Nuclear Magnetic Resonance Studies of Intramolecular Motions and Side-Chain Interactions in Water-Soluble Polyamino Acids*

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ABSTRACT: High-resolution nuclear magnetic resonance (100 Mc) studies have been carried out on polymers of the N^5-(ω-hydroxyalkyl)-L-glutamines, *viz.*, poly-N^5-(2-hydroxyethyl)-L-glutamine, poly-N^5-(3-hydroxypropyl)-L-glutamine, and poly-N^5-(4-hydroxybutyl)-L-glutamine. In aqueous solution, the helix content of these polymers depends on the length of the side chain, the main chain, and temperature. In methanol, all three polymers assume the fully α-helical conformation. Hence, it is possible to obtain various combinations of solvent composition and temperature at which the helix content is the same. Since the polyhydroxyalkyl-glutamine derivatives contain no ionizable side-chain groups, they are better models for studying changes throughout the helix–coil transition than polymers where the ionization of the side chain plays a role during this transition. Randomly coiled polymers show well-developed nuclear magnetic resonance peaks with a noticeable splitting pattern. Polymers of high helix content show very broad peaks for the inner side-chain protons, sharper ones for the outermost side-chain protons, and (at the signal amplification used) such a broad peak for the α-CH proton that it cannot be observed. The splitting patterns and line widths of the proton resonance peaks are discussed in terms of side-chain and backbone interactions and mobility; also, it was possible to distinguish between different average conformations of randomly coiled polymers. For a partially helical polymer in aqueous solvents, the observed chemical shift of the α-CH proton depends on both the temperature and helix content. As the temperature is raised, the observed chemical shift contains contributions from two different effects: the decrease in helix content and changes in the average conformation of the random coil. The conversion of a complete helix of poly-N^5-(4-hydroxybutyl)-L-glutamine into a completely nonhelical form is accompanied by a downfield chemical shift of the α-CH peak of about 0.16 ppm. An explanation is provided for the appearance of two separate peaks of comparable width for the α-CH resonance reported for certain polymers in organic solvents containing strong acids.

It is well established that the side chains make an important contribution to the stabilities of the various conformations of polyamino acids in solution. For example, poly-L-alanine, which has CH_3 groups as side chains, can form an α helix, whereas polyglycine, with only H atoms as side chains, does not form an α-helical structure (Schellman, 1955; Gratzer and Doty, 1963; Bixon *et al.*, 1963; Ingwall *et al.*, 1968). Under comparable conditions, poly-L-glutamic acid is more helical than poly-L-aspartic acid (Brahms and Spach, 1963; Jacobson, 1965), and their benzyl and methyl esters are of opposite helix sense (Karlson *et al.*, 1960; Bradbury *et al.*, 1960, 1968a; Goodman *et al.*, 1962, 1963; Fasman, 1967; Ooi *et al.*, 1967; Yan *et al.*, 1968). Under conditions where poly-L-lysine is almost fully helical, poly-L-ornithine, which has one less methylene group in the side chain of each residue, is only about 25% helical (Chaudhuri and Yang, 1968). In aqueous solutions, the helix content of N^5-(ω-hydroxyalkyl)-L-glutamine polymers, having the structure $[NHCH(CO)CH_2CH_2CONH(CH_2)_mOH]_n$, also depends on the number of methylene groups in the side chain; thus, at 0°, the ethyl (m = 2, DP 200), *n*-propyl (m = 3, DP 750), and *n*-butyl (m = 4, DP 500) derivatives showed helix contents of 0, 40, and 80%, respectively, while, at 70°, the corresponding values were 0, 10, and 20% (Lotan *et al.*, 1966), where DP is the average degree of polymerization.

The present study was undertaken in order to assess the influence of the side chains on the conformation of the polyhydroxyalkyl-L-glutamines. For this purpose, high-resolution nuclear magnetic resonance spectroscopy was used, since this technique has found wide application in studies of the conformations of polyamino acids (Stewart *et al.*, 1967; Markley *et al.*, 1967; Bradbury *et al.*, 1968b; Bradbury and Crane-Robinson, 1968); also, it appears to be a sensitive technique for detecting changes in the mobility and interactions of both the backbones and the side chains under various conditions.

The polyhydroxyalkyl-L-glutamines are excellent polymers for a nuclear magnetic resonance study of side-chain interactions, since polymers of various helix contents (under similar conditions) can be obtained by varying the lengths of both the main chain and the side chain.

Experimental Section

Materials

Ethanolamine and 3-amino-1-propanol were products of Fluka, Switzerland. 4-Amino-1-butanol was obtained from

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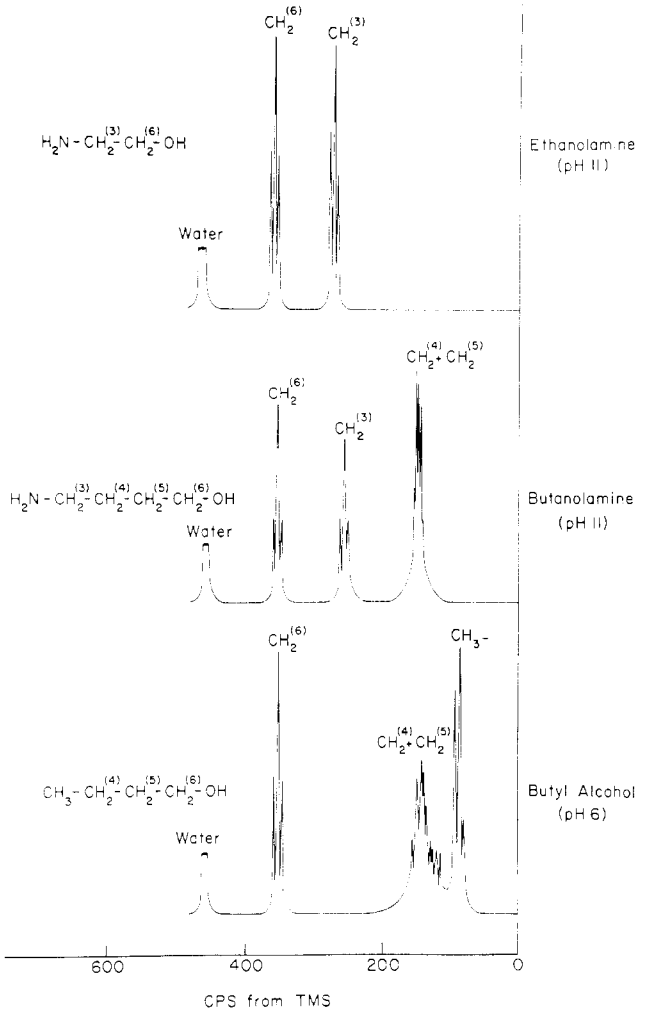

FIGURE 1: Nuclear magnetic resonance spectra of three model compounds in D_2O at 35°.

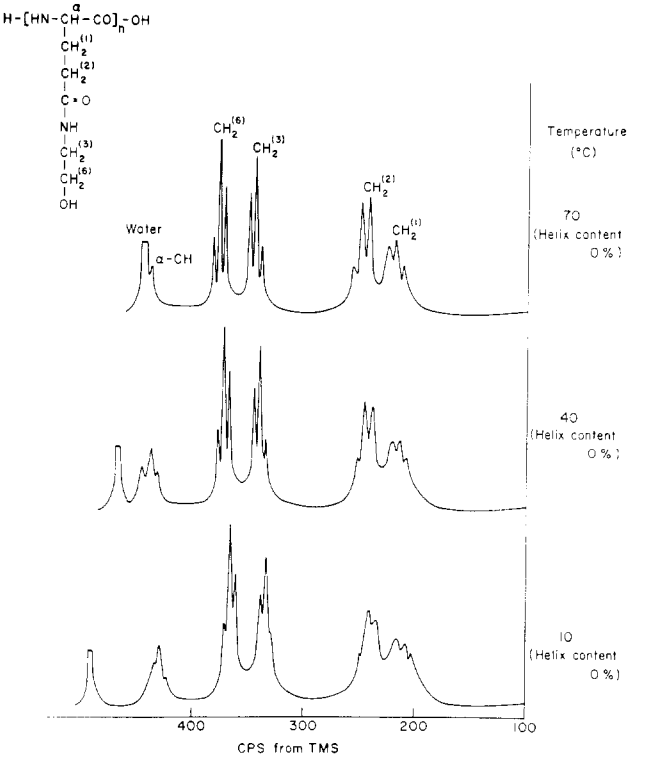

FIGURE 2: Nuclear magnetic resonance spectra of PHEG (DP 200) in D_2O at the temperatures indicated. The helix content is 0% at all three temperatures.

Fluka and also from City Chemical Corp., New York, N. Y. All of the aminoalkanols were of pure grade, and were dried over barium oxide and redistilled at atmospheric pressure before use. 1-Butanol (analytical reagent) was obtained from Mallinckrodt Chemical Works.

Deuterium oxide, D_2O (International Chemical and Nuclear Corp.), was of 99.8% isotopic purity, and the deuterated methyl alcohol, CD_3OD (Merck Sharp & Dohme), of 99% isotopic purity

PHEG,[1] PHPG, and PHBG were synthesized, and their degrees of polymerization were determined, as described previously (Lupu-Lotan *et al.*, 1965).

For convenience, the methylene groups in the side chains of the amino acid residues were designated in a manner which always yields the same identification number for the homologous groups in all of the polymers investigated. Thus, the β-CH_2 is $CH_2^{(1)}$, the γ-CH_2 is $CH_2^{(2)}$, the CH_2 next to the hydroxyl is $CH_2^{(6)}$ and, therefore, $CH_2^{(5)}$ is missing in PHPG and $CH_2^{(4)}$ and $CH_2^{(5)}$ are both absent from PHEG. This procedure was also applied to the model compounds (Figure 1); thus, the numbering identifies the positions of the methylene groups in the corresponding polymers (see Figures 2–4).

Methods

The helix content of the polymers was determined by optical rotatory dispersion measurements (Lupu-Lotan *et al.*, 1965; Lotan *et al.*, 1966; Von Dreele *et al.*, 1970)[2] of solutions in water, methanol, and water–methanol mixtures,

High-resolution nuclear magnetic resonance spectra were obtained on a Varian HA 100 spectrometer. The temperature of the samples was adjusted to within ±0.5°, with a standard Varian control unit. For the comparison of spectra, precision-bore nuclear magnetic resonance cells (Wilmad Glass Co.) were used, with coaxial inserts containing a solution of 10% tetramethylsilane and 5% chloroform in carbon tetrachloride, as an external standard. The peak from the chloroform proton served as a control for optimum adjustment of the spectrometer, and enabled corrections to be made for comparison of different spectra. There is a difference in the temperature dependence of the bulk magnetic susceptibility between the external standard and the unknown solution. This resulted in a slight downfield shift of the resonance peaks with increasing temperature and a marked upfield shift (about 40 cps) when the solvent was changed from D_2O to CD_3OD. When it was required for proper compar-

[1] Abbreviations used are: PHEG, poly-N^5-(2-hydroxyethyl)-L-glutamine; PHPG, poly-N^5-(3-hydroxypropyl)-L-glutamine; PHBG, poly-N^5-(4-hydroxybutyl)-L-glutamine; PBLG, poly-γ-benzyl-L-glutamate; PGA, poly-L-glutamic acid; TMS, $(CH_3)_4Si$.

