Laser Raman Spectroscopy of Polypeptides. II. Spectra of Random Poly(hydroxybutylglutamine-*co*-glycine) in the Solid State[1]

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ABSTRACT: Laser Raman spectra of random copolymers of hydroxybutylglutamine and glycine in the solid state have been obtained. The spectra in the region 200 to 1700 cm^{-1} were interpreted in terms of the influence of glycyl residues on the helix–coil transition of poly(hydroxybutylglutamine), and were shown to be sensitive to the conformational states of individual residues in the random copolymer. The spectra in the region 0–200 cm^{-1}, obtainable with the use of an iodine absorption cell, indicate the presence of helical modes previously predicted in normal coordinate treatments.[3-5] In particular, a sharp band at 61 cm^{-1} is thought to arise from specific effects of the incorporation of *single* glycyl residues in the helical sections of the copolymers.

In the first paper[6] of this series, we showed that laser Raman spectroscopy could be used as an effective structural tool in the study of block copolymers of L-alanine and D,L-lysine. That investigation illustrated that the spectra were sensitive to the conformational states of the individual amino acid residues and, in addition, that the technique could be used as a probe for internal interactions such as hydrophobic bonding. In the present paper, we have applied the technique to random binary (host–guest) copolymers to obtain information about the conformational states of the two kinds of residues in the copolymer and also about the behavior of the guest residue among its host neighbors.

The system chosen for this investigation is a random copolymer [P(HBG:Gly)] of hydroxybutylglutamine (HBG) as the host and glycine as the guest residue. The copolymers had been synthesized, fractionated, and characterized for a previous study[8] in which the host–guest technique was used to obtain the helix–coil stability constants for glycine. It was our hope that the laser Raman method would give a detailed picture of glycine and its environment in PHBG, thereby providing spectroscopic evidence for the model used to interpret the data obtained in the previous study.[7]

Experimental Section

(A) Materials. The synthesis, fractionation, and characterization of the random copolymers P(HBG:Gly) were described earlier.[7] The average mole percent of glycine and the weight-average degree of polymerization DP_w (based on the weight-average molecular weight $\bar{M}_w$) of the fractions used here are: (a) 3.1%, 510; (b) 3.0%, 282; (c) 3.0%, 168; (d) 4.1%, 521; (e) 6.5%, 334; (f) 7.9%, 100; (g) 8.9%, 99; and (h) 18.0%, 92. The homopolymer PHBG was fraction VIC, DP_w = 120, of a previous investigation.[8]

Monomeric glycine was obtained from Mann Research Laboratories and was used without further purification. Poly(glycine I) was also obtained from Mann and had a stated molecular weight of between 10,000 and 25,000. It was used without further fractionation or purification. Hexafluoroacetone sesquihydrate (HFA) was a spectral grade product from Eastman Kodak Co. The polyglycine from Mann was dissolved in HFA to make sure that it was in form I and then, after filtration, it was precipitated by pouring it into a large volume of water. The precipitate was washed with water and air-dried. A saturated LiBr solution was then used to redissolve some of the dried precipitate, and the resulting solution was filtered and poured into excess water to convert it to a precipitate of form II which was centrifuged, washed with water, and air-dried.

(B) Preparation of Samples. The samples were used as solids, and pressed into disks using a pellet press.

(C) Apparatus. The apparatus was the same as that described previously.[6] The Coherent Radiation Laboratory Model 52A argon-ion laser was single moded with an etalon. In order to obtain spectra close to the exciting line, a 10-cm iodine cell[9] (filled with iodine gas and heated) was placed between the sample and the monochromator. A temperature of 65° in the iodine cell was found to be optimal for elimination of the Rayleigh scattering of the 5145 > Å line of the argon-ion laser, there being a discrete absorption band at this wavelength in the spectrum of molecular iodine. With the use of the iodine cell, it was possible to obtain spectra as close as 5 cm^{-1} to the exciting line, even though the tail of the exciting line extends to ~15 cm^{-1}. This is of special importance in polymeric systems which exhibit Raman scattering at low frequencies.

It was fortunate that the fluorescence exhibited by the samples decayed (without degradation of the sample) on prolonged exposure to the laser beam, allowing excellent detectability of low-intensity modes. The high intensity of the signals allowed slits of 20 μ to be used. The monochromator was stepped at a wavelength interval of 0.1 Å, and integration times of up to 60 sec at each step were used.

