figure 11 appear to be slightly off the extrapolated straight lines. Instead of postulating changes in the heat capacity with

[2] For low molecular weight data (DP = 51), Gaskin and Yang (1971) have shown that the influence of polydispersity ($\bar{M}_z/\bar{M}_w = 1.34$) changed the thermodynamic parameters σ and ΔH by at most 17 and 5%, respectively. Lower polydispersity is usually found in higher molecular weight samples, so deviations in these parameters would be expected to be less.

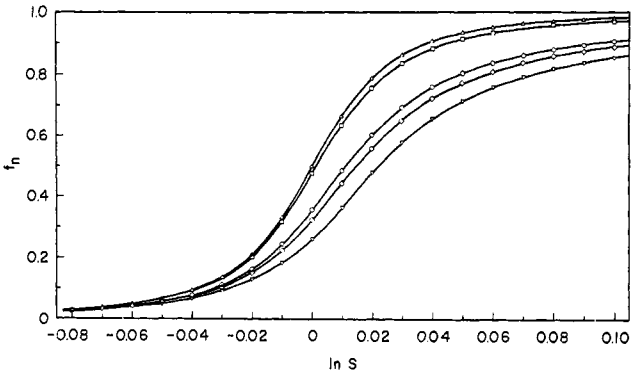

FIGURE 10: Theoretical curves for fraction helix (f_n) as a function of ln s for homopolymers with different DP based on $\sigma = 2 \times 10^{-4}$ according to eq 7 and 8. DP: ∞ (△), 1500 (□), 250 (○) 200 (◇), 150 (▽).

temperature for these copolymers, it is believed more likely that the discrepant ΔG values are due to errors in determining f_n. The cumulative error in f_n arising from Nessler determinations (±2%), CD measurements of $[\theta]_{222}$ (±4%), DP estimates from viscosity (±3%) and per cent leucine composition in copolymers from amino acid analysis (±2%) is estimated at about 11%. In the case of PHPG, the helical content is only 4% at 70°, and as evident from Figure 10, ln s is a very sensitive function of f_n in regions of both low and high helicity. Thus small errors in f_n will lead to large errors in ln s, which may account for the discrepant ΔG values at the low- and high-temperature ends. Possible errors in ΔG at 0° may also be due to the difficulty of temperature control (±0.5° as compared to ±0.2° at higher temperatures) as noted in the CD experimental section.

From Figure 11 it can be seen that ΔG decreases as the temperature is lowered for a given P(HPG^{x}Leu^y) copolymer, and also as the leucine content in a copolymer is increased at any given temperature. The ΔG of PHPG also decreases with increasing per cent MeOH in the solvent (Figure 12). The results reported here are in good agreement with literature values (Lotan *et al.*, 1969; Okita *et al.*, 1970). A decrease in ΔG implies that helix formation is favored for the P(HPG^x-Leu^y) system at low temperatures with high leucine content as well as in high methanol concentration. Hence the qualitative conclusions drawn from the CD spectral studies are confirmed by these thermodynamic calculations.

The temperature at the midpoint of transition ($f_n = 1/2$, $s = 1$) for a polymer of infinite N is defined by $T_c = \Delta H/\Delta S$ (Applequist, 1963) and values are tabulated for the various polymers in Table V. As expected the transition temperature rises for copolymers with greater percentages of leucine, since this amino acid acts as a helix stabilizer. It may also be seen from Table V that ΔH and ΔS become more positive upon the initial incorporation of leucine in the copolymer. However, one cannot distinguish whether this increase is due to hydrophobic stabilization of the helix or the dilution effect on ΔH and ΔS upon incorporation of leucine into the homopolymer.

Thermodynamic Parameters for Leucine. When ΔG is plotted against per cent leucine in P(HPG^{x}Leu^y) and extrapolated to 100% leucine (not shown), a value of $\Delta G^{20°}_{Leu} = -110$ cal/mole. This is in close agreement with the average $\Delta G^{20°}_{Leu} = -149$ cal/mole for poly(Leu) as determined by Ostroy *et al.* (1970). In order to check whether the result herein is fortuitous due to extrapolation, it was decided to calculate ΔG_{Leu} ex-

