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# Determination of Rotational Mobilities of Backbone and Side-Chain Carbons of Poly( $\gamma$ -benzyl L-glutamate) in the Helical and Random-Coil States from Measurements of Carbon-13 Relaxation Times and Nuclear Overhauser Enhancements<sup>†</sup>

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ABSTRACT: We have determined the <sup>13</sup>C spin-lattice relaxation times  $(T_1)$  of the various types of carbons in helical and random-coil poly( $\gamma$ -benzyl L-glutamate) ((BzlGlu)<sub>n</sub>) samples of molecular weight 7,000, 17,000, and 46,000 in  $CDCl_3$ -CF<sub>3</sub>-COOH mixtures, by means of proton-decoupled naturalabundance <sup>13</sup>C partially relaxed Fourier transform nuclear magnetic resonance (nmr) spectra at 14.2 kG. We have also measured the nuclear Overhauser enhancement (NOE) of the various carbons by means of integrated intensities in decoupled and undecoupled <sup>13</sup>C spectra. The  $T_1$  values of  $C^{\alpha}$  change by less than a factor of 2 when going from helical to random-coil  $(BzlGlu)_n$ . However, the NOE changes from about 1.1 (10%) intensity enhancement) to more than 2 (100% intensity enhancement). The very low NOE for the  $\alpha$ -carbons of helical  $(BzlGlu)_n$  is evidence for a rotational correlation time too slow to satisfy the extreme narrowing condition. The combination of  $T_1$  and NOE values yields effective rotational correlation times ( $\tau_{eff}$ ) for C<sup> $\alpha$ </sup> of 24–32 nsec for the helix and only about 0.8 nsec for the random coil. These results suggest that

**P**roton nuclear magnetic resonance (nmr) has been used extensively for studying the helix-coil transition of homopolymeric polypeptides (Bovey, 1972; Bradbury *et al.*, 1973). Recently, <sup>13</sup>C nmr has also been used for this purpose (Paolillo *et al.*, 1972; Boccalon *et al.*, 1972). The usual approach has been to observe chemical shift variations between the helical and random-coil species. However, theoretical calculations of differences in chemical shifts between helical and

 $\tau_{\rm eff}$  of C<sup> $\alpha$ </sup> of the helical species is dominated by overall rotation with little or no contribution from segmental motion. When going to the random-coil species,  $\tau_{eff}$  of  $C^{\alpha}$  decreases by a factor of about 30 as a result of the onset of rapid segmental motion. The  $\alpha$ -carbon  $T_1$  values of helix and coil differ only slightly because of their particular positions on different sides of the minimum in the functional relation between the spin-lattice relaxation time and the correlation time. In both the helical and random-coil molecules, there is a progressive increase in  $T_1$  of the protonated carbons of the side chains when going away from the polymer backbone, as a result of fast internal rotations. In the helical molecules, there is a large increase in NOE when going from  $C^{\alpha}$  to  $C^{\beta}$ , because  $\tau_{eff}$ acquires contributions from internal motions with rates that satisfy the extreme narrowing condition. In contrast, in random-coil (BzlGlu)\_n  $\tau_{\rm eff}$  of  $C^{\alpha}$  is already in the extreme narrowing limit, and changes in NOE along the side chains are small.

random-coil environments would be extremely difficult. Thus, the observed chemical shifts must first be *empirically* assigned to the helical and random-coil environments on the basis of other measurements.

We show here that measurements of <sup>13</sup>C spin-lattice relaxation times ( $T_1$ ), spin-spin relaxation times ( $T_2$ ), and nuclear Overhauser enhancements (NOE) of  $\alpha$ -carbons provide a probe for studying transitions from rigid to flexible forms of a polymer without a priori knowledge about conformations. The <sup>13</sup>C relaxation and NOE measurements should distinguish the relatively immobile  $C^{\alpha}$ -H<sup> $\alpha$ </sup> groups of a helical polypeptide from the mobile  $C^{\alpha}$ -H<sup> $\alpha$ </sup> groups of a random-coil polymer undergoing fast segmental motion. We present <sup>13</sup>C  $T_1$ ,  $T_2$ , and NOE measurements for the  $\alpha$ -carbons of poly( $\gamma$ -benzyl L-glutamate) ((BzlGlu)<sub>n</sub> Figure 1) of various molecular

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FIGURE 1: Structure of poly( $\gamma$ -benzyl L-glutamate).

weights. In addition, we have measured  $T_1$ ,  $T_2$ , and NOE values of various side-chain carbons. These results should be useful in analyzing the more complex <sup>13</sup>C spectra of proteins (Allerhand *et al.*, 1970, 1971a, 1973; Glushko *et al.*, 1972; Moon and Richards, 1972).