Results

The spectra of samples a, e, and h from 200 to 1700 cm^{-1} are shown in Figure 1, and the spectra of all the samples from 0 to 200 cm^{-1} are given in Figure 2. Tables I and II summarize the data from the spectra of Figures 1 and 2, respectively. Raman spectra (not shown here) were also obtained for PHBG, monomeric glycine, poly(glycine I), and poly-

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(3) K. Itoh and T. Shimanouchi, *Biopolymers*, **9**, 383 (1970).

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(5) B. Fanconi, E. W. Small, and W. L. Peticolas, *Biopolymers*, **10**, 1277 (1971).

(6) A. Lewis and H. A. Scheraga, *Macromolecules*, **4**, 539 (1971).

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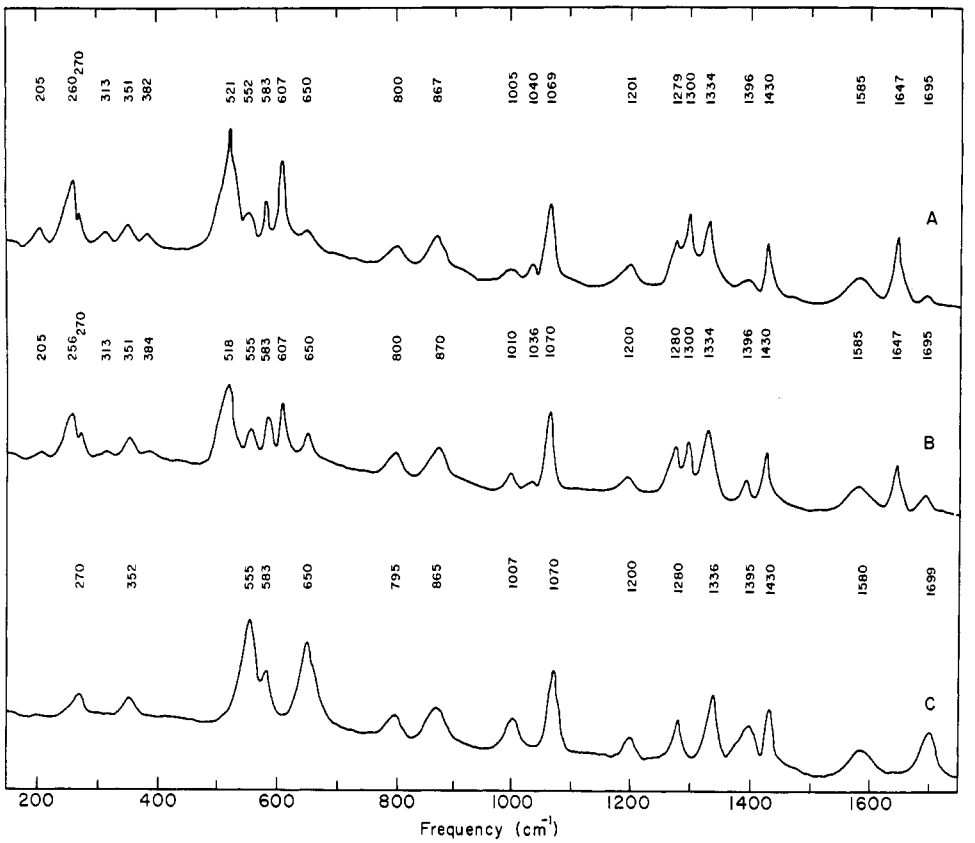

Figure 1. Raman spectrum of P(HBG:Gly) in the solid state from 200 to 1700 cm^{-1}: (A) 3.1% Gly, DP_w = 510 (sample a); (B) 6.5% Gly, DP_w = 334 (sample e); (C) 18.0% Gly, DP_w = 92 (sample h).