[2] To be submitted.

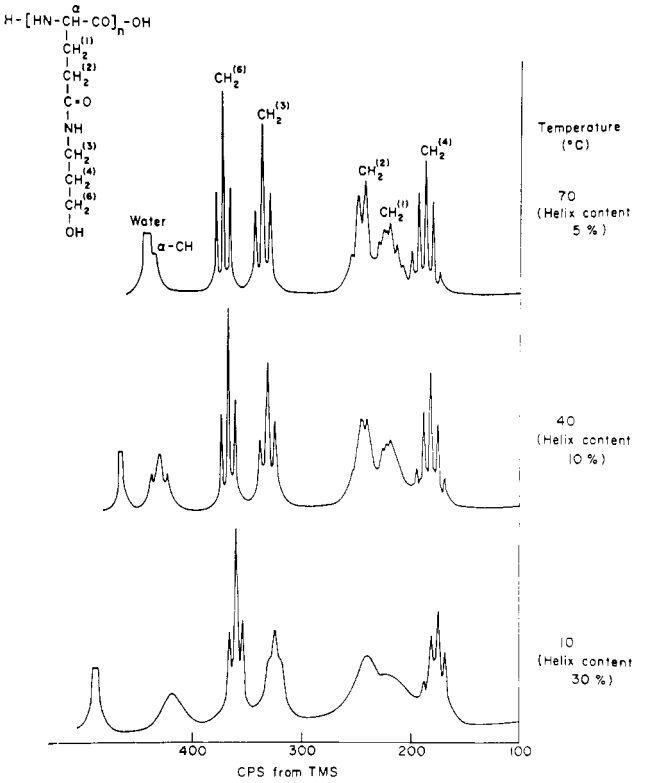

FIGURE 3: Nuclear magnetic resonance spectra of PHPG (DP 270) in D_2O at the temperatures indicated. The helix content at each temperature is stated.

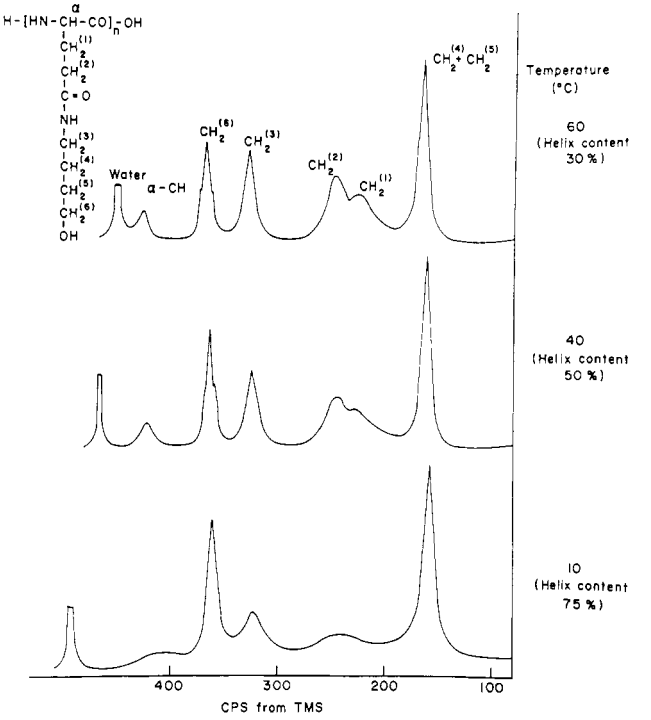

FIGURE 4: Nuclear magnetic resonance spectra of PHBG (DP 650) in D_2O at the temperatures indicated. The helix content at each temperature is stated.

isons, chemical shifts in cycles per second were, therefore, obtained by using the internal standard, $(CH_3)_3SiCD_2CD_2COONa$ (E. Merck AG., Germany). The standard was added to the polymer solutions to give a final concentration of 0.3% (w/v).

All polyamino acid samples were lyophilized from D_2O solutions prior to dissolution in the appropriate solvent for nuclear magnetic resonance measurements; the polymer concentration was 2% (w/v) for all of the nuclear magnetic resonance measurements.

Nuclear magnetic resonance measurements were made at sufficiently low radio frequency fields to avoid saturation effects.

Results

Model Compounds. In order to be able to assign the resonance peaks to specific protons of the polymers, various model compounds (as 2% solutions in D_2O and CD_3OD, respectively) were studied; the nuclear magnetic resonance spectra of some of them are shown in Figure 1. The assignments indicated in Figure 1 follow directly from a comparison of the spectra of these model compounds.

Polymers in D_2O. The nuclear magnetic resonance spectra of PHEG (DP 200), PHPG (DP 270), and PHBG (DP 650) in D_2O at several temperatures are shown in Figures 2, 3, and 4, respectively.

In the temperature range investigated, PHEG (DP 200) exists in the random coil conformation (Lotan *et al.*, 1966), and well-developed resonance peaks are observed. As the temperature increases from 10 to 70°, the resolution of the splitting patterns is improved.

On the other hand, PHPG (DP 270) is 30% helical at 10° and 5% helical at 70° (Lupu-Lotan *et al.*, 1965). This conformational change produces a marked change in the spectrum (Figure 3), mostly in the resonance peaks corresponding to the α-CH, $CH_2^{(1)}$, $CH_2^{(2)}$, and $CH_2^{(3)}$ protons.

Similarly, PHBG (DP 650) is 75% helical at 10° and 30% helical at 60° (Lotan *et al.*, 1966; Von Dreele *et al.*, 1970). At 60°, the resonance peaks of all the protons are clearly visible (Figure 4). As the temperature is decreased through the coil-to-helix transition region, marked changes appear in the spectrum. The α-CH peak has almost disappeared (*i.e.*, broadened) at 10°, marked broadening of the side-chain methylene peaks $CH_2^{(1)}$, $CH_2^{(2)}$, and $CH_2^{(3)}$ is observed but the $[CH_2^{(4)} + CH_2^{(5)}]$ and $CH_2^{(6)}$ resonance peaks show little change. While the α-CH resonance peak of PHBG (DP 650) shows a gradual downfield shift of about 10 cps as the temperature is increased from 10 to 60°, the chemical shifts for the side-chain protons show no temperature dependence. These chemical shifts are summarized in Figure 5, together with those for PHEG (DP 200). Even though PHEG is randomly coiled while PHBG undergoes a substantial helix–coil transition in this temperature range (Lotan *et al.*, 1966; Von Dreele *et al.*, 1970), the two polymers nevertheless exhibit no temperature dependence of the chemical shifts of the side-chain protons. However, for the α-CH protons of the two polymers, the temperature dependence of the chemical shift is quite different.

The helix content of a polyamino acid can be altered, not only by changing the temperature, but also by changing

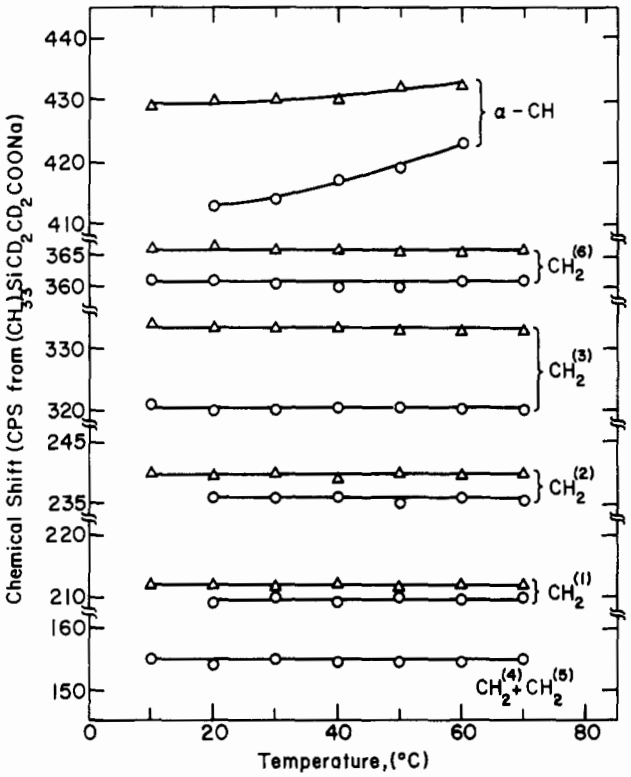

FIGURE 5: Temperature dependence of chemical shifts in D_2O for the protons indicated. (△) PHEG (DP 200); (○) PHBG (DP 650).

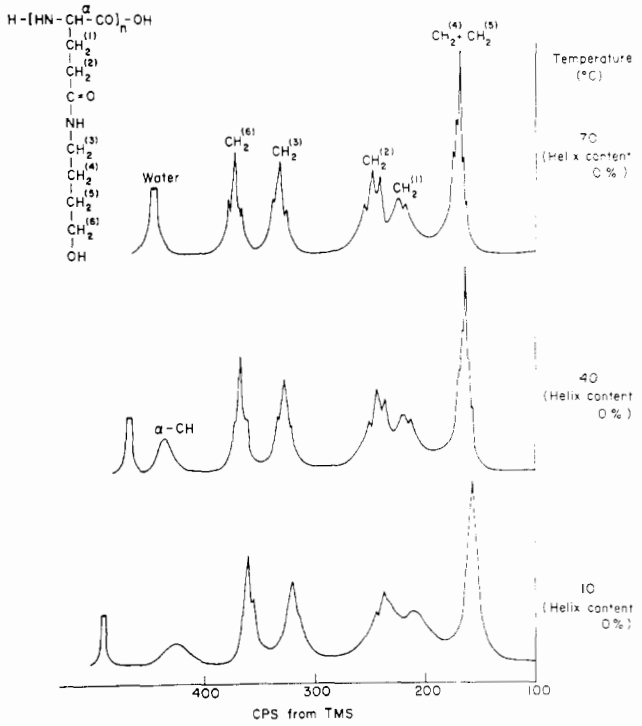

FIGURE 7: Nuclear magnetic resonance spectra of PHBG (DP 20) in D_2O at the temperatures indicated. The helix content at each temperature is stated.