### **Experimental Section**

Samples of  $(BzlGlu)_n$  were purchased from the Research Products Division of Miles Laboratories, Kankakee, Ill., and had the following reported molecular weights: 6,970 (code 71-110A, lot GL90), 17,300 (code 71-110A, lot GL101), and ~46,000 (code 71-110B, lot GL103) as determined by ultracentrifugation (mol wt 6970 and 17,000) and viscosity (mol wt 46,000). Trifluoroacetic acid was obtained from Matheson, Coleman & Bell, Norwood, Ohio, and deuteriochloroform (99.8% <sup>2</sup>H) was purchased from Mallinkrodt Chemical Works, St. Louis, Mo.

The nmr spectrometer has been described (Allerhand *et al.*, 1971b, 1972, 1973). All spectra were recorded on samples with natural-abundance <sup>13</sup>C content, at 15.18 MHz (14.2 kG) in spinning samples tubes of 20-mm o.d. (Allerhand *et al.*, 1972). All spectra were obtained at  $40 \pm 2^{\circ}$ . For studies of the helical species, samples were 0.8 M  $\gamma$ -benzyl glutamate residue, in a deuteriochloroform-trifluoroacetic acid solvent mixture (97:3, v/v). For studies of the random-coil form, samples were 0.6 M in residue, in a deuteriochloroform-trifluoroacetic acid mixture (71:29, v/v).

Carbon-13 spin-lattice relaxation times were measured by means of partially relaxed Fourier transform (PRFT) spectra (Vold et al., 1968), as described previously (Allerhand et al., 1971b), with an estimated accuracy of  $\pm 10\%$  for narrow resonances and about  $\pm 15\%$  for broad ones. Spin-spin relaxation times were determined from measured line widths. corrected for instrumental broadening. The estimated accuracy is  $\pm 25\%$  for C<sup> $\beta$ </sup> and C<sup> $\gamma$ </sup> in helical (BzlGlu)<sub>n</sub> of mol wt 46,000 (where non-Lorentzian lines overlap) and for  $C^{\alpha}$  in helical  $(BzlGlu)_n$  of mol wt 17,000 and 46,000 (where the line shape is asymmetric). All other line widths were measured with an estimated accuracy of  $\pm 15\%$ . Nuclear Overhauser enhancements were determined as follows. Undecoupled and fully proton-decoupled spectra were acquired using recycle times equal to or greater than five times the longest pertinent  $T_1$ . Integrated intensities were obtained digitally. Coupled spectra were recorded by offsetting the proton-decoupling frequency 50-kHz off-resonance and switching off the noise modulation (Schaefer and Natusch, 1972). In spectra where poor base lines could cause inaccuracies in measurements of integrals, base-line corrections and integrations were performed in parts. Peaks used for calculation of coupled singlecarbon intensities are given in the footnotes to Table II. The estimated errors are  $\pm 10-20\%$ .



FIGURE 2: Log-log plot of  $NT_1$  and  $NT_2$  vs.  $\tau_R$ , at 14.2 kG, for a <sup>13</sup>C nucleus relaxing by a dipolar interaction with N protons 1.09 Å away (typical C-H bond length), in the case of isotropic rotational reorientation, and under conditions of complete proton decoupling.

#### Theoretical Considerations

It has been shown (Allerhand et al., 1971b) that because of the very short C-H bond length, the <sup>13</sup>C relaxation of protonated carbons in large molecules is overwhelmingly dominated by dipole-dipole interactions with directly bonded hydrogens. This fact greatly simplifies the interpretation of <sup>13</sup>C relaxation times of protonated carbons in large molecules, because one does not have to be concerned with contributions to the relaxation from dipolar interactions with nonbonded hydrogens, and from nondipolar relaxation mechanisms (Lyerla and Grant, 1972). Equations have been given elsewhere (Doddrell et al., 1972) that relate the measured <sup>13</sup>C  $T_1$  and  $T_2$  values, under conditions of complete proton decoupling, with the rotational correlation times of the pertinent C-H vectors. In Figure 2 we show theoretical <sup>13</sup>C NT<sub>1</sub> and  $NT_2$  values (at 14.2 kG) of a carbon with N directlyattached hydrogens, which is part of a rigid molecule rotating isotropically with a correlation time  $\tau_{\rm R}$ . From the measured <sup>13</sup>C  $T_1$  and  $T_2$  values one can, in principle, extract rotational correlation times. In practice,  $T_2$  values are difficult to measure. Moreover, even though accurate  $T_1$  values of resolved resonances can be obtained, the interpretation can be clouded by the fact that  $T_1$  is not a monotonic function of the rate of rotation.