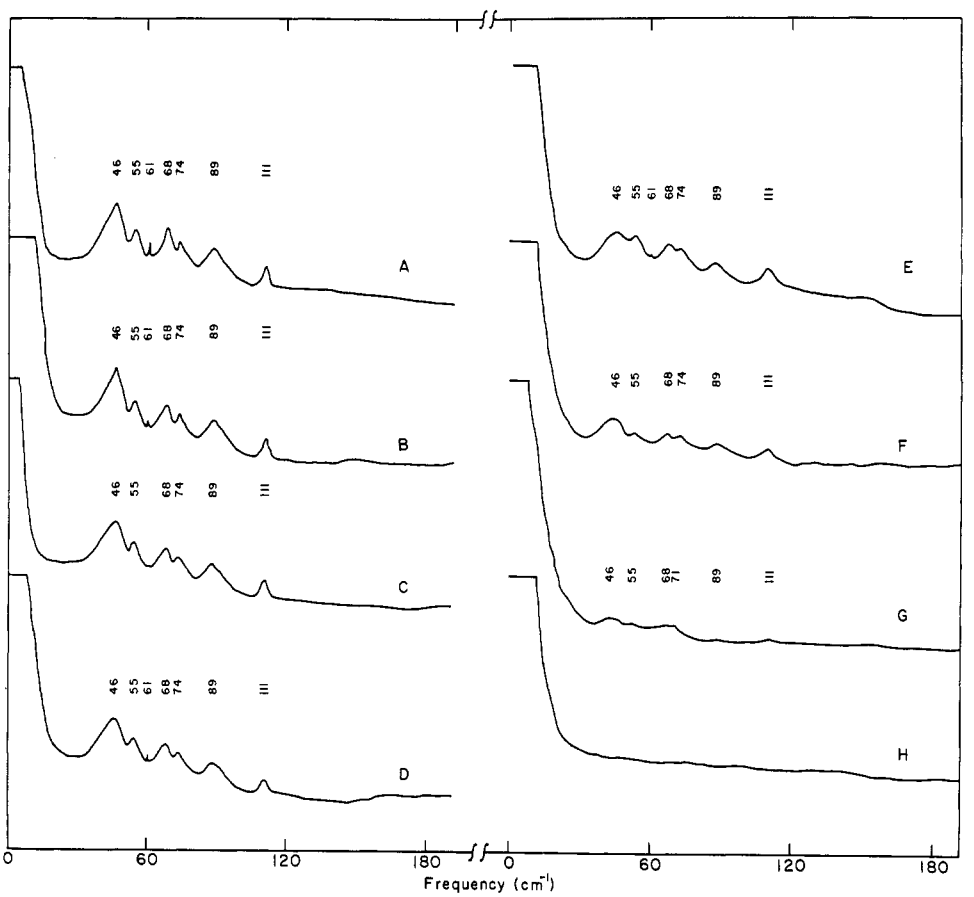

Figure 2. Raman spectrum of P(HBG:Gly) in the solid state from 0 to 200 cm^{-1}: (A) 3.1% Gly, DP_w = 510; (B) 3.0% Gly DP_w = 282; (C) 3.0% Gly, DP_w = 168; (D) 4.1% Gly, DP_w = 521; (E) 6.5% Gly, DP_w = 334; (F) 7.9% Gly, DP_w = 100; (G) 8.9% Gly, DP_w = 99; (H) 18.0% Gly, DP_w = 92. These correspond to samples a–h in the order given.

TABLE I
RAMAN FREQUENCIES OF P(HBG : Gly) IN THE SOLID STATE FROM 200 TO 1700 cm^{-1}

Frequency, cm^{-1}			Approximate group modes[a]
Sample a	Sample e	Sample h	
205 (w)[b]	205 (vw)		Helix def
260 (m)	256 (m)		Helix def
270 (w)	270 (w)	270 (w)	C^{α}—C^{β} bend
313 (w)	313 (vw)		Helix def
351 (w)	351 (w)	352 (w)	C^{α}—C^{β} bend
382 (w)	384 (w)		Helix def
521 (vs)	518 (m)		C=O in-plane bend
552 (w)	555 (w)	555 (s)	C=O in-plane bend
583 (w)	583 (w)	583 (w)	
607 (s)	607 (m)		Amide VI
650 (vw)	650 (w)	650 (m)	Amide VI
800 (w)	800 (w)	795 (w)	
867 (w)	870 (w)	865 (w)	C^{α}—C^{β} stretch + skeletal
1005 (w)	1010 (w)	1007 (w)	Glycine skeletal stretch
1040 (w)	1036 (vw)		Glycine skeletal stretch
1069 (m)	1070 (m)	1070 (m)	C^{α}—C^{β} stretch
1201 (w)	1200 (w)	1200 (w)	
1279 (m)	1280 (m)	1280 (m)	C—H_2 int rot.
1300 (m)	1300 (m)		Amide III
1334 (m)	1334 (m)	1336 (m)	C—H bend
1396 (vw)	1396 (w)	1395 (w)	Amide III
1430 (m)	1430 (m)	1430 (w)	CH_2 bend
1585 (w)	1585 (w)	1580 (w)	Amide II
1647 (m)	1647 (m)		Amide I
1695 (vw)	1695 (w)	1699 (w)	Amide I

[a] These group modes were obtained from ref 6, 10, 11, and 12 and from the spectra which we obtained for PHBG, monomeric glycine, poly(glycine I) and poly(glycine II). See Table II of ref 6 for assignments of the amide bands. [b] Qualitative estimates of relative intensity: vw = very weak, w = weak, m = medium, s = strong, vs = very strong.