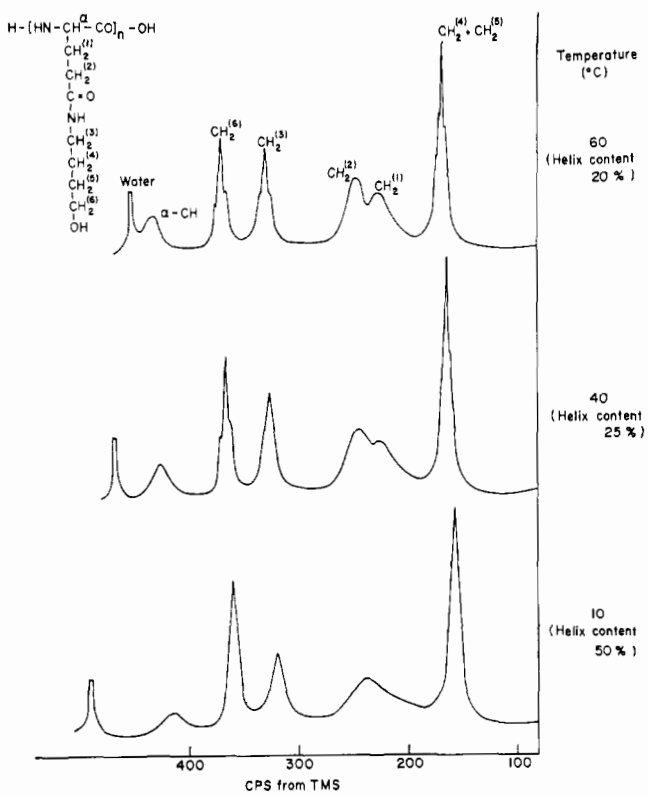

FIGURE 6: Nuclear magnetic resonance spectra of PHBG (DP 70) in D_2O at the temperatures indicated. The helix content at each temperature is stated.

the chain length or solvent at a given temperature. Figures 6 and 7 show the nuclear magnetic resonance spectra of PHBG samples of DP 70 and 20, respectively, in D_2O. These, together with the data of Figure 4 (DP 650) cover a range of helix content from 0 to 75%; at any given temperature, PHBG (DP 70) has a somewhat lower helix content than the DP 650 sample, while the DP 20 fraction shows no helix content in the temperature range investigated (Von Dreele *et al.*, 1970). The nmr spectra for the DP 650 and 70 samples are similar at each temperature (compare Figures 4 and 6). However, at 10°, the resonance peaks of the α-CH, $CH_2^{(1)}$, $CH_2^{(2)}$, and $CH_2^{(3)}$ groups of the DP 70 sample are somewhat sharper than those for the DP 650 polymer. Furthermore, at 60°, the resolution of the splitting patterns is slightly better for the DP 70 polymer than for the DP 650 polymer. In contrast, the DP 20 polymer (Figure 7) shows well-resolved resonance peaks at all temperatures in the range of 10–70° (the α-CH peak being masked by the water peak at 70°). However, the splitting patterns of the individual peaks for PHBG (DP 20) are less developed than those observed (Figure 2) for the corresponding protons of another nonhelical polymer, *viz.*, PHEG (DP 200) in D_2O.

Polymers in CD_3OD and CD_3OD–D_2O Mixtures. In methanol–water mixtures, the helix content of PHEG, PHPG and PHBG depends on the solvent composition (Lupu-Lotan *et al.*, 1965; Lotan *et al.*, 1966). At methanol concentrations higher than 80%, the three polymers are largely in the α-helical conformation, and this is confirmed by their nuclear magnetic resonance spectra (Figure 8). The α-CH resonance peak is so broad that it is not observable, and considerable broadening of the $CH_2^{(1)}$ and $CH_2^{(2)}$

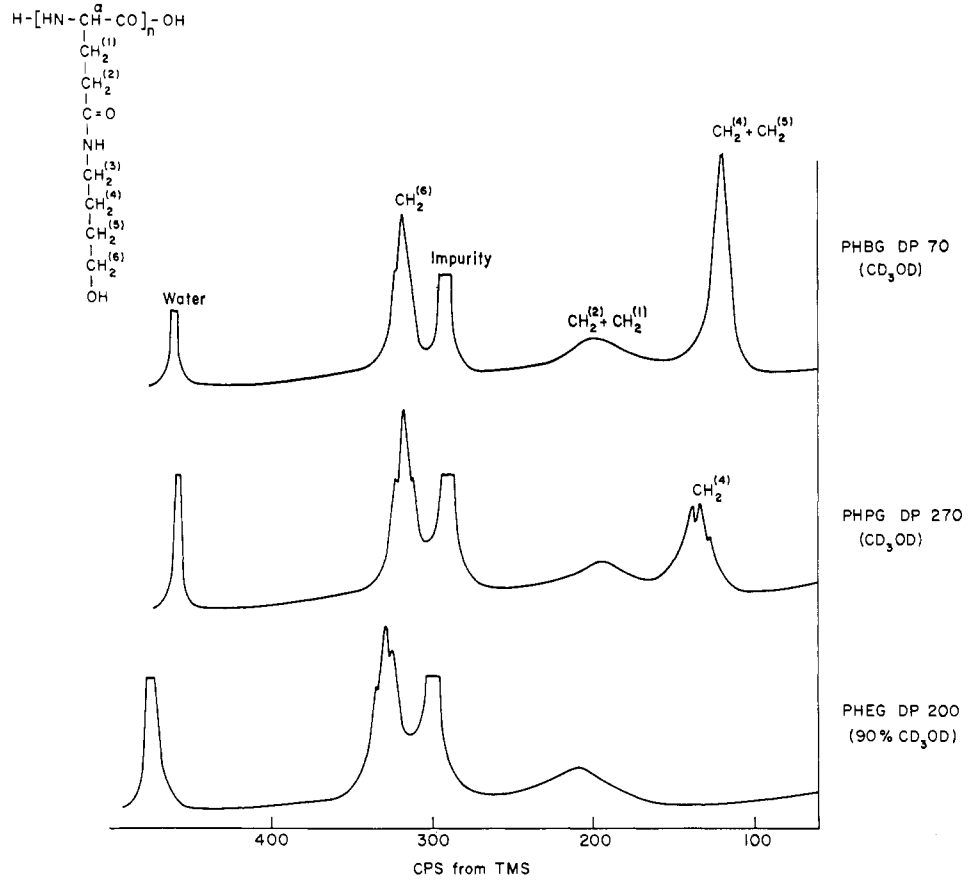

FIGURE 8: Nuclear magnetic resonance spectra, at 5°, of the polymers and solvents indicated. In all samples, the helix content is 95–100%. The "impurity" peak, in the position where the $CH_2^{(3)}$ peak would be expected to lie, arises from an impurity in the CD_3OD solvent. Since PHEG is insoluble in absolute CD_3OD, the spectrum was obtained in 90% CD_3OD.

resonance peaks is apparent. The $CH_2^{(3)}$ signal is masked by a peak arising from an impurity present in the deuterated methanol, and cannot be observed. The $CH_2^{(6)}$ resonance peak is visible for all three polymers, but the peak heights and resolution are reduced (especially for PHEG and PHPG) compared with the spectra in D_2O (Figures 2, 3, and 6); this is also the case for the $[CH_2^{(4)} + CH_2^{(5)}]$ resonance peak of PHBG and the $CH_2^{(4)}$ peak of PHPG.

When the temperature of the solutions containing highly helical polymers was increased, only minor changes in the spectra were observed; typical results are shown in Figure 9 for PHPG (DP 270) in CD_3OD. As the temperature increases from 5 to 55°, a somewhat better resolution of the resonance peaks of the outermost side-chain methylene groups is observed; however, the $CH_2^{(1)}$ and $CH_2^{(2)}$ peaks are not resolved, even at 55°, and the α-CH peak is not visible at all.

The influence of solvent composition on the nuclear magnetic resonance spectra was studied with the PHPG (DP 270) polymer in CD_3OD–D_2O mixtures. The spectra in a 7:3 (v/v) mixture (Figure 10) are similar to those in anhydrous CD_3OD (Figure 9) at comparable temperatures. The resonance peak of the α-CH proton is not observable, while those of the $CH_2^{(1)}$ and $CH_2^{(2)}$ protons are considerably broad. Also, an increase in temperature from 5 to 55° causes only minor changes in the conformation of the polymer, as indicated by the relatively small changes in the helix content and in the nuclear magnetic resonance spectra. At lower methanol concentrations (CD_3OD–D_2O, 3:7, v/v), a marked temperature dependence in the helix content and in the nuclear magnetic resonance spectra is observed (Figure 11). At 5°, only the $CH_2^{(4)}$ and $CH_2^{(6)}$ protons show well-developed resonance peaks while, at elevated temperatures, marked increases in peak heights and resolution of the side-chain proton peaks can be seen; also, the α-CH peak becomes visible at higher temperatures. These changes are associated with the large decrease in the helix content of the polymer.

Changes in solvent composition give rise to changes in the size and shape of the resonance peaks, but practically no change in their positions in the spectra. The chemical shifts observed for the side-chain protons of PHPG (DP 270) (Figures 3 and 9–11) in D_2O, CD_3OD, and CD_3OD–D_2O mixtures, show no temperature dependence (when measured with respect to the internal standard $(CH_3)_3SiCD_2CD_2COONa$), are in good agreement for comparable resonance peaks, and are summarized in Table I. The α-CH peak is not observable at high methanol concentrations because of the high helix content. This behavior is not unique for PHPG; similar results (summarized in Table I) were obtained for PHEG and PHBG, when the solvent is changed from D_2O to CD_3OD.

The relationship between helix content and the chemical shift of the α-CH proton has been considered previously.

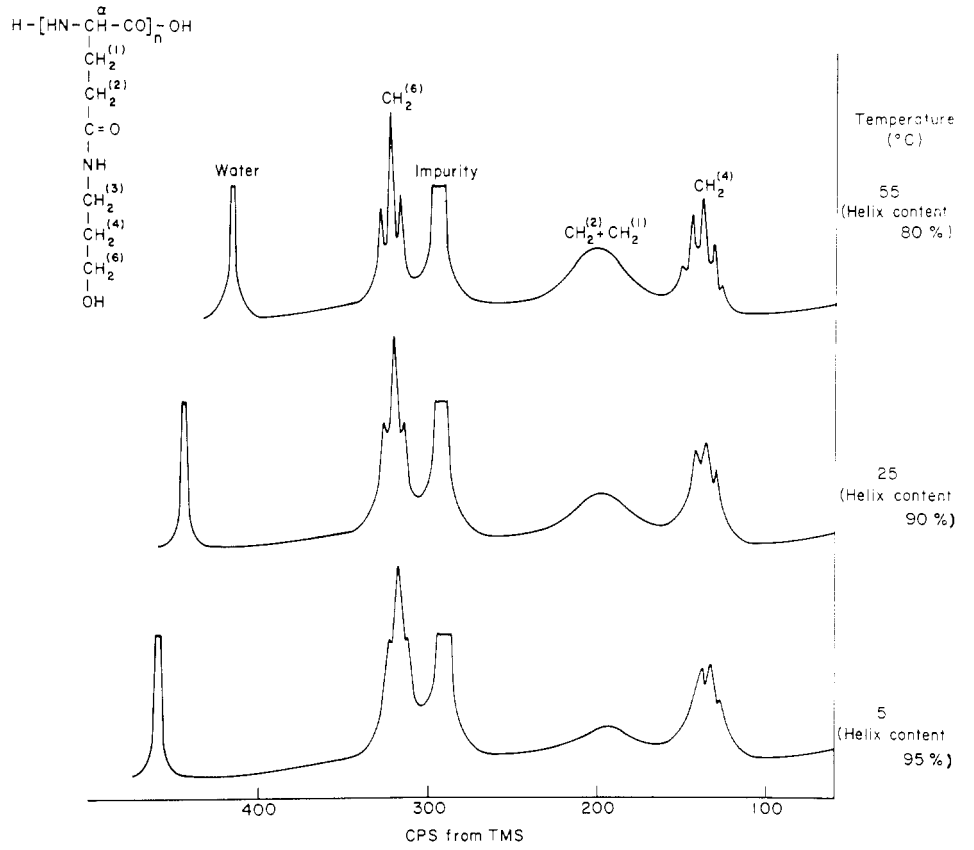

FIGURE 9: Nuclear magnetic resonance spectra of PHPG (DP 270) in CD_3OD at the temperatures and helix content indicated. See Figure 8 for origin of "impurity" peak.