As an application of Figure 2, if a methine carbon has a  $T_1$  of 50 msec,  $\tau_R$  is either 1.1 or 37 nsec. The corresponding  $T_2$  values are 49 and 5.9 msec, respectively. In practice, it is desirable to obtain first the two solutions for  $\tau_R$  from  $T_1$  measurements, and then to choose between the two values on the basis of the usually less accurate  $T_2$  and NOE values (see below). We follow this practice here.



FIGURE 3: Semilog plot of the NOE  $vs. \tau_R$ , at 14.2 kG, for a <sup>13</sup>C nucleus relaxing exclusively by dipolar interactions with one or more protons, in the case of isotropic rotational reorientation.



FIGURE 4: Natural-abundance <sup>13</sup>C Fourier transform nmr spectra of poly( $\gamma$ -benzyl L-glutamate) of molecular weight 7,000 in CDCl<sub>3</sub>-CF<sub>3</sub>COOH solvent mixtures at 40°, recorded at 15.18 MHz with 4096 points in the time domain and 250-ppm spectral widths. Vertical presentations of spectra and their integrals vary arbitrarily. Integrated intensities were measured digitally and normalized for variations in number of accumulations per spectrum. (A) Fully proton-decoupled spectrum of 0.8 м (in residue) (BzlGln)<sub>n</sub> in 97% CDCl<sub>3</sub>-3% CF<sub>3</sub>COOH (v/v), after 2048 accumulations with a recycle time of 15.8 sec. Assignments are those of Paolillo et al. (1972). Carbon designations are those of Figure 1. (B) Same as part A, but without proton-decoupling, after 4096 accumulations. (C) Fully proton-decoupled spectrum of 0.6 M (in residue)  $(BzlGln)_n$  in 71 % CDCl<sub>3</sub>-29 % CF<sub>3</sub>COOH (v/v) after 256 accumulations with a recycle time of 21.1 sec. (D) Same as part C, but without proton decoupling, after 2048 accumulations.

The nuclear Overhauser enhancement (NOE) may also be useful for determining rotational mobility of C-H groups. The NOE in proton-decoupled 13C spectra is the increase in integrated intensity of the <sup>13</sup>C resonance of a given carbon when going from the undecoupled spectrum to the proton-decoupled spectrum (Kuhlmann and Grant, 1968; Kuhlman et al., 1970). We define the NOE as the ratio of the integrated intensities in the decoupled and undecoupled spectra (Kuhlman et al., 1970). In Figure 3 we show theoretical NOE values as a function of  $\tau_{\rm R}$  for a carbon undergoing  ${}^{13}\text{C}{}^{-1}\text{H}$  dipolar relaxation at 14.2 kG. As in Figure 2, we assume here that the rotational motion is isotropic and that the pertinent C-H vector does not have internal motions. Unlike  $T_1$  and  $T_2$ , the NOE is independent of the number of directly attached hydrogens (Kuhlmann et al., 1970). The results of Figure 3 apply even for a nonprotonated carbon, but only if it is undergoing purely <sup>13</sup>C-<sup>1</sup>H dipolar relaxation (Lyerla and Grant, 1972). This is the case for nonprotonated carbons in many large molecules (Allerhand et al., 1971b).

Figure 3 predicts the maximum NOE of 2.988 when <sup>13</sup>C relaxation is purely dipolar *and* when the rotational motion is



FIGURE 5: Effect of molecular weight on the saturated carbon region in the proton-decoupled natural-abundance <sup>13</sup>C spectra of helical and random-coil (BzlGln)<sub>n</sub> at 40° and 14.2 kG. Solvents and concentrations are the same as in Figure 4A, C, respectively, for helical and random-coil (BzlGln)<sub>n</sub>. X indicates an impurity. Number of accumulations and recycle times for the helical systems were (molecular weight, accumulations, recycle time in sec): 7000, 2048, 1.1; 17,000, 512, 1.1; 46,000, 2048, 1.1. The corresponding values for the random-coil systems were: 7000, 256, 21; 17,000, 512, 1.6; 46,000, 512, 1.1.

sufficiently fast to satisfy the "extreme narrowing" condition (Kuhlmann *et al.*, 1970)

$$4\pi^2(\nu_{\rm H} + \nu_{\rm C})^2 \tau_{\rm R}^2 \ll 1 \tag{1}$$

Here  $\nu_{\rm H}$  and  $\nu_{\rm C}$  are the resonance frequencies, in hertz, of <sup>1</sup>H and <sup>13</sup>C, respectively. Even when the relaxation is purely dipolar, less than the full NOE is expected when eq 1 is not satisfied. In the limit of very slow rotation, defined by

$$4\pi^2 (\nu_{\rm H} + \nu_{\rm C})^2 \tau_{\rm R}^2 \gg 1 \tag{2}$$

the NOE will only be 1.153 (Kuhlmann et al., 1970).