(glycine II). Our spectra for the last three compounds agreed well with those reported in the literature.[10-12]

Consider first the range from 200 to 1700 cm^{-1}. Without discussing all the frequencies in Table I, we point out a few pertinent ones. In accord with the observation[7] that samples a, e, and h have partial, less, and no helix content, respectively, in aqueous solution, sample h in the solid state exhibits none of the modes corresponding to helix deformations, and a shifting and vanishing of those modes associated with amide bands and C=O motions. For example, the C=O in-plane bend occurs at 521 and 518 cm^{-1} in samples a and e and is of strong and moderate intensity, respectively, but disappears completely in sample h. However, a mode at 552 cm^{-1}, which is quite weak in samples a and e becomes strong in sample h. Similar changes occur in the amide III and I vibrations which lose their intensity at 1300 and 1647 cm^{-1}, respectively, in sample h, but seem to reappear at 1395 and 1699 cm^{-1} with enhanced intensity. The band at 583 cm^{-1} occurs in monomeric glycine, oligomers of glycine, and poly(glycine I and II). Its appearance in the copolymers with noticeable intensity, where it is independent of glycine content and helix content, is puzzling; therefore, it remains unassigned. Bands at 800 and 1200 cm^{-1} have not been observed previously, and thus are not assigned.

(10) S. Suzuki, Y. Iwashita, T. Shimanouchi, and M. Tsuboi, *Biopolymers*, **4**, 337 (1966).
(11) M. Smith, A. G. Walton, and J. L. Koenig, *ibid.*, **8**, 29 (1969).
(12) E. W. Small, B. Fanconi, and W. L. Peticolas, *J. Chem. Phys.*, **52**, 4369 (1970).

The two bands at 1005 and 1040 cm^{-1} are of interest. In going from sample a to h, the band at 1040 cm^{-1} becomes weaker and eventually disappears, while that at 1005 cm^{-1} gains in intensity. From studies[10-12] of the homopolymers (poly(glycine I and II)), it is clear that these bands should be assigned to intra-residue skeletal stretching modes of glycine.

Turning next to the range from 0 to 200 cm^{-1}, we consider the frequencies in Table II. Again, in accord with the observation[7] that the helix content decreases (reaching zero in sample h which has the highest glycine content) in going from sample a to h, all of the modes except the one at 61 cm^{-1} decrease progressively in intensity and eventually disappear in the spectrum of sample h. Our assignment of the modes at 46, 74, 89, and 111 cm^{-1} to a helical origin is supported by normal-coordinate calculations.[3-5] These calculations, which were performed for α-helical poly(L-alanine), gave frequencies within a few wave numbers of the above observed frequencies. However, we observe two additional bands at 55 and 68 cm^{-1}; we feel that our assignment of these bands to some helical motion is justified because they show the same dependence on helix content as that exhibited by the four other bands. The band at 61 cm^{-1} is not observed in either of the homopolymers (poly(glycine I or II) or PHBG) or in monomeric glycine, and seems to depend on the glycine content and on the polymer chain length. Sample a has one of the lowest glycine contents (3.1%) and largest chain lengths (DP_w = 510), and the band is strongest in the spectrum of this compound. The intensity decreases in sample b (same glycine content but smaller chain length), and the band disappears in sample c (same glycine content, but still smaller chain length). The intensity at 61 cm^{-1} reappears in sample d, with a higher glycine content (4.1%) and higher chain length (DP_w = 521), and decreases again in sample e (6% glycine, DP_w = 334). The band is absent from samples f, g, and h, which have high glycine contents and short chain lengths. A characteristic feature of this band is its unusual sharpness compared to the other bands in the same spectral region; this sharpness is a clue to its origin.

Discussion

The spectra of Figures 1 and 2 may be considered in terms of the effect of glycine content and chain length on the helix content, and of the behavior of an isolated glycyl residue among those of hydroxybutylglutamine in helical and coil sequences, respectively.

(1) The 200–1700-cm^{-1} Region. The spectra of samples a, e, and h in Figure 1 are representative of those for samples with extreme (3.1 and 18.0%) and intermediate (6.5%) glycine contents. As indicated above, the disappearance of the bands at 205, 260, 313, and 382 cm^{-1} in sample h, which is randomly coiled, is consistent with the assignment of these modes as helix deformations. This assignment is further supported by the similarity of these modes to the bands observed in the study of poly(L-alanine) by Koenig and Sutton[13] and in the calculation by Fanconi, *et al.*[5] The C=O in-plane bend at 521 cm^{-1} is very strong in sample a, and is accompanied by a weak band at 552 cm^{-1}. We attribute the former to a C=O group in a helix conformation and the latter to one in a random-coil conformation. In sample e, with less helix content, the helix band (at 518 cm^{-1}) is weaker. In sample h, the helix band is absent, but the coil band at 555 cm^{-1} is strong. The amide VI band, which occurs at 607 cm^{-1} for the helix and at 650 cm^{-1} for the coil, exhibits this same intensity variation in going from sample a to h. Similarly, the amide III

(13) J. L. Koenig and P. L. Sutton, *Biopolymers*, **8**, 167 (1969).