TABLE I: Chemical Shifts Observed for PHEG, PHPG, and PHBG at 30°.[a]

Polymer	Solvent	Helix Content (%)	Chemical Shift (cps from $(CH_3)_3SiCD_2CD_2COONa$)						
			α-CH	$CH_2^{(1)}$	$CH_2^{(2)}$	$CH_2^{(3)}$	$CH_2^{(4)}$	$CH_2^{(4)}$ + $CH_2^{(5)}$	$CH_2^{(6)}$
PHEG (DP 200)	D_2O	0	430	209	239	334			366
	CD_3OD–D_2O (9:1, v/v)[b]	90		c	225[c]	330–340[d]			364
PHPG (DP 270)	D_2O	15	422	215	238	327	174		362
	CD_3OD–D_2O (3:7, v/v)	65	413	c	230[c]	325–335[d]	172		360
	CD_3OD–D_2O (7:3, v/v)	90		c	230[c]	325–335[d]	173		361
	CD_3OD	95		c	230[c]	325–335[d]	171		359
PHBG (DP 70)	D_2O	30	419	209	236	320		155	360
	CD_3OD	95		c	220[c]	315–325[d]		155	358

[a] For identification of the protons, see Figures 2, 3, and 4. [b] The polymer is insoluble in anhydrous methanol. [c] The resonance peaks of $CH_2^{(2)}$ and $CH_2^{(1)}$ are not separated. [d] Not clearly observed because the peak is masked by impurities present in the solvent.

Ferretti (1967) suggested that a downfield shift of 0.5 ppm is associated with the helix-to-coil transition for polyamino acids in organic solvents; however, Markley *et al.* (1967) found that, in aqueous solutions, the shift was only 0.15 ppm, while Bradbury *et al.* (1968b) found no shift. We have investigated the relationship between helix content and the chemical shift of the α-CH proton for the PHBG–D_2O system, in which different helix contents (between 0 and

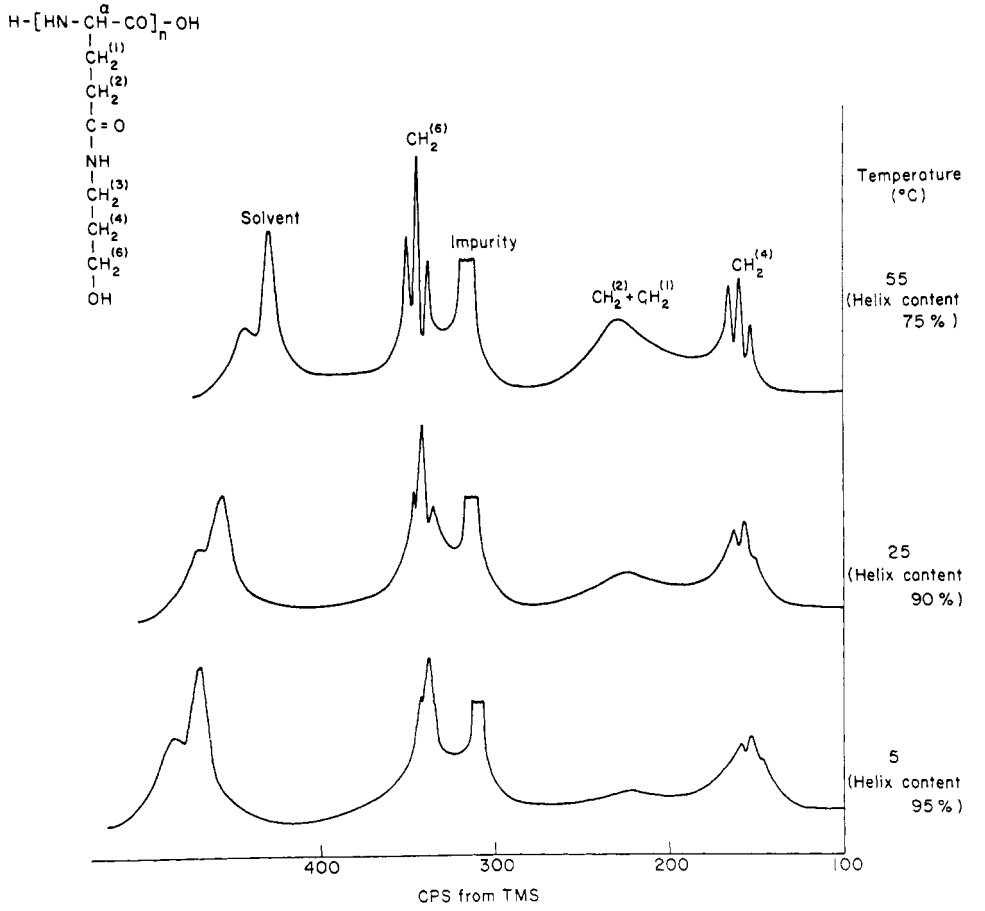

FIGURE 10: Nuclear magnetic resonance spectra of PHPG (DP 270) in CD_3OD–D_2O (7:3, v/v) at the temperatures and helix content indicated. The "solvent" peak arises from proton exchange between methanol and water. See Figure 8 for origin of the "impurity" peak.

75%) could be obtained by appropriate selection of chain length and temperature. The results are shown in Figure 12, together with data for PHPG (DP 270) and PHEG (DP 200) for comparison. When the temperature was increased from 10 to 60°, a change of about 3 cps (0.03 ppm) was observed for the two nonhelical polymers (PHBG, DP 20; PHEG, DP 200); the polymers undergoing a helix-to-coil transition (PHPG, DP 270; PHBG, DP 70; PHBG, DP 650) show somewhat larger changes of 7–10 cps (0.07–0.10 ppm) and, in these cases, the resonances of the α-CH occur consistently at higher fields, at any given temperature.

Discussion

General Considerations. In correlating nuclear magnetic resonance spectra with helix content, we first consider the fact that the optical rotatory dispersion measurements were made in protiated solvents while the nuclear magnetic resonance measurements were made in deuterated ones. Calvin *et al.* (1959) observed that there is a difference in the helix content of PBLG in these two types of solvents at a given temperature, and that this difference is more pronounced in the transition region. Therefore, such an effect can also be expected for other systems, especially if the transition is fairly sharp, as it was in the system studied by Calvin *et al.* (1959). However, in the systems under consideration here, the thermal- and solvent-induced transitions are much broader (Lupu-Lotan *et al.*, 1965; Lotan *et al.*, 1966; Von Dreele *et al.*, 1970), and only an insignificant error is committed by neglecting the effect of D–H substitution on helix content.

As a second point, it is of interest to compare the conformational information obtained from optical rotatory dispersion and nuclear magnetic resonance measurements, respectively, on polyamino acids. Optical rotatory dispersion measurements discriminate between different backbone conformations (*e.g.*, helix and coil) without, in general, providing information about the conformations or mobilities of the side chains attached to either backbone conformation. On the other hand, nuclear magnetic resonance measurements can detect differences in conformation and mobility in both the backbone and side chains (and can detect mobility of the side chains, in the helical as well as in the coil forms). While relatively small variations are observed in the optical rotatory dispersion properties of the random coil forms of different polyamino acids (Tanford, 1967; Adler *et al.*, 1968; Ingwall *et al.*, 1968), we will show here that nuclear magnetic resonance provides a more sensitive probe of such differences among various "random coils." Thus, the two techniques both complement and supplement each other, as far as the information they provide about polyamino acid conformation is concerned.

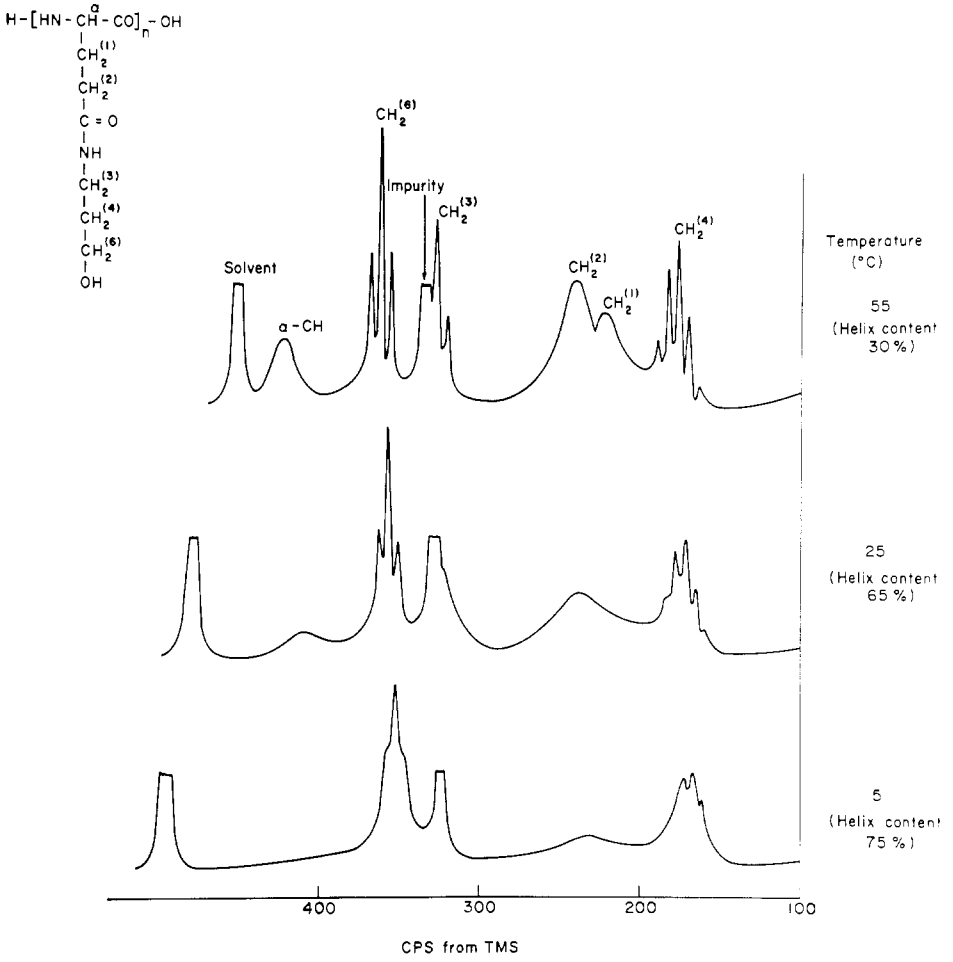

FIGURE 11: Nuclear magnetic resonance spectra of PHPG (DP 270) in CD_3OD–D_2O (3:7, v/v) at the temperatures and helix content indicated. See Figure 8 for origin of the "impurity" peak.