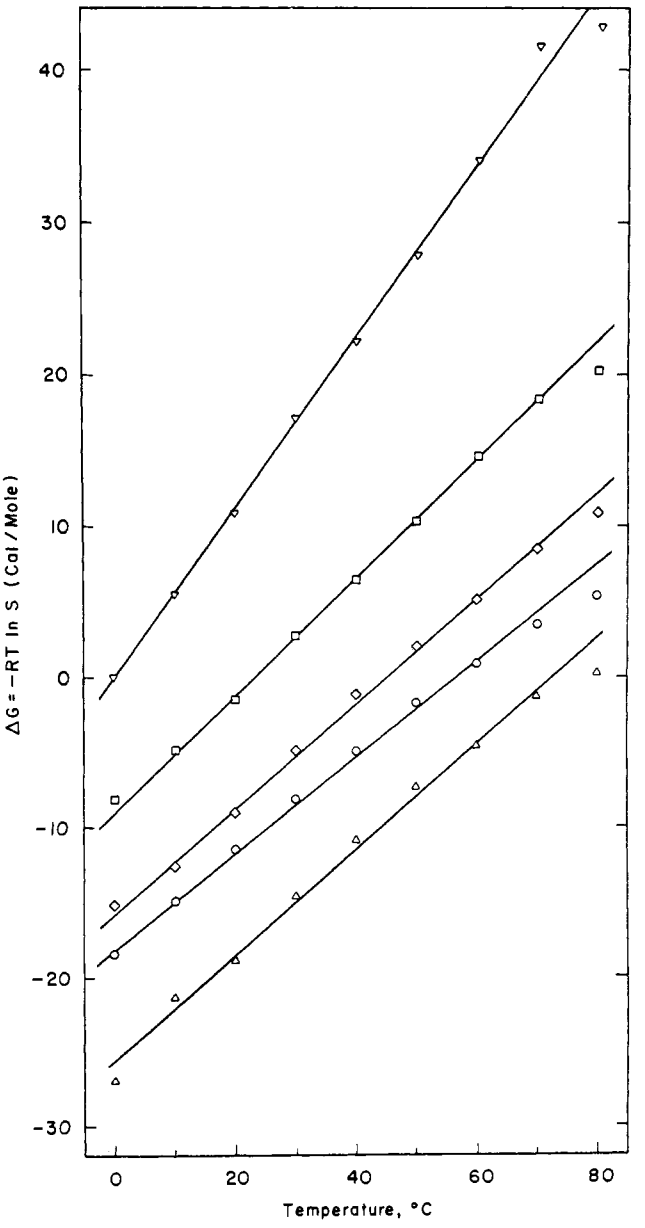

FIGURE 11: Dependence of ΔG on temperature for the P(HPG^x-Leu^y) series. 100:0 (▽), 92:8 (□), 83:17 (◇), 80:20 (○), 76:24 (△).

plicitly from s_{Leu}, the equilibrium constant for coil → helix formation for a leucine residue. The equilibrium constant for helix formation in a copolymer may be expressed as the mole-fraction-weighted arithmetic mean (Reiss *et al.*, 1966)

$$s = f_a s_a + f_b s_b \qquad (9)$$

where f_a and f_b are the mole fractions of A and B in the chain, and s_a and s_b are the equilibrium constants for helix formation in the homopolymers A and B. Equation 9 has also been used recently by Roig and Cortijo (1971) in analyzing the helix–coil transition of random copolypeptides of γ-benzyl-L-glutamate and γ-methyl-L-glutamate. Letting A = hydroxypropyl-L-glutamine (HPG) and B = leucine (Leu), one can solve for s_{Leu} knowing s for the copolymers, the mole fractions f_{HPG} and f_{Leu} in each copolymer, and s_{HPG} obtained from the PHPG homopolymer data. In this manner s_{Leu} values from 0 to 80° were isolated from the s values of the