TABLE II
RAMAN FREQUENCIES OF P(HBG:Gly) IN THE SOLID STATE FROM 0 TO 200 cm^{-1}

Frequency, cm^{-1} [a]								Approximate group modes
a	b	c	d	e	f	g	h	
46 (m)	46 (m)	46 (m)	46 (m)	46 (w)	46 (w)	46 (vw)		Helical[b]
55 (w)	55 (w)	55 (w)	55 (w)	55 (w)	55 (vw)	55 (vw)		Helical
61 (w)	61 (vw)		61 (vw)	61 (vw)				Not obsd in homopolymers
68 (w)	68 (w)	68 (w)	68 (w)	68 (w)	68 (vw)	68 (vw)		Helical
74 (w)	74 (w)	74 (w)	74 (w)	74 (w)	74 (vw)	71 (vw)		Helical
89 (w)	89 (w)	89 (w)	89 (w)	89 (w)	89 (vw)	89 (vw)		Helical
111 (w)	111 (w)	111 (w)	111 (w)	111 (w)	111 (vw)	111 (vw)		Helical

[a] The letters a–h refer to the sample designation. [b] Helical refers to some motion of the helical structure of the polymer (*e.g.*, deformation of the helix).

band (1300 cm^{-1} for helix and 1395 cm^{-1} for coil) and the amide I band (1647 cm^{-1} for helix and 1695 cm^{-1} for coil) exhibit this same behavior. All of these observations support the conclusions[7] from optical rotatory dispersion measurements that the helix content of these polymers decreases in going from sample a to h.

The bands at 1005 and 1040 cm^{-1} are of specific interest. These are skeletal stretching modes of glycine, and probably reflect the state of the glycyl residues. In sample a, in which some of the glycyl residues are in the helical and some in the coil positions of PHBG, both bands are observed, with that at 1040 cm^{-1} a bit more intense. In sample e, with 6.5% glycine and lower helix content, the band at 1010 cm^{-1} is of higher intensity and that at 1036 cm^{-1} of lower intensity. When the polymer is completely in the random-coil conformation (sample h), the band at 1040 cm^{-1} is not detectable, while that at 1007 cm^{-1} is now of larger intensity. First of all, it appears that the bands at 1005 and 1040 cm^{-1} pertain to glycyl residues in coil and helical sections, respectively; the difference in the nature of the helix (α helix in the P(HBG:Gly) copolymers and polyglycine helices in forms I and II, respectively, in the glycine homopolymer) causes some variation in the frequency of the 1040-cm^{-1} band. Secondly, as discussed elsewhere,[14,15] an occasional glycyl (*i.e.*, helix breaking) residue was predicted to accommodate to the conformational states of its neighbors (but affecting the statistical weight of a group of residues in a given sequence), being able to participate in both the helical and coil regions; *i.e.*, glycyl residues are *not* sites of preferential melting (to coil) but adapt to both the helix and coil regions according to the dictates of the conformational states of their neighbors (in the sense of the one-dimensional Ising model). The spectra of Figure 1 provide experimental evidence to verify this prediction, *viz.*, that an occasional glycyl residue can appear in both the helix and coil regions. Most importantly, the laser Raman technique has enabled us to detect both conformational states of glycyl residues in a given copolymer.

(2) The 0–200-cm^{-1} Region. As indicated in Table II, the spectra of Figure 2 contain low-frequency bands which arise from deformation motions in the helical regions of the polymers, and hence provide some insight as to the conformational states of the copolymers and of their constituent residues. The bands at 46, 55, 68, 74, 89, and 111 cm^{-1} all disappear progressively as the helix content (determined by optical rotatory dispersion measurements[7] on these polymers) decreases.