In general, randomly coiled polymers show well-developed resonance peaks with a noticeable splitting pattern (Figure 2), as observed for the corresponding protons in low molecular weight model compounds (Figure 1). On the other hand, polymers of high helix content show very broad peaks for the inner side-chain protons, sharper ones for the outermost side-chain protons, and (at the signal amplification used) such a broad peak for the α-CH protons that it cannot be detected (Figure 8). The shapes of the peaks for the side-chain protons reflect averages over helical and nonhelical residues, these peaks being detectable (in some cases) even with highly helical polymers (Figure 8). The appearance of these peaks depends on the nature of the polymer and on the effect of the experimental conditions on the helix content and side-chain conformation. Similar observations have been reported previously for other systems, such as: poly-γ-ethyl-L-glutamate in trifluoroethanol (Goodman and Masuda, 1964), poly-γ-benzyl-L-glutamate in chloroform (Marlborough *et al.*, 1965; Bovey, 1968), poly-β-benzyl-L-aspartate in chloroform (Bovey, 1968), poly-L-lysine-HBr at pD 12.9 and 5° (Bradbury *et al.*, 1968b), copoly (L-glutamic acid[42]-L-lysine-HBr[28]-L-alanine[30]) at pD 2.5 and 2° (Bradbury *et al.*, 1968b), copoly (β-ethyl-L-aspartate[49]-β-benzyl-L-aspartate[51]) in chloroform at 33.5° (Bradbury *et al.*, 1968a), and poly-L-lysine-HBr in CD_3OD–D_2O (9:1, v/v) (Joubert *et al.*, 1969).

Effect of Rotary Brownian Motion. In order to draw conclusions about internal mobility from line widths, it is first necessary to consider the effect of rotary Brownian motion of the whole molecule on line width (Woessner, 1962, 1964a,b; Huntress, 1968). At a given temperature, T, the rotational diffusion time depends on the viscosity, η, of the solvent, and varies with temperature in the same manner as η/T (Perrin, 1934; Scheraga and Signer, 1960). If the rotation of the whole molecule were isotropic, and if the line width depended only on this rotational diffusion time, then we would expect the peaks from all protons in a given sample to have similar line widths at a given temperature. Since random coils have essentially spherical symmetry, their rotary Brownian motion may be considered to be isotropic with a rotational diffusion time which is shorter the lower the molecular weight. Thus, for example, a sample of PHBG (DP 20) would be expected to have a shorter rotational diffusion time (and hence narrower line widths, if the latter were determined only by rotary Brownian motion) than a sample of PHEG (DP 200); however, from a comparison of Figures 2 and 7, the opposite is the case. Hence, we conclude that, while the rotary Brownian motion does

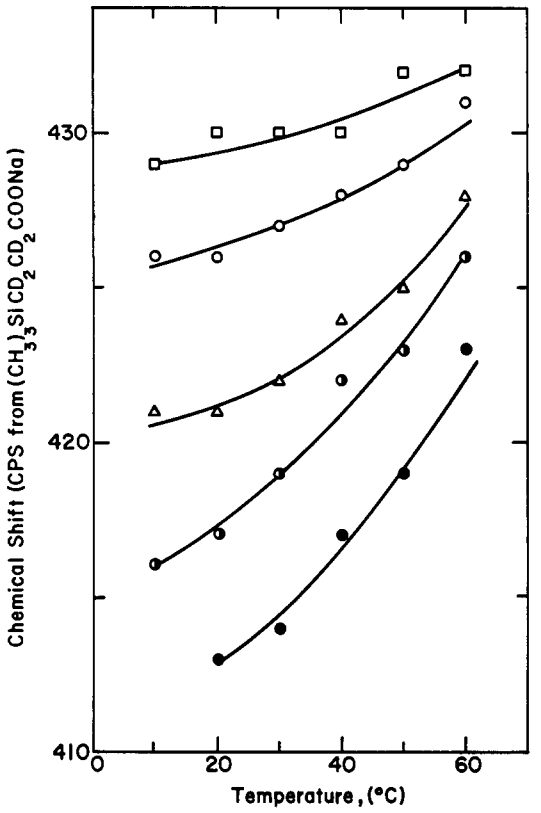

FIGURE 12: Temperature dependence of chemical shifts of the α-CH protons in D_2O, for (□) PHEG (DP 200), (△) PHPG (DP 270), (○) PHBG (DP 20), (◑) PHBG (DP 70), and (●) PHBG (DP 650).

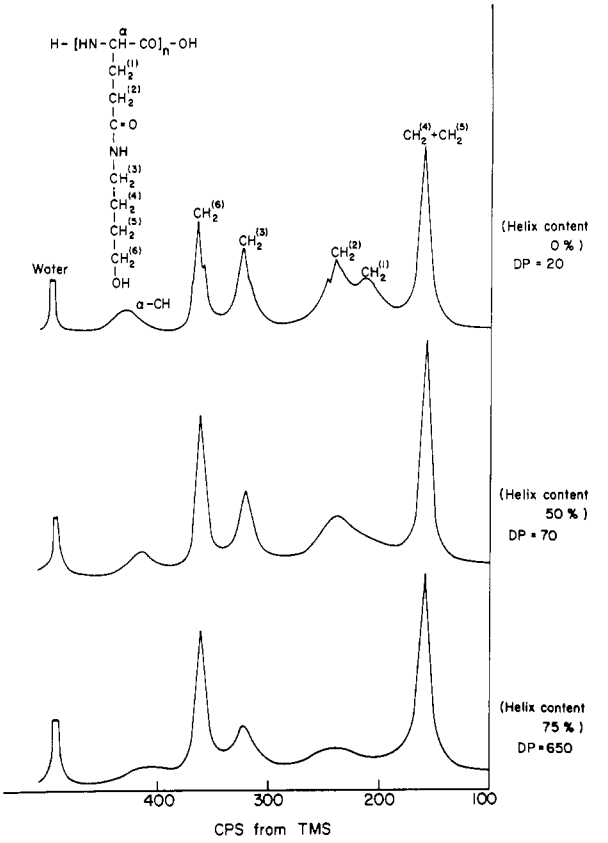

FIGURE 13: Nuclear magnetic resonance spectra of PHBG in D_2O at 10° for the degree of polymerization and helix content indicated. The spectra are the same as those in Figure 7 (DP 20), Figure 6 (DP 70) and Figure 4 (DP 650).

contribute to the line width, the broader lines of Figure 7 arise from the reduced mobility of the side chains of PHBG compared with those of PHEG. Further, the peaks of Figure 2, for PHEG (DP 200), have only *slightly* increased line widths, compared with those of Figure 1, for the model compounds, even though the polymer and the model compounds differ considerably in their rotational diffusion times. Hence, the sharpness of the peaks in Figure 2 must be due, in first approximation, to the internal mobility of the side chains; the *slight* increase in line width probably reflects the longer diffusion time of rotary Brownian motion of the polymer and also some reduced mobility of the side chains of the polymer compared with the model compounds. Finally, the fact that the line widths of, say, the $CH_2^{(1)}$ protons and the $CH_2^{(3)}$ protons of PHEG (DP 200), in Figure 2, differ, indicates that the internal mobilities of these protons differ; if the line width were due only to the rotary Brownian motion, the peaks for both kinds of protons should have similar line widths.

When the molecule becomes rodlike (*e.g.*, when the helix content approaches 100%), the rotational diffusion times about its principal axes differ, and the line widths reflect a more complicated averaging process in which the line widths of different protons in the same molecule are affected in different ways. However, a *partially* helical polymer may still be regarded as a random coil, with an increased root-mean-square end-to-end distance as the helix content increases *in the transition region* (Nagai, 1961); hence, as a first approximation, we may regard the partially helical polymer as isotropic with respect to its rotary Brownian motion. Therefore, we would expect all the side-chain peaks to show similar line widths, if the latter were influenced only by rotary Brownian motion, and will attribute differences in line widths to differences in internal mobility, as a first approximation.

Effect of Temperature. Consider first the effect of temperature on a randomly coiled polymer (PHEG in D_2O). Since this polymer shows no helix content between 10 and 70° in D_2O, the sharpening of the resonance peaks with increasing temperature (Figure 2) must be due to increased freedom of internal motion of the side chains and/or a decrease in the rotational diffusion time of the whole molecule. On the other hand, when an increase of temperature also causes a decrease in helix content, the changes in the spectra are more profound, as observed, for example, with PHPG (DP 270) and PHBG (DP 650) in D_2O (Figures 3 and 4). However, although the helix content of PHBG (DP 650) changes from 75 to 30% between 10 and 60° (Figure 4), the chemical shifts of the side-chain protons do not change with temperature, as is also the case for the nonhelical PHEG (DP 200) (Figure 5). Therefore, the chemical shifts of the side-chain protons are not related to the helix content (see section on Differences in Random Coils). A different behavior was observed for the α-CH proton of both PHBG (DP 650) and PHEG (DP 200). The chemical shift of this resonance

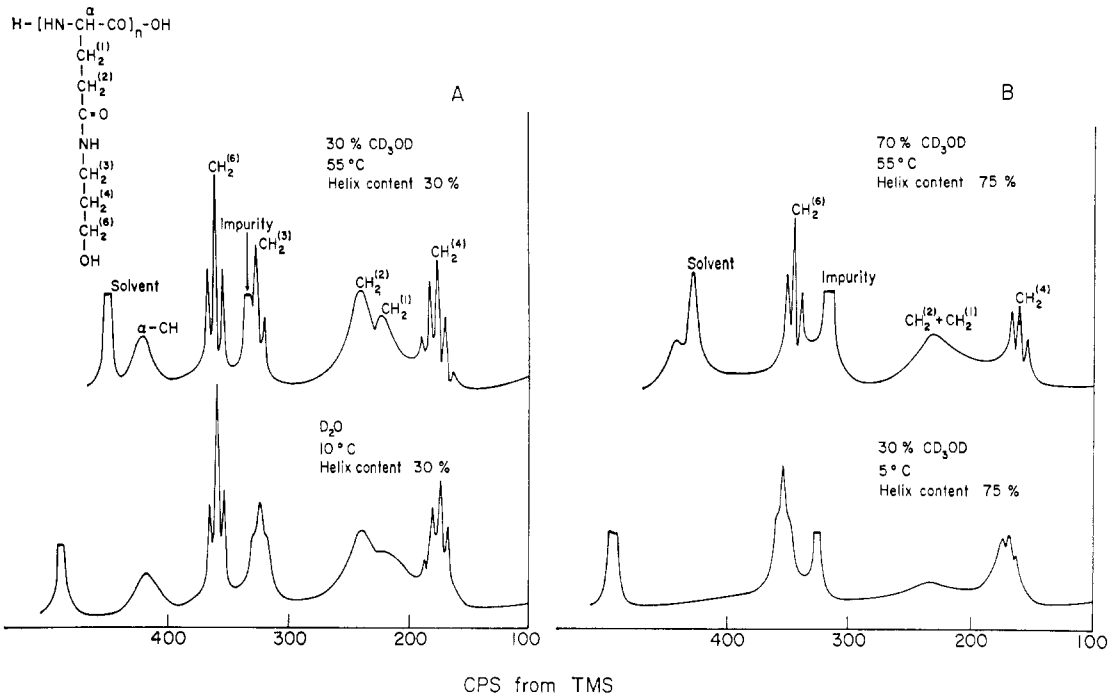

FIGURE 14: Nuclear magnetic resonance spectra of PHPG (DP 270) for the solvents and temperature indicated, at (A) 30% helix content, and (B) 75% helix content. The spectra were reproduced from Figures 3, 10, and 11.

peak is temperature dependent (Figure 5); as will be shown below, this is indeed due to the decrease in helix content, or to effects in the random coil.