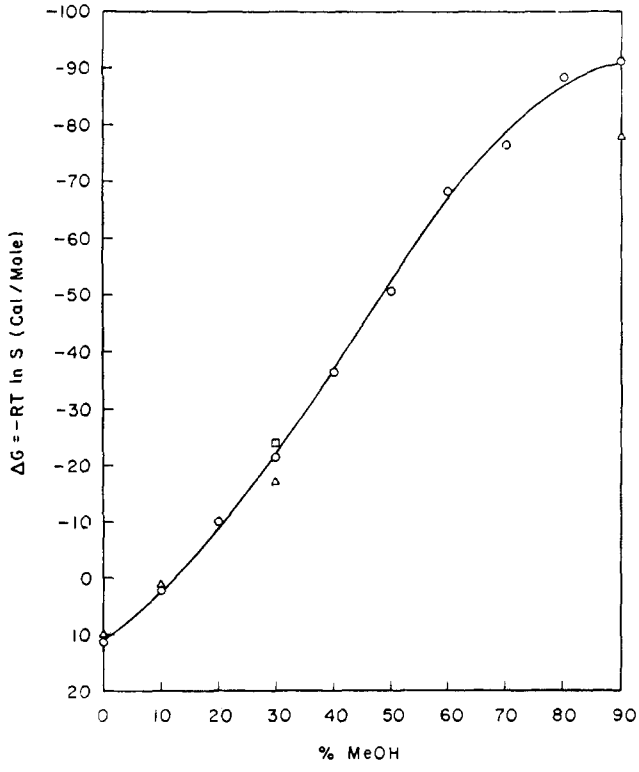

FIGURE 12: Dependence of ΔG of PHPG on per cent MeOH at 23°. Present work (○); data of Okita *et al.* (1970) (△); data of Lotan *et al.* (1969) (□).

four copolymers and averaged at each temperature. The value of $s_{Leu}^{20°}$ was found to be 1.195 by using eq 9 from which the value of $\Delta G_{Leu}^{20°} = -106$ cal/mole was obtained. In addition, when the mole-fraction-weighted geometric mean, $\bar{s} = (s_a)^{f_a}(s_b)^{f_b}$ (Reiss *et al.*, 1966), was used, the $s_{Leu}^{20°}$ value obtained was 1.217 and $\Delta G_{Leu}^{20°} = -114$ cal/mole. The close agreement in $\Delta G_{Leu}^{20°}$ obtained by the three methods gives confidence to the $\Delta G_{Leu}^{20°}$ value found by linear extrapolation to 100% leucine in a ΔG *vs.* per cent leucine plot, as well as the calculated s_{Leu} values.[3] Plots of s and ΔG *vs.* T for (leucyl)$_n$ isolated from copolymers in H_2O are shown in Figure 13. A plot of s *vs.* T for PHPG in H_2O is included for comparison. Since s is slightly below unity for PHPG, helical formation is unfavorable in this homopolymer, especially at higher temperatures, where s continues to decrease. On the other hand, the greater than unity value for s for leucine indicates that this amino acid is a helix former (Fasman *et al.*, 1964; Miller and Nylund, 1965; Auer and Doty, 1966; Ostroy *et al.*, 1970). The increase in s and the decrease in ΔG with temperature shown in Figure 13 imply that the leucyl helix is more stable at higher temperatures due to stronger hydrophobic interactions (Némethy and Scheraga, 1962). This was demonstrated earlier by Fasman *et al.* (1964) for Glu:Leu random copolymers, and by Auer and Doty (1966), who found the values of $-b_0$ for a block poly(Leu) to increase (indicating increased helix content) with temperature in 8 M urea and in 7 M guanidinium chloride al-

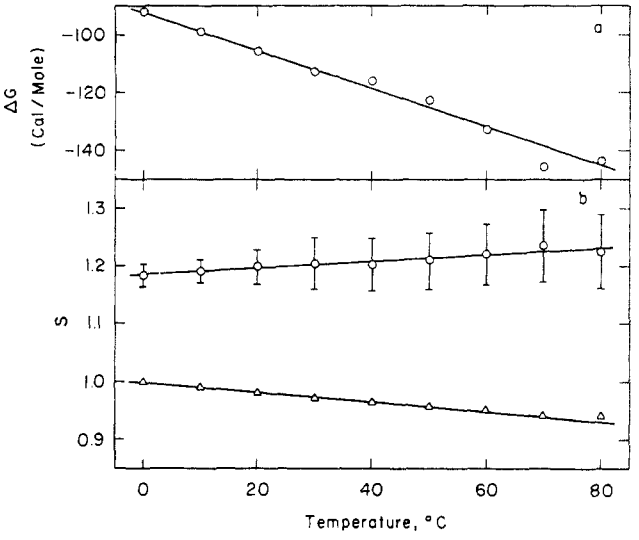

FIGURE 13: ΔG and the Zimm–Bragg parameter, s, *vs.* temperature for (Leu)$_n$ isolated from P(HPG^{z}Leu^y) in H_2O (○); and s *vs.* temperature for PHPG in H_2O (△). The error bars represent standard deviations in s calculated from four different P(HPG^{z}Leu^y) samples.