Itoh and Shimanouchi,[3] Miyazawa, *et al.*,[4] and Fanconi, *et al.*,[5] have carried out normal-coordinate analyses of poly(L-alanine) and obtained similar low-frequency normal modes. They suggested that these low-frequency modes should have great potential in enabling one to interpret the structural features of polypeptides and proteins. However, heretofore, this low-frequency region was unobservable because of the lack of available techniques which would permit spectra to be obtained at such low frequencies. The introduction of the iodine cell has opened this region of the Raman spectrum to observation, and the spectra of Figure 2 along with studies by Peticolas, *et al.*,[16] provide a verification of the predictions.[3–5] Thus, through our observation of bands at 46, 74, 89, and 111 cm^{-1} and our demonstration of their sensitivity to helix content, we have corroborated experimentally the calculations of several authors[3–5] who predicted these bands and suggested that they should be highly dependent on helix content.

The band in this region at 61 cm^{-1} is of sufficient importance to warrant a more detailed consideration of its origin.

(3) The 61-cm^{-1} Band. The band at 61 cm^{-1} is of considerable interest. First of all, it is not an emission line of the argon-ion laser or a grating ghost. While there is an emission line of the argon laser at 5162.2 Å,[17] it would have been eliminated by the interference filter (transmitting at 5145 Å) which was used in our spectrometer. However, spectra were obtained both with and without this filter, and the emission line was observed at 69 cm^{-1} along with the 61-cm^{-1} Raman band (without any increase in the intensity of the latter) when the filter was removed. This clearly indicates that the 61-cm^{-1} band is not an emission line. Further, since the 61-cm^{-1} band could be obtained (with no difference in intensity) both with and without the use of the iodine cell, it cannot be a grating ghost. A grating ghost is really (Rayleigh scattered) light of the frequency of the laser (but appearing at another position in the spectrometer because of grating imperfections), and thus would have been eliminated (or, at least, partially reduced in intensity) by the iodine cell which absorbs light of the laser frequency. Therefore, we assume that the 61-cm^{-1} band is truly a Raman one. Not only is it absent from the spectra of monomeric glycine and of the homopolymers (poly(glycine I or II) or PHBG), but its dependence on glycine content and polymer chain length seems to indicate that the polymer must be highly helical in order for this band to be observed. It seems reasonable to assume that the band arises from some new motion present in the (helical) *copolymer*.

(14) D. Poland and H. A. Scheraga, *Biopolymers*, 7, 887 (1969).
(15) P. H. Von Dreele, D. Poland, and H. A. Scheraga, *Macromolecules*, **4**, 396 (1971).
(16) W. L. Peticolas, G. W. Hilber, J. L. Lippert, A. Peterlin, and H. Olf, *Appl. Phys. Lett.*, **18**, 87 (1971).
(17) W. F. Meggers and C. J. Humphreys, *J. Res. Nat. Bur. Stand., Sect. A*, **13**, 293 (1934).

A comparison of curves A, B, and C of Figure 2, all corresponding to the same glycine content (3%) but decreasing chain length, shows that the 61-cm^{-1} band seems to disappear at low chain length. Since the helix content decreases[7] somewhat, but not extensively, in going from sample a to c, this decrease in helix content cannot be the primary cause of the decrease in intensity of the 61 cm^{-1}. We believe that the main reason for this decrease is that glycyl residues tend to be at the ends of chains.[7] In the preparation of these water-soluble polymers,[7] selective cleavage takes place at glycyl residues, thereby leading to a high probability that the end residues are glycines (especially in the shorter chains). Thus, such end residues would not lie in the helical portions of the chain and could not take part in the helical motion which is postulated to be the origin of this band (even though the other helical frequencies from nonglycine interior residues do appear in curve C); presumably, only glycyl residues in the *interior* of helical regions contribute to the 61-cm^{-1} band.

As mentioned above, the sharpness of the 61-cm^{-1} band is a clue to its origin. Such sharpness in a spectral band could arise for two reasons, *viz.*, (a) if the band originates from a specific type of interaction (*e.g.*, an interaction between a *single* glycyl residue and its neighboring HBG residues in a helical section) or (b) if it originates from a long-lived energy state (in essence, a vibrational state of glycine (in the helical section) having a frequency higher than the frequencies of the HBG residues in the perfect PHBG homopolymer).