Effect of Helix Content. At any given solvent composition and temperature, the helix content of a polyamino acid depends on the molecular weight, up to a limit. Advantage was taken of this property in order to demonstrate how the nuclear magnetic resonance spectra depend on helix content alone, when all other experimental variables are kept constant. The helix content of PHBG in D_2O at 10° is 75, 50, and 0% for samples having DP's of 650, 70, and 20, respectively (Von Dreele *et al.*, 1970), and the corresponding nuclear magnetic resonance spectra are compared in Figure 13. Despite the large differences in molecular weight and helix content (and, hence, rotational diffusion time), the three polymers show almost no differences in the properties of the $[CH_2^{(4)} + CH_2^{(5)}]$ and $CH_2^{(6)}$ peaks. On the other hand, the α-CH, $CH_2^{(1)}$, $CH_2^{(2)}$, and $CH_2^{(3)}$ peaks exhibit a decrease of peak height and an increase of line width, as the helix content increases; in addition, the α-CH peak is shifted upfield.

Under the experimental conditions of Figure 13, the increase in the degree of polymerization is accompanied by an increase in the macroscopic viscosities of the solutions. However, as in the discussion of rotary Brownian motion, if the viscosity had a substantial effect on the observed nuclear magnetic resonance properties of PHBG, one would expect a *similar* broadening for *all* the resonance peaks. Yet, as was already pointed out, essentially no change is observed in the appearance of the peaks of the outermost side-chain protons; as a result, the width of the peaks for the outermost side-chain protons may be used as a reference with which to compare the effect of viscosity on the peaks of other protons. Hence, we conclude that the differences observed for the α-CH and for the inner side-chain protons must reflect differences (in mobility) due to the variation in helix content.

A comparable study has been reported recently (Bradbury and Stubbs, 1968) for PBLG (DP 20–1200) in dimethylformamide, and the main features described here for PHBG (Figure 13) are similar to those observed with PBLG (Bradbury and Stubbs, 1968). However, unlike PHBG, the broadening of the resonance peaks with increasing molecular weight was observed for all of the protons of PBLG, including the outermost (aromatic) ones; this indicates that, in the case of PBLG, the mobility of the whole side chain becomes largely restricted when the backbone assumes the α-helical conformation. Also, no mention was made (Bradbury and Stubbs, 1968) of any change in the chemical shift of the α-CH protons of PBLG.

Bovey *et al.* (1959) have studied the nuclear magnetic resonance properties of randomly coiled polystyrene in carbon tetrachloride, and observed that the spectra are identical for samples having degrees of polymerization in the range of 20,000–10,000. Also, poly-D-alanine samples of different degrees of polymerization (in the range above which the helix content becomes independent of molecular weight) in trifluoroacetic acid–$CDCl_3$ (40:60, v/v) were found to have identical nuclear magnetic resonance spectra (Bradbury *et al.*, 1967). All of these observations suggest that the macroscopic viscosity (and rotary Brownian motion) have little effect on line width in these systems; our results are in agreement with this conclusion.

Side-Chain Mobility. In most theories of the helix–coil transition (*e.g.*, those of Zimm and Bragg (1959) and Lifson and Roig (1961)), the interactions involving the side chains

are included with the parameters characteristic of hydrogen-bond formation in the backbone, implying that the backbone amide hydrogen bonds *and* the interactions involving side chains are both disrupted simultaneously when the helix is converted into the random coil. However, the results obtained here suggest that side-chain interactions may be disrupted (and hence side-chain mobility may change) *without* a change in helix content.

It is possible to obtain various combinations of solvent composition and temperature at which the helix content is the same; this is illustrated for PHPG (DP 270) at two different helix contents in Figure 14A,B. At low helix content (*e.g.*, 30%, Figure 14A), there is little difference in the appearance of the spectra, presumably because the relatively non-helical backbone does not place much limitation on the mobility of the side chains. The value of η/T for 30% methanol at 55° is about one-half that of H_2O at 10°. This (together with a possible influence of the solvent and temperature on the end-to-end distance) would be expected to produce a significant effect on the rotational diffusion time and hence on the line width for all the protons. Since such a change in line width is not observed, we may assume that the rotational diffusion of the whole molecule does not play the dominant role in determining the line width. On the other hand, at high helix content (*e.g.*, 75%, Figure 14B), markedly different spectra are observed. In Figure 14B, η/T is four times higher for the lower spectrum compared with the upper one. While this larger difference in η/T (compared with Figure 14A) might be expected to reflect a more important influence of rotational diffusion on line width at the higher temperature, the fact that the line widths of a given sample at a given temperature still differ (*e.g.*, the large breadth of the α-CH peak) indicates that the various protons have different internal mobilities. Hence, presumably the mobilities and interactions between the side chains are different under the two sets of experimental conditions of Figure 14B, despite the fact that the helix content is the same in both cases. This is the justification for introducing side-chain interactions specifically in statistical mechanical theories of the helix–coil transition, as was done by Bixon *et al.* (1963), and by Poland and Scheraga (1965), rather than including these interactions together with those characteristic of the backbone; the point here is that interactions involving side chains can be modified without changing the helix content of the backbone significantly.

Hydrophobic Interactions. We are particularly interested in the restrictions imposed on the mobility of the side chains when the latter are involved in hydrophobic interactions, since the previous results (Lotan *et al.*, 1966) suggested that, in aqueous solutions, these interactions seem to play an important role in endowing PHBG with a higher helix content than PHPG and PHEG. If this were so, one would expect these interactions to involve the outermost part of the side chains, where the three polymers differ from each other. However, even in this case, the nonpolar parts of the outer alkyl portions of the side chains may still retain the opportunity to roll or slide relative to each other (Steinberg and Scheraga, 1963); hence, while such hydrophobic bonding will contribute to the stability of the helix, it will not cause a complete restriction of the motion within the molecule. This is illustrated in Figure 15, where we compare the spectra of aqueous solutions of two polymers having

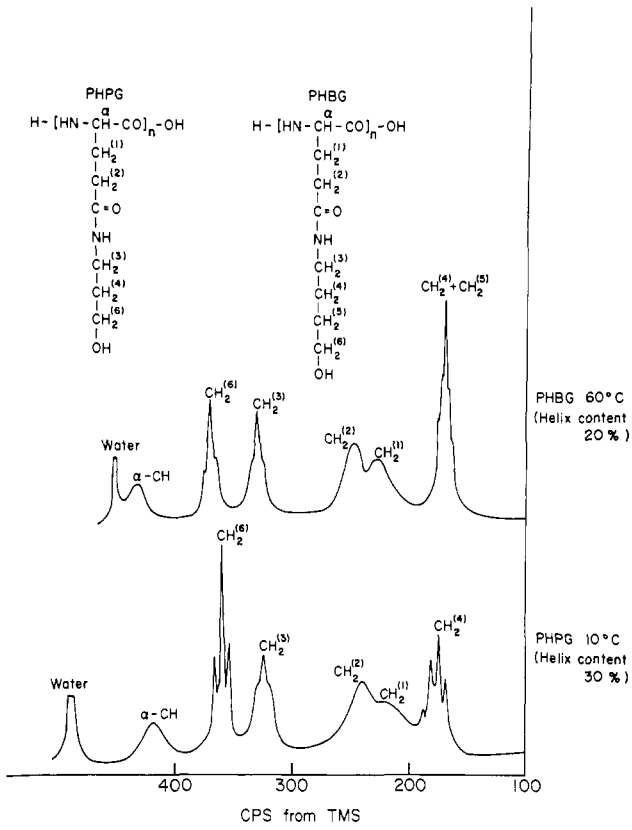

FIGURE 15: Nuclear magnetic resonance spectra of PHBG (DP 70) and PHPG (DP 270) in D_2O at similar helix content at the temperatures indicated. The spectra were reproduced from Figures 6 and 3.

essentially the same helix content (but at different temperaturs), PHBG (DP 70) being 20% helical at 60°, and PHPG, (DP 270) being 30% helical at 10°. The peaks for the α-CH, $CH_2^{(1)}$, $CH_2^{(2)}$, and $CH_2^{(3)}$ protons are similar, with the resolution of the $CH_2^{(1)}$ and $CH_2^{(2)}$ peaks being better for PHBG, presumably because of the higher temperature. While the resolution of the $CH_2^{(4)}$ peak for PHPG and the ($CH_2^{(4)}$ + $CH_2^{(5)}$) peak for PHBG differ, this arises from different coupling constants in the amino alcohols themselves. However, the resolution of the splitting pattern for the $CH_2^{(6)}$ protons is better for PHPG than for PHBG, even though the latter is at a higher temperature and has a slightly lower helix content and lower DP than the former. Presumably, the hydrophobic interactions in PHBG place restrictions on the movements of the outermost groups, thus providing additional stability to the α-helical conformation of PHBG in water.

Differences in Random Coils. In this section we provide evidence that, under similar experimental conditions, the random coil forms of various polymers differ from each other.

The restricted motion of the side chains of PHBG, discussed above in connection with Figure 15, is observed not only in a partially helical polymer, but also in a sample with no detectable helix content. This is illustrated in Figure 16, where the spectra of two nonhelical polymers, PHEG (DP 200) and PHBG (DP 20), are compared at the same temperature, and in the same solvent. Both polymers are random coils by optical rotatory dispersion criteria; yet

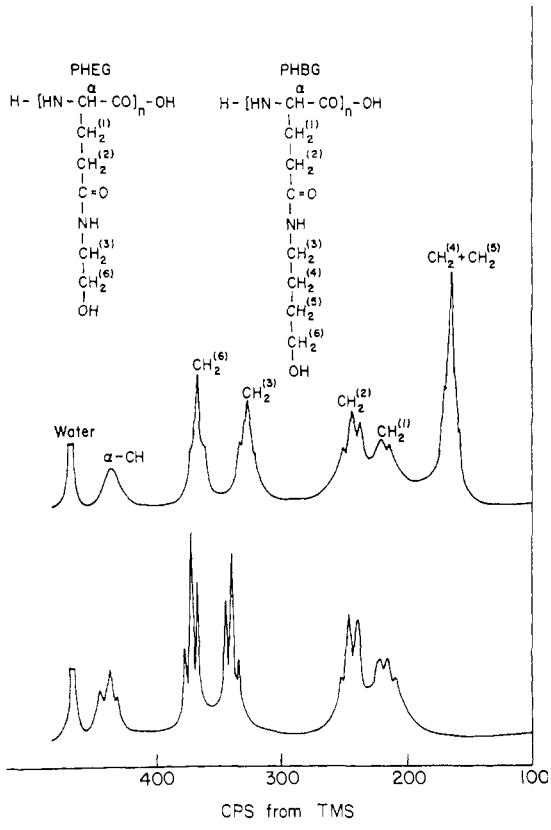

FIGURE 16: Nuclear magnetic resonance spectra of two polyamino acids in D_2O at 40°, both having 0% helix content. Top: PHBG (DP 20); bottom: PHEG (DP 200). The spectra were reproduced from Figures 7 and 2.

their spectra show different line widths, those for PHEG being sharper even though its DP is higher (also the chemical shifts of the side-chain protons of PHEG and PHBG differ, as shown in Figure 5 and Table I). The differences can be accounted for by considering the conformational energy diagrams of the various (interacting) amino acid residues (Brant and Flory, 1965; Go *et al.*, 1968; Scheraga, 1968). The relative energies (and hence the populations of states at a given temperature) differ from one amino acid to another and, thus, the distribution of populations for the corresponding randomly coiled polymers will not be similar. Further, as the temperature is increased, the populations of the various states change (but in a different way for each polymer); however, at *very high* temperature, the population distributions of the various randomly coiled polyamino acids should become similar to each other. Thus, the physical properties, which are related to the conformation, can be expected to show a similar behavior; indeed, this was detected (but to a small extent) in the optical rotatory dispersion properties of various random coils (Tanford, 1967; Ingwall *et al.*, 1968; Adler *et al.*, 1968). A more pronounced effect was observed here for the nuclear magnetic resonance spectra (Figure 16). From these considerations, we can understand the greater similarities in the spectra (in water at 70°) of PHEG (DP 200, helix content 0%), of PHPG (DP 270, helix content 5%), and of PHBG (DP 20, helix content 0%) (Figures 2, 3, and 7), although a temperature much higher than 70° would be required to make these spectra completely similar to each other; we can also understand the similar trend in the change of the spectra of the nonhelical PHEG (DP 200) and PHBG (DP 20) with temperature.