though its value in H_2O was essentially constant up to 90°. Likewise, Ostroy *et al.* (1970) showed from b_0 measurements that the helical content of block copolymers of poly(Leu) in water increases slightly with increasing temperature. These latter authors obtained s values of 1.28 and 1.33 for poly(Leu) at 0 and 80°, respectively, which compare favorably to the s_{Leu} values of 1.19 and 1.23 at these temperatures reported in this paper. Since the modified Zimm–Bragg homopolymer theory was used herein, it was expected that the thermodynamic parameters for PHPG would be in close agreement with literature values. However, it is surprising that the thermodynamic results for (leucyl)$_n$ obtained here from the copolymer data, by using the homopolymer theory, should be in such good agreement with values calculated by more rigorous methods. This could be attributed to the similar values of σ_{leu} and σ_{HPG} as explained earlier.

Due to the strengthening of hydrophobic interactions of leucine residues with increasing temperature, the slope of the ΔG *vs.* T plot for (leucyl)$_n$ in Figure 13 is opposite in sign to the slopes of ΔG *vs.* T for PHPG and P(HPG^{z}Leu^y) in Figure 11. Upon close inspection of the data in Figure 11, it is seen that for copolymers with greater than 17% leucine, the values of ΔG above 60° fall off the extrapolated straight lines toward more negative ΔG values, due to greater contributions of $-\Delta G_{H\varphi}$ from leucine hydrophobic bonding. That the hydrophobic effect does not produce greater helicity in P(HPG^{z}Leu^y) at higher temperatures (see Figure 8) is due to the small percentage of leucine in the copolymers, as well as the decreasing s function with temperature for PHPG (see Figure 13) which offsets the increasing s values with T for (leucyl)$_n$. One may speculate that if leucine is copolymerized with an amino acid which is a helix former, one can expect the copolymer to have greater helicity at elevated temperatures. Fasman *et al.* (1964) found this to be indeed the case in their studies of poly(Glu^{80}Leu^{20}) and poly(Glu^{77}Leu^{23}) at pH 4.88 in 0.2 M NaCl. They observed that the $-b_0$ values for these two copolymers decrease with increasing temperature, but show an inversion at 60° indicating an increase in helical content. These results as well as the more negative ΔG values above 60° for P(HPG^{z}Leu^y) in the present study confirm the

[3] Using $\sigma_{Leu} = 1 \times 10^{-3}$($\bar{\sigma} = 5 \times 10^{-4}$) for calculating s_{Leu} in the P(HPG^{76}Leu^{24}) copolymer, values of $s_{Leu}^{20°} = 1.203$ (0.7% deviation) and $s_{Leu}^{80°} = 1.147$ (3.2% deviation) were obtained, which are well within the experimental error.

TABLE VI: Thermodynamic Parameters of Coil–Helix Transition of Polypeptides in Aqueous Solutions at 25°.

Poly-(α-amino Acid)	σ	s	ΔG°	ΔH°	ΔS°	Ref
PLDBA			+			b
Poly(Orn)	8.2×10^{-4}	0.93	+41	−675	−2.4	b
Poly(Lys)		1.26	−138	−505	−1.2	b
	2.9×10^{-3}	1.27	−140			c
		1.15	−80	−885	−2.7	d
Poly(Glu)	5×10^{-5}	1.19	−105	−1120	−3.4	d
	3×10^{-3}	1.25	−130	−1000	−2.8	e
	5×10^{-3}	1.06	−34	−630	−2.0	f
PHPG	2.0×10^{-4}	0.98	+12.2	−119	−0.44	g
	2.8×10^{-4}	0.98	+12.3	−107	−0.40	h
	2.2×10^{-4}	0.97	+15.7	−168	−0.62	i
	2.0×10^{-4}	0.98	+15.0	−155	−0.57	a
PHBG	6.7×10^{-4}	1.01	−8.1	−195	−0.63	i
Poly(Gly)	2.6×10^{-4}	0.47	+451	−488	−3.15	j
Poly(Ala)	1.4×10^{-4}	1.04	−24	−188	−0.55	k
	1.7×10^{-2}	1.09	−50			c
Poly(Leu)	3×10^{-3}	4.13	−840		+	e
	1.3×10^{-3}	1.30	−153	+100	+0.85	j
	2.0×10^{-4}	1.20	−109	+88	+0.66	a

[a] This work. [b] Grourke and Gibbs (1971). [c] Sugiyama and Noda (1970). [d] Hermans (1966). [e] Miller and Nylund (1965). [f] Rifkind and Applequist (1964). [g] Lotan *et al.* (1969). [h] Okita *et al.* (1970). [i] von Dreele *et al.* (1971). [j] Ostroy *et al.* (1970). [k] Ingwall *et al.* (1968).

importance of hydrophobic bonding in helix stabilization, especially at elevated temperatures.