Considering first possibility a, we recall that the Zimm–Bragg parameter[18] s is much less than unity for glycine in water,[7] *i.e.*, glycyl residues must be classified as helix breakers. According to theory,[14,15] a single glycyl residue can be incorporated into a helical section, but would lower the statistical weight of the helical section. Two consecutive glycyl residues in the helical section of a copolymer would lower the statistical weight even more (see especially Figure 10 of ref 7). In fact, empirical observations on the behavior of glycyl (and other helix breaking) residues in proteins[19,20] indicate that two consecutive helix-breaking residues will cause a break in the helix. If there is a specific interaction (*i.e.*, a discrete interaction energy) between a glycyl and an HBG residue in an α helix, then the 61-cm^{-1} band would be expected to be quite sharp. However, if two or more consecutive glycyl residues appear within the PHBG helix, then the interactions are not so discrete, since the border HBG residue interacts (with different strengths) with the several glycyl residues in the run of glycines; these multiple interactions would cause a variation in the force constant of the glycine–HBG interaction and hence increase the width of the 61-cm^{-1} band beyond the observed 1.2-cm^{-1} band width. If this explanation is correct, then the sharpness of the 61-cm^{-1} band implies that, in the samples where this band is observed, glycyl residues occur discretely in helical sections and not in runs of more than one. This conclusion agrees with the prediction[19,20] that the helical section cannot contain more than one consecutive helix breaker.

Turning to possibility b, consider first the vibrational modes of a helical polymer.[3-5,21-23] In a homopolymer the frequencies of these modes depend on the relative motions of the monomers in the polymer and can vary between two extremes, *viz.*, that in which there is completely in-phase motion of the monomeric units, resulting in a long-wavelength standing wave, and that in which there is completely out-of-phase movement of the monomeric residues (resulting in a wavelength as short as the rise per helical turn). These extreme, and intermediate, cases, *i.e.*, the variation or dispersion of frequency with relative motion of the monomeric units in a helical homopolymer, have been treated by several authors.[3-5,21-23] The relative motion of the monomeric units is represented by the wave vector (usually given the symbol k), which is inversely proportional to the wavelength of the standing wave; thus, k approaches zero for long standing waves.

Of course, for absorption to occur in the spectral region corresponding to the vibrational modes of a helical polymer, the incident radiation must have a wavelength corresponding to that of the standing wave of the given vibrational mode. Since the wavelength of the incident visible radiation is long, we can observe only those vibrational frequencies corresponding to in-phase motion of the monomeric units[24] (*i.e.*, $k \sim 0$).

Now, suppose we incorporate an impurity (guest) amino acid residue into a formerly pure helical homopoly(amino acid) (host). If the guest residue has a lighter mass than those of the host (as is the case here with glycine compared to hydroxybutylglutamine), then the frequencies of the guest residue would be expected to be higher than those of the host because of the lighter mass of the glycyl residues, since both the host and guest residues have the same –NH–CH–CO– backbone. In other words, in the extreme of a large difference in masses, we would have two sets of frequencies, one set corresponding to the HBG residues in the homopolymer PHBG helix and the other corresponding to the glycyl residue incorporated into the copolymer helix. Since the glycyl residue exhibits a different, slightly higher frequency for its motions than those of the host HBG residues, the motions of the glycyl residue have no way to couple (*i.e.*, have no way to interact energetically) with the motions of the HBG residues. Thus, they have a very long lifetime and, through the uncertainty principle, must therefore have an extremely discrete energy. This would result in a very sharp band similar to the one we have observed at 61 cm^{-1}.

Such phenomena have often been observed in solids doped with impurities, and the reader is referred to reviews dealing with the experimental[25] and theoretical[26] aspects of these phenomena and to an original article on lattice defects[27] for a more detailed discussion. In spectroscopic terminology, these modes are referred to as localized modes since they are a result of localized impurities.

It is our feeling that the drastic sharpness of the band at 61 cm^{-1} tends to support this latter explanation of its origin. Although the first possibility cannot be completely discounted, we feel that the specificity of the interaction that would be required to yield the discrete force constant needed in possibility a is unlikely to be encountered under the conditions of normal thermal fluctuations at room temperature.

(18) B. H. Zimm and J. K. Bragg, *J. Chem. Phys.*, **31**, 526 (1959).
(19) D. Kotelchuck and H. A. Scheraga, *Proc. Nat. Acad. Sci. U. S.*, **61**, 1163 (1968); **62**, 14 (1969).
(20) D. Kotelchuck, M. Dygert, and H. A. Scheraga, *ibid.*, **63**, 615 (1969).
(21) W. L. Peticolas and M. W. Dowley, *Nature (London)*, **212**, 400 (1966).
(22) B. Fanconi, B. Tomlinson, L. A. Nafie, W. Small, and W. L. Peticolas, *J. Chem. Phys.*, **51**, 3993 (1969).
(23) B. Fanconi and W. L. Peticolas, *Biopolymers*, **10**, 2223 (1971).
(24) We have restricted the above discussion to helical polymers, since the vibrations of randomly coiled polymers would be expected to be relatively insensitive to the relative motions of the monomeric units, because the latter are essentially uncorrelated in the random coil.
(25) M. V. Klein in "Physics of Color Centers," M. V. Klein, Ed., Academic Press, New York, N. Y., 1968, p 429.
(26) C. Kittel, "Introduction to Solid State Physics," 3rd ed, Wiley New York, N. Y., 1967, p 156.
(27) E. W. Montroll and R. B. Potts, *Phys. Rev.*, **100**, 525 (1955).