Based on the evidence presented above, and on the fact that the spectrum of PHEG (DP 200) at 70° is more like the one observed for ethanolamine (Figure 1) than is the one for PHBG (DP 20), we may consider the conformation of PHEG in water to be similar to that of a statistical random coil, and more so than for the so-called random coil forms of other polyamino acids. This conclusion is supported by the results of a comparative study of the optical rotatory dispersion properties of PHEG and PGA (Adler *et al.*, 1968), and by the investigations of Tiffany and Krimm (1968).

Consideration of the α-CH Peak. In general, we have seen that the α-CH resonance peak shifts upfield, broadens, and ultimately becomes unobservable (at the signal amplification used) as the helix content of the various polymers increases. However, despite the fact that the correlation between helix content and the nuclear magnetic resonance properties of the α-CH proton has been considered frequently, the results are still confusing. In some cases (Markley *et al.*, 1967), the α-CH peak was a single one whose position changed by about 0.15 ppm during the helix–coil transition while, in other cases (Ferretti, 1967), two widely separated (0.5 ppm) peaks, which were assumed to correspond to helical and randomly coiled residues, were observed. In still another case (Bradbury *et al.*, 1968b), a single peak, which exhibited no shift as the helix content increased from 10 to 60%, was observed. All of these results were obtained with systems in which other important factors, in addition to the conformational changes, were playing a role; these are the ionization of side-chain functional groups (in polylysine and polyglutamic acid), or the extensive solvation of peptide bonds by the strong acids used as solvents.

Since the polyhydroxyalkylglutamine derivatives contain no ionizable groups, and since strong acids were not used in the experiments reported here, an examination of the chemical shifts, δ^{α}, of their α-CH resonances in aqueous systems is, therefore, of interest. From Figure 12, we see that δ^{α} increases with increasing temperature, and that the trend of the temperature dependence is not the same in all cases. In interpreting these results, it should be recalled that, in the temperature range of 10–60°, both PHEG (DP 200), and PHBG (DP 20) are nonhelical, whereas PHPG (DP 270), PHBG (DP 70), and PHBG (DP 650) undergo a temperature-induced helix–coil transition. A general feature of the data of Figure 12 is that the α-CH peaks appear at higher fields (*i.e.*, at lower δ^{α} values) when these protons are in a more compactly packed structure than in a looser one. When the helix content is higher, more C^{α} protons are not only in a more compact structure, but more of them are in the specific α-helical magnetic environment of the amide group which leads to lower δ^{α} values (Markley *et al.*, 1967). For any one of the *nonhelical* polyamino acids, the structure is transformed from a compactly packed one to a looser one as the temperature is increased, because of the effect of temperature on the intramolecular librational motions of the chain. Also, from the fact that the magnitudes of δ^{α} differ for these two (nonhelical) polymers (PHEG, DP 200; PHBG, DP 20) at any temperature, we reach the same conclusion obtained from the discussion of Figure 16, *viz.*, that the two

nonhelical conformations are not identical. Moreover, considering the temperature effect mentioned above, we conclude that the random coil conformation of PHEG is less compact than the one of PHBG, since the value of δ^{α} is higher for PHEG than for PHBG at any given temperature. The same conclusion was deduced from the resonance properties of the *side-chain* protons (see section on Difference in Random Coils).

On the other hand, for a partially helical polymer, the environment of the α-CH group would be expected to be more tightly packed, the higher the helix content, and we may regard the value of δ^{α} as being an average of the chemical shifts for all coil states. Therefore, if this interpretation is correct, we would expect δ^{α} to contain contributions from *two* different effects, as the temperature is raised: (1) the decrease in helix content; and (2) changes in the properties of the random coil. In other words, we can envisage the overall conformational change as taking place in two *hypothetical* steps, *viz.*

rigid helix, low temperature (HLT)
↓ isothermally
partially rigid random coil, low temperature (CLT)
↓ Δ
less rigid random coil, higher temperature (CHT)

Our results for the three partially helical polymers of Figure 12 seem to support such an interpretation. The values of δ^{α} for PHBG at 20, 40, and 60° (Figure 12) are replotted in Figure 17 as a function of the corresponding helix content of the samples, and we see that δ^{α} depends on both the temperature and the helix content. For example, the conformational transition of PHBG (DP 650) from 20° (helix content 67%, δ^{α} = 413 cps) to 60° (helix content 32%, δ^{α} = 423 cps) can be decomposed (see Figure 17) into the two hypothetical steps mentioned above, where the conformations are: HLT (20°, helix content 67%, δ^{α} = 413 cps), CLT (20°, helix content 32%, δ^{α} = 418 cps), and CHT (60°, helix content 32%, δ^{α} = 423 cps). By extrapolating the 20° isotherm (Figure 17) to essentially 100% helix content, we obtain a value of about 410 cps for δ^{α} of a coil residue in a largely helical polymer; the value of δ^{α} for a completely nonhelical polymer at this temperature is 426 cps. Hence, the conversion of an essentially complete helix of PHBG into a completely nonhelical form at 20° is accompanied by a downfield shift ($\Delta\delta^{\alpha}$) of the α-CH peak of a coil residue of about 16 cps (0.16 ppm). Extrapolation of the 40 and 60° isotherms gives roughly the same values of δ^{α} for the essentially complete helix, as obtained at 20°. However, the δ^{α} values in the randomly coiled polymer are temperature dependent, being 426, 428, and 431 cps at 20, 40, and 60°, respectively; this variation arises from temperature-induced changes in the properties of the random coil.

As shown above, the chemical shift of the α-CH proton changes during the helix–coil transition because of changes in the environment of this proton. Both the α-helix and the low-temperature nonhelical conformation are tight compact structures, which are transformed into looser ones as the temperature is increased. This change of conformation may also bring the α-CH proton closer to the bulk of solvent. However, we cannot attribute the increase of δ^{α} during the helix–coil transition (Figure 17) solely to changes in the state

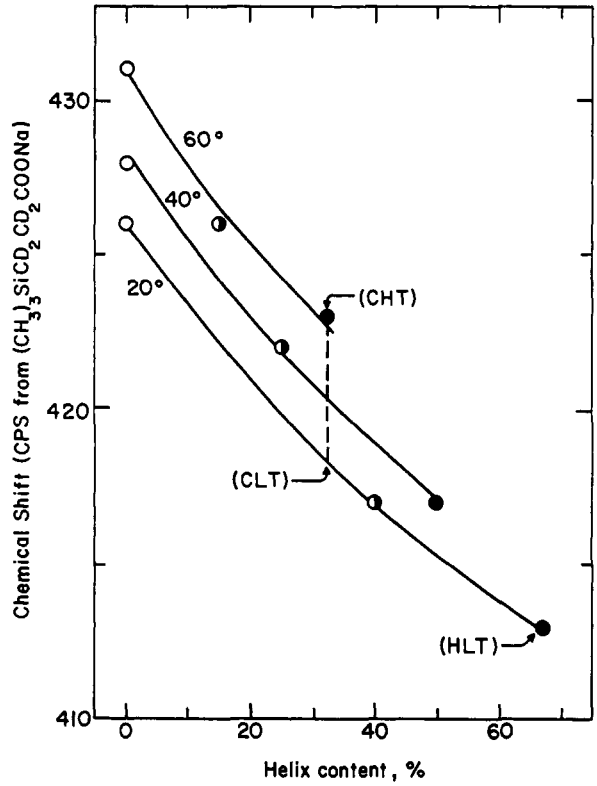

FIGURE 17: Dependence of chemical shifts of the α-CH protons of PHBG in D_2O on helix content at several temperatures: (○) DP 20, (◑) DP 70, and (●) DP 650. This Figure was obtained by replotting the data of Figure 12, taking into account the known helix content of the various samples at each temperature.

of hydration of the peptide bond. Since the binding of water occurs with a negative enthalpy change (Morrison and Hanlan, 1957), an increase of temperature would reduce the extent of solvation of the peptide bond; therefore, as the temperature is increased at 0% helix, one would expect to observe a change in δ^{α} similar to the one associated with the formation of the helical conformation. As can be seen in Figure 17 the opposite is observed. Such a behavior is also shown by the randomly coiled PHEG (DP 200) (Figure 12). These considerations are consistent with the observation (Figure 13) that the outermost side-chain protons do not show such a shift, since their environment changes to a much lesser extent; they are also consistent with the fact that the α-CH resonance for coiled PHBG occurs at a higher field than for coiled PHEG (Figure 12), the latter having a less compact (*i.e.*, less shielded) conformation. In summary, we conclude that: (1) The observed resonance peak of the α-CH proton is attributed to an average over those protons which are attached to nonhelical residues; (2) when this peak is sufficiently narrow to be observed in a thermally induced helix–coil transition, the changes in the chemical shift of this peak reflect modifications in the environment of the α-CH protons; these modifications of environment are, in turn, due to changes in both the helix content of the polymer and to the thermally induced changes in the conformation of the random coil; the resonance peak of the α-CH proton of pure helical residues is not observed at the signal amplification used, because of its appreciable broadness.

Our interpretation of the properties of the α-CH resonance differs from the one (Ferretti, 1967) which assumed the existence of two separate peaks of comparable width, one for helical (H peak) and one for randomly coiled residues (R peak). This explanation is not acceptable, mostly because we cannot assume the existence of an H peak in partially helical polymers when, at the same signal amplification, we observe no peak with comparable width for the fully helical ones. Also, the two peaks (H and R) were observed only in the presence of dichloroacetic acid or trifluoroacetic acid (see Ferretti, 1967, and references cited therein), *but not in other solvents*. We thus propose an alternative explanation for the observation of two α-CH peaks in dichloroacetic acid and trifluoroacetic acid.