Comparison to Literature Values. The thermodynamic parameters of helix formation of various polypeptides in aqueous solutions are summarized in Table VI. In the PLDBA, poly(Orn), and poly(Lys) series, Grourke and Gibbs (1971) demonstrated that helix stability of these polypeptides is a direct function of the number of methylene groups on the side chain. This is reflected thermodynamically by $-\Delta G_{\text{poly(Lys)}} > -\Delta G_{\text{poly(Orn)}} > -\Delta G_{\text{PLDBA}}$, as the more negative free-energy changes imply necessarily greater ease in helix formation. Furthermore, the ΔH and ΔS values for poly(Lys) are more positive than for poly(Orn) due to greater hydrophobic stabilization of the helix as the size of the nonpolar side chains is increased.

In the PHEG, PHPG, and PHBG series, Lotan *et al.* (1966) showed that these polymers in water at 24° have helical contents of 0, 20, and 65%, respectively. This again illustrates the increase in helix stability with increasing number of methylene groups in the side chains due to hydrophobic bonding.

This trend is also observed in the series of nonpolar poly(α-amino acids) in water, as it is found that $-\Delta G_{\text{Leu}} > -\Delta G_{\text{Ala}} > -\Delta G_{\text{Gly}}$. In addition it is seen from Table VI how ΔH and ΔS become increasingly positive as the more bulky side chains contribute to greater hydrophobic bonding. In order to estimate the contribution from hydrophobic bonding to the thermodynamic parameters during helix formation, the following equations, similar to those employed by Ostroy *et al.* (1970), can be used

$$(\Delta H_{\text{H}\varphi})_{\text{Leu}} = \Delta H_{\text{Leu}} - \Delta H_{\text{Gly}} \quad (10)$$

$$(\Delta S_{\text{H}\varphi})_{\text{Leu}} = \Delta S_{\text{Leu}} - \Delta S_{\text{Gly}} \quad (11)$$

where ΔH_{Leu} represents the observed enthalpy change in forming the leucyl helix and ΔH_{Gly} (see Table VI) represents the enthalpy change in forming the poly(Gly) helix (Ostroy *et al.*, 1970) (*i.e.*, interactions arising only from the backbone atoms). The difference between these two quantities should then give the enthalpy change associated with the side chains of the leucyl helix due to hydrophobic interactions, $(\Delta H_{\text{H}\varphi})_{\text{Leu}}$. Likewise $(\Delta S_{\text{H}\varphi})_{\text{Leu}}$ and $(\Delta G_{\text{H}\varphi})_{\text{Leu}}$ are calculated in this manner, and the results tabulated in Table VII. The thermodynamic parameters obtained with the data herein for the hydrophobic interactions of $(\text{leucyl})_n$ are in good agreement with both experimental (Ostroy *et al.*, 1970) and theoretical values (Némethy and Scheraga, 1962). The more negative value of

TABLE VII: Net Contribution from Hydrophobic Bonding to the Thermodynamic Parameters for the Transition of a Residue from a Coil to a Helical State at 25°.

	$\Delta G^\circ_{\text{H}\varphi}$	$\Delta H^\circ_{\text{H}\varphi}$	$\Delta S^\circ_{\text{H}\varphi}$
Theoretical			
Alanine[a]	−400	+300	+2.45
Leucine[a]	−400	+700	+3.75
Experimental			
Alanine[b]	−475	+300	+2.60
Leucine[c]	−605	+588	+4.00
Leucine[d]	−560	+576	+3.81

[a] Némethy and Scheraga (1962). [b] Ingwall *et al.* (1968). [c] Ostroy *et al.* (1970). [d] This work.

$\Delta G^{\circ}_{H\varphi}$ for poly(Leu) (isobutyl side chains) when compared to poly(Ala) (methyl side chains) arises from the stronger hydrophobic interactions between leucine residues than alanine residues during helix formation.