As far as we are aware, the 61-cm^{-1} band is the first observed localized mode in a helical biopolymer. Aside from its spectroscopic interest, this band should be of importance in the study of localized modes in biopolymers, since such modes provide a detailed probe of internal interactions in such systems. For example, the sharpness of the 61-cm^{-1} band was attributed to weak (but *not* zero) coupling between glycyl and HBG residues. In fact, it is presumably this weak coupling which forces the glycyl residue to adopt the helical conformation of its HBG neighbors.[14,15] Finally, if this band indeed arises from a localized mode, then it appears that only one glycine is incorporated into any particular region of the PHBG helix. Since the observation of the localized mode rests on the mass difference[26] between helical glycine and its helical neighbors, this mass difference would be reduced drastically if more than one consecutive glycine were incorporated into any region of the PHBG helix, and this should preclude the observation of the local mode.

In order to provide a quantitative basis for these ideas, detailed calculations similar to those on solid systems will have to be undertaken. Such calculations may indicate the need for additional experiments (*e.g.*, the effect of substituting deuteriums for the hydrogens of glycine) to verify our assignment and to try to determine the vibrational origin of the local mode.

Summary

The laser Raman technique has yielded an understanding of the conformational behavior of a guest residue (glycine) among the host residues (hydroxybutylglutamine) in the random copolymer P(HBG:Gly). The conformationally sensitive nature of very low-frequency helical motions has been observed experimentally for the first time, and the spectra have provided direct experimental evidence for the incorporation of glycine residues into the PHBG helix. In addition, we may have observed the first localized mode in a polymeric system. If the explanation is corroborated, detailed studies of such localized modes should provide much information on the structure, coupling, and energetics of biopolymers.[27]

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(27) NOTE ADDED IN PROOF. After submitting this paper, we obtained spectra of sample a (3.1% Gly, DP_w = 510) using the 4880-Å line of the argon-ion laser. The 61-cm^{-1} band appeared exactly as it does in curve A of Figure 2. This provides additional proof that the 61-cm^{-1} band is not an emission line argon ion laser or a grating ghost.

On the Possible Existence of α-Helical Structures of Regular-Sequence D,L Copolymers of Amino Acids. Conformational Energy Calculations[1]

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ABSTRACT: Conformational energy calculations have been carried out to consider the question of whether alternating regular-sequence copolymers of D- and L-amino acids can adopt an α-helical conformation. Several other regular structures were also considered, in addition to the α helix. The copolymers treated were poly(D,L-alanine), poly(D,L-valine), poly(D,L-phenylalanine), and poly(D,L-α-aminoheptanoic acid) (a model for poly(D,L-lysine)). The energies of the right- and left-handed α-helical forms of poly(D,L-alanine) are essentially the same, comparable to that of the right-handed α-helical form of poly(L-alanine), and several kilocalories per residue lower than that of some other structures (the LD ribbon and the LD helix) previously proposed for the D,L copolymer. For valine, the energy of the α-helical form of the D,L copolymer is even lower than that of the all-L homopolymer because of favorable side chain–side chain interactions, and again several kilocalories per residue lower than that of the LD helix. For poly(D,L-phenylalanine) and poly(D,L-α-aminoheptanoic acid), α-helical structures were found with energies comparable to that of the right-handed α helix of the corresponding all-L homopolymers. In conclusion, no steric hindrance to α-helix formation was found for any of these D,L copolymers. Of course, the side-chain conformations of the low-energy α-helical copolymers are different from those in the corresponding all-L homopolymers.

Considerable attention has been paid to the question of whether regular-sequence D,L copolymers of amino acids can exist in the α-helical conformation;[3] presumably, steric hindrance between the side chains of D and L residues disrupts the helix. For example, it has been stated[4] that helix formation should be absent or minimal for these copolymers in any solvent. The experimental evidence to support this view appears to be inconclusive. Often, the experiments are carried out with low molecular weight material, for which the helix content would be expected to be low; also, the solvent may differ from one experiment to another, thus making comparison of results difficult. For low molecular weight random poly(D,L-alanine) in water, the conformation appears to be a random coil according to hydrogen-exchange[5,6] studies, but partially (30–35%) helical from ultraviolet hypochromism

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