On the basis of observations using a variety of methods, including infrared (Bradbury and Rattle, 1968; Hanlon and Klotz, 1965) and the Hammett acidity function (Hammett and Deyrup, 1932), it is known that amides interact with strong acids. Also, the helix–coil transition of polyamino acids was found to be dependent on the polymer concentration (presumably because the polymer binds the acid and reduces its activity) in dichloroacetic acid containing solvents, when this transition was studied by optical rotatory dispersion (Goodman *et al.*, 1962; Liu *et al.*, 1967), nuclear magnetic resonance (Liu *et al.*, 1967), or calorimetry (Ackermann and Neumann, 1967). Further, a viscosity study of PBLG (Bradbury and Fenn, 1968) clearly indicates that the polymer behaves as a polyelectrolyte (*i.e.*, is solvated by the dichloroacetic acid) in dichloroacetic acid containing solvents. The acid–amide interaction has also been demonstrated by nuclear magnetic resonance studies on low molecular weight amides in the presence of hydrochloric acid, perchloric acid, or trifluoroacetic acid (Berger *et al.*, 1959; Stewart *et al.*, 1967), as well as on small peptides in $CDCl_3$–trifluoroacetic acid mixtures (Bradbury and Fenn, 1969). The differences between the nuclear magnetic resonance properties of low molecular weight amides, *e.g.*, the system *N*-methylacetamide–trifluoroacetic acid–chloroform (Stewart *et al.*, 1967), and those observed with polyamino acids–trifluoroacetic acid–chloroform (Ferretti, 1967; Bradbury *et al.*, 1968c) can be understood if one considers that the rate of solvation is faster for the small molecules than for large polymers. As a matter of fact, such a difference in rate has been reported (Scarpa *et al.*, 1967; Klotz and Mueller, 1969) for a similar solvation process, *viz.*, the H–D exchange in poly-*N*-isopropylacrylamide (whose amides are not hydrogen bonded).

Recognizing that the peptide bond can be solvated by strong acids, we can explain the experimental results, which have heretofore been presented as support for the "two peaks" assumption, without encountering the contradictions mentioned above. We consider that the H peak corresponds to an average for the α-CH groups in the unsolvated nonhelical residues, the R peak is associated with the α-CH groups in the proximity of solvated nonhelical peptide bonds, and that the α-CH proton of a *helical* residue does not exhibit a separate resonance line having a width comparable with those of the H or R peaks at the signal amplification used. Thus, for example, in the case of the temperature-induced coil-to-helix transition of PBLG in trifluoroacetic acid–chloroform (Bradbury *et al.*, 1968c), the changes in the spectrum can be interpreted as being due to the dissociation of trifluoroacetic acid from the nonhelical parts of the polymer. As pointed out by the authors (Bradbury *et al.*, 1968c), this conformational change is produced by heating without changing the *overall* composition of the system. However, it must be realized that the degree of solvation of the polymer is altered by changing the temperature; as a matter of fact, the desolvation process, induced by increasing the temperature, is the very origin of the observed "inverted" transition (Bradbury *et al.*, 1968c).

The same reasoning can be used to account for the observed (Bradbury *et al.*, 1968c) variation in the behavior of the α-CH peak of PBLG, when samples of different degrees of polymerization are compared at the same helix content (50%) and temperature, but at different $CDCl_3$–trifluoroacetic acid ratios; the DP 92 fraction shows two peaks (R and H), and the spectrum is changed only slightly when the degree of polymerization of the sample and the trifluoroacetic acid content of the solvent are simultaneously lowered, in such a manner that the helix content (by optical rotatory dispersion) is kept constant. Disregarding the fact that the conversion factor (for optical rotatory dispersion to helix content) is a constant for high polymers (DP >100) but varies for the low molecular weight ones (DP ~10–20), the above observations can be understood by considering the characteristics of the solvation of the nonhelical part of the polymer by trifluoroacetic acid. Since the process is presumably one of random, noncooperative binding, the fraction of solvated coil residues, f_s, depends on the concentration of the binding agent (*i.e.*, trifluoroacetic acid), and not on the molecular weight of the polymer. For the DP 13, 21, and 92 samples, the concentrations of trifluoroacetic acid required to produce the same (50%) helix content were very similar to each other (about 6–8%). Within such a narrow range of solvent composition, f_s is almost constant; this accounts for the similarity in the spectra of the three samples. At the same helix content, a larger polymer (DP 640) showed only one wide peak for the α-CH proton, instead of the two observed for the low molecular weight samples. However, a higher concentration of trifluoroacetic acid (about 25%) was required in order that this high-DP polymer have the same helix content as the low-DP ones; under these conditions of a higher trifluoroacetic acid concentration, the rate of solvation is higher and, as a result, only one peak is observed. This interpretation is also supported by the observation (Bradbury *et al.*, 1967) that only one peak is obtained for the α-CH proton of the high molecular weight poly-D-alanine in trifluoroacetic acid-$CDCl_3$ (40:60, v/v).

The observed linear relationship (Bradbury *et al.*, 1968c) between helix content determined by optical rotatory dispersion and helix content determined from the areas under the H and R peaks for PBLG of DP 13, 21, and 92 can be explained as follows. Since the trifluoroacetic acid concentration changes very little (and, therefore, most importantly, f_s is essentially constant) in the transition range in which the helix content changes markedly, the area under the H and R peaks for these samples would be expected to vary linearly with the *absolute* number of solvated coil units.

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References

Ackermann, T., and Neumann, E. (1967), *Biopolymers 5*, 649.
Adler, A. J., Hoving, R., Potter, J., Wells, M., and Fasman, G. D. (1968), *J. Am. Chem. Soc. 90*, 4736.
Berger, A., Loewenstein, A., and Meiboom, S. (1959), *J. Am. Chem. Soc. 81*, 62.
Bixon, M., Scheraga, H. A., and Lifson, S. (1963), *Biopolymers 1*, 419.
Bovey, F. A. (1968), *Pure Appl. Chem. 16*, 417.
Bovey, F. A., Tiers, G. V. D., and Filipovich, G. (1959), *J. Polymer Sci. 38*, 73.
Bradbury, E. M., Carpenter, B. G., and Goldman, H. (1968a), *Biopolymers 6*, 837.
Bradbury, E. M., and Crane-Robinson, C. (1968), *Nature 220*, 1079.
Bradbury, E. M., Crane-Robinson, C., Goldman, H., and Rattle, H. W. E. (1968b), *Biopolymers 6*, 851.
Bradbury, E. M., Crane-Robinson, C., Goldman, H., and Rattle, H. W. E. (1968c), *Nature 217*, 812.
Bradbury, E. M., Crane-Robinson, C., and Rattle, H. W. E. (1967), *Nature 216*, 862.
Bradbury, E. M., Downie, A. R., Elliott, A., and Hanby, W. E. (1960), *Proc. Roy. Soc. (London) A259*, 110.
Bradbury, E. M., and Rattle, H. W. E. (1968), *Polymer 9*, 201.
Bradbury, J. H., and Fenn, M. D. (1968), *J. Mol. Biol. 36*, 231.
Bradbury, J. H., and Fenn, M. D. (1969), *Australian J. Chem. 22*, 357.
Bradbury, J. H., and Stubbs, G. J. (1968), *Nature 218*, 1049.
Brahms, J., and Spach, G. (1963), *Nature 200*, 72.
Brant, D. A., and Flory, P. J. (1965), *J. Am. Chem. Soc. 87*, 2791.
Calvin, M., Hermans, J., Jr., and Scheraga, H. A. (1959), *J. Am. Chem. Soc. 81*, 5048.
Chaudhuri, S. R., and Yang, J. T. (1968), *Biochemistry 7*, 1379.
Fasman, G. D. (1967), *in* Poly-α-Amino Acids, Fasman, G. D., Ed., New York, N. Y., Marcel Dekker, p 551.
Ferretti, J. A. (1967), *Chem. Commun.*, 1030.
Go, N., Go, M., and Scheraga, H. A. (1968), *Proc. Natl. Acad. Sci. U. S. 59*, 1030.
Goodman, M., Boardman, F., and Listowsky, I. (1963), *J. Am. Chem. Soc. 85*, 2491.
Goodman, M., and Masuda, Y. (1964), *Biopolymers 2*, 107.
Goodman, M., Schmitt, E. E., and Yphantis, D. A. (1962), *J. Am. Chem. Soc. 84*, 1288.
Gratzer, W. B., and Doty, P. (1963), *J. Am. Chem. Soc. 85*, 1193.
Hammett, L. P., and Deyrup, A. J. (1932), *J. Am. Chem. Soc. 54*, 2721.
Hanlon, S., and Klotz, I. M. (1965), *Biochemistry 4*, 37.
Huntress, W. T., Jr. (1968), *J. Chem. Phys. 48*, 3524.
Ingwall, R. T., Scheraga, H. A., Lotan, N., Berger, A., and Katchalski, E. (1968), *Biopolymers 6*, 331.
Jacobson, A. L. (1965), *Biopolymers 3*, 249.
Joubert, F. J., Lotan, N., and Scheraga, H. A. (1969), *Physiol. Chem. Phys. 1*, 348.
Karlson, R. H., Norland, K. S., Fasman, G. D., and Blout, E. R. (1960), *J. Am. Chem. Soc. 82*, 2268.
Klotz, I. M., and Mueller, D. D. (1969), *Biochemistry 8*, 12.
Lifson, S., and Roig, A. (1961), *J. Chem. Phys. 34*, 1963.
Liu, K.-J., Lignowski, J. S., and Ullman, R. (1967), *Biopolymers 5*, 375.
Lotan, N., Yaron, A., and Berger, A. (1966), *Biopolymers 4*, 365.
Lupu-Lotan, N., Yaron, A., Berger, A., and Sela, M. (1965), *Biopolymers 3*, 625.
Markely, J. L., Meadows, D. H., and Jardetzky, O. (1967), *J. Mol. Biol. 27*, 25.
Marlborough, D. I., Orrell, K. G., and Rydon, H. N. (1965), *Chem. Commun.*, 518.
Morrison, J. L., and Hanlan, J. F. (1957), *Nature 179*, 528.
Nagai, K. (1961), *J. Chem. Phys. 34*, 887.
Ooi, T., Scott, R. A., Vanderkooi, G., and Scheraga, H. A. (1967), *J. Chem. Phys. 46*, 4410.
Perrin, F. (1934), *J. Phys. Radium (7)5*, 497.
Poland, D. C., and Scheraga, H. A. (1965), *Biopolymers 3*, 283, 305, 315, 335.
Scarpa, J. S., Mueller, D. D., and Klotz, I. M. (1967), *J. Am. Chem. Soc. 89*, 6024.
Schellman, J. A. (1955), *Compt. Rend. Trav. Lab. Carlsberg 29*, 230.
Scheraga, H. A. (1968), *Advan. Phys. Org. Chem. 6*, 103.
Scheraga, H. A., and Signer, R. (1960), *in* Technique of Organic Chemistry, Weissberger, A., Ed., Vol. I, Part III, New York, N. Y., Interscience, p 2387.
Steinberg, I. Z., and Scheraga, H. A. (1963), *J. Biol. Chem. 238*, 172.
Stewart, W. E., Mandelkern, L., and Glick, R. E. (1967), *Biochemistry 6*, 150.
Tanford, C. (1967), *Abstr. 7th Intern. Congr. Biochem., Tokyo, Symp 1–3, 5*, 33.
Tiffany, M. L., and Krimm, S. (1968), *Biopolymers 6*, 1379.
Woessner, D. E. (1962), *J. Chem. Phys. 36*, 1.
Woessner, D. E. (1964a), *J. Chem. Phys. 40*, 2341.
Woessner, D. E. (1964b), *J. Chem. Phys. 41*, 84.
Yan, J. F., Vanderkooi, G., and Scheraga, H. A. (1968), *J. Chem. Phys. 49*, 2713.
Zimm, B. H., and Bragg, J. K. (1959), *J. Chem. Phys. 31*, 526.