While it is generally true that increasing the size of the nonpolar side chain results in greater helix stability as shown in the polypeptides above, there is a point of diminishing return as the side chain becomes too bulky. This is the case with poly(Phe) which was found to form a less stable helix than poly(Leu), due to steric interference between the bulky aromatic side chain and the α-helical backbone (Auer and Doty, 1966). Likewise the ORD and CD curves of poly(Arg) (Hayakawa *et al.*, 1969) showed that this polymer was less helical than poly(Lys) and poly(Glu). While these authors did not offer an explanation for this, it is believed that the bulkiness of the arginine side chain with its three amino groups was the main factor in destabilizing the helical backbone. In this connection, it may be pointed out that PLDBA which has two methylene groups as compared to one methyl side-chain group in poly(Ala) is nevertheless a weaker helix than the latter. Hatano and Yoneyama (1970) as well as Grourke and Gibbs (1971) found that PLDBA in aqueous solutions at 25° was almost completely random even at pH 11. This may not be so surprising when one considers that the amino groups at the tail end of the PLDBA side chains may interact with the nearby carbonyl groups in the backbone. This reduces hydrogen bonding along the main chain which plays a greater role in helix stability than the hydrophobic interactions of the side-chain methylene groups. However, as the amino group at the tail end is further removed from the backbone as in the case of poly(Orn) and poly(Lys) where it cannot compete with the hydrogen bonding in the backbone, the methylene groups in the nonpolar region of the side chains could contribute to helix stability due to hydrophobic bonding.

While the thermodynamic parameters in Tables VI and VII show that poly(Leu) is a more stable helix than poly(Ala) in aqueous solution, this is not necessarily the case in nonaqueous solvents where hydrophobic interactions play a less important role in helix stabilization. Fasman (1962) showed that in chloroform, poly(Ala) appears more stable than poly(Leu) to the disruptive effect of trifluoroacetic acid since the midpoint of the former transition occurs at 80% trifluoroacetic acid, and that for the latter is at 50% trifluoroacetic acid. Working with the same solvent system, Perlmann and Katchalski (1962) showed that the transition of poly(L-methionine) occurs at 45% trifluoroacetic acid while Auer and Doty (1966) found that only 3% trifluoroacetic acid was needed to break the poly(Phe) helix. These studies show the importance of polymer–solvent interaction in influencing the transition region as well as conformational stability of polypeptides.

Role of Leucine in Protein Conformation. From the thermodynamic calculations it was seen that the leucine helix has greater stability than the alanine helix due to its stronger hydrophobic interactions. While these interactions may be stronger in phenylalanine, methionine, and arginine, experimental evidence from homopolymer and copolymer studies with these residues cited above show that they form weaker helices than poly(Ala) and poly(Leu) due to their bulkiness and steric hindrance with the backbone. While poly(Glu) and poly(Lys) form stable helices, random copolymers of poly($Glu^{z}Leu^{y}$)and poly($Lys^{z}Leu^{y}$) yield helices of still greater stability as the leucine content in these copolymers is increased. A comparison of the *s* parameter in Table VI leads to the conclusion that leucine may possibly be the strongest helical-forming amino acid residue. If this is true one should expect to find a greater percentage of leucine residues in proteins to be in the helical regions. A survey was made on 11 proteins whose amino acid sequence and conformation are known (myoglobin, lysozyme, ribonuclease A, α-chymotrypsin, α-hemoglobin, carboxypeptidase A, papain, subtilisin, insulin, ferricytochrome *c*, and staphylococcal nuclease). It was found that of the 1886 amino acid residues in these proteins, 663 of them are in helical regions (35%). When this analysis was carried out for the 20 amino acids, Glu, Ala, and Leu were found to have the highest percentages in helical regions with 53, 52, and 52%, respectively. However, when the inner helical cores in these proteins (17%) are considered (neglecting the three helical residues on both N-terminal and C-terminal of a helical region), Leu, Ala, Trp, and Glu are found most frequently with 35, 27, 27, and 25%, respectively. This implies that leucine is clearly the most favorable residue of the 20 amino acids to be found in the inner helical regions of globular proteins. These results will be published in greater detail (P. Y. Chou and G. D. Fasman, unpublished data).

Thus, in summary it can be stated that the illustrations discussed above demonstrate the importance of elucidating the nature of side chains to understand their contribution to helical stabilization in polypeptides. In particular, it was clearly shown in this paper that the helicity of $P(HPG^{z}Leu^{y})$ increases with increasing incorporation of leucine in the copolymers. The thermodynamic calculations confirmed the strong helical-forming power of leucine as evidenced from the CD spectral studies. Furthermore it is encouraging to find that careful studies of conformational changes in polypeptides and copolypeptides can provide much insight into the factors controlling protein structure. This in turn may lead to greater understanding of biological control mechanisms.

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