#### Experimental Results and Discussion

In Figure 4 we show proton-decoupled and undecoupled <sup>13</sup>C spectra and the corresponding integrals for helical (in

TABLE 1: <sup>13</sup>C Spin–Lattice Relaxation Times of Helical (in CDCl<sub>3</sub> with 3% CF<sub>3</sub>COOH) and Random Coil (in CDCl<sub>3</sub> with 29% CF<sub>3</sub>COOH) Poly( $\gamma$ -benzyl L-glutamate) at 40°.

	$NT_1 \text{ (msec)}^b$						
	7000°		17,000°		46,000°		
Carbon <sup>a</sup>	Helix	Coil	Helix	Coil	Helix	Coil	
α	35.5	52	39	70	44	64	
β	65	85	71	107	72	91	
$\gamma$	108	139	127	159	109	143	
Bzl	264	419	279	480	281	397	

<sup>a</sup> Carbon designations of Figure 1. <sup>b</sup> N is the number of directly attached hydrogens. Estimated accuracy of  $T_1$  values is  $\pm 10$  to  $\pm 15\%$  (see Experimental Section). <sup>c</sup> Molecular weight.

TABLE II: Nuclear Overhauser Enhancements of Protonated Carbons of Helical and Random-Coil Poly( $\gamma$ -benzyl L-glutamate) at 40°.

	NOE <sup>b</sup>					
	70	00°	17,000°		46,000°	
Carbon <sup>a</sup>	Helix <sup>d</sup>	Coil <sup>d</sup>	Helix	Coil <sup>e</sup>	Helix <sup>d</sup>	Coil
	1.0	2.3	1.2	2.6	1.1	2.2
β	1.6	2.4	2.0	2.8	1.9	2.7
γ	2.1	2.8	2.4	3.1	2.2	3.1
Bzl	2.0	2.7	1.9	2.5	1.9	2.6
2–6	2.6	2.9	2.6		2.9	

<sup>a</sup> Carbon designations of Figure 1. <sup>b</sup> Estimated accuracy ranges from  $\pm 10$  to  $\pm 20\%$ , depending on the extent of peak overlap (see Figures 4 and 5). <sup>c</sup> Molecular weight. <sup>d</sup> Average of all measurable integrated intensities in the undecoupled spectrum was used as the single-carbon undecoupled intensity. <sup>e</sup> Average of saturated-carbon integrated intensities in the undecoupled spectrum was used as the single-carbon undecoupled intensity. <sup>f</sup> Average of integrated intensities of  $C^{\alpha}$ ,  $C^{\beta}$ , and  $C^{\gamma}$  in the undecoupled spectrum was used as the single-carbon undecoupled intensity.

CDCl<sub>3</sub> with 3% CF<sub>3</sub>COOH) and random-coil (in CDCl<sub>3</sub> with 29% CF<sub>3</sub>COOH) poly( $\gamma$ -benzyl L-glutamate) of mol wt 7000. Chloroform is a commonly used "helix-promoting" solvent for polypeptides. The addition of small amounts of an organic acid prevents aggregation (Doty et al., 1956). The presence of 3% CF<sub>3</sub>COOH is not sufficient to destroy the helical form of  $(BzlGlu)_n$  (Marlborough et al., 1965). When the concentration of CF<sub>3</sub>COOH is raised to 29%,  $(BzlGlu)_n$  exists in the random-coil form (Marlborough et al., 1965). The spectra of  $(BzlGlu)_n$  of higher molecular weight have chemical shifts nearly identical with those of Figure 4. However, the resonances of  $C^{\alpha}$ ,  $C^{\beta}$ , and  $C^{\gamma}$  of the helical molecules get broader as the molecular weight is raised (Figure 5). In contrast, the line widths in the random-coil state are practically independent of molecular weight (Figure 5). A further discussion of these effects is given below.

In Table I we show <sup>13</sup>C relaxation times of protonated carbons (excluding aromatic ones), measured by means of PRFT spectra (Vold *et al.*, 1968; Allerhand *et al.*, 1971b). In Figure 6 we show portions of a typical series of PRFT spectra.

 $\alpha$ -Carbons. The  $T_1$  values of the  $\alpha$ -carbons increase by only about 50% when going from helix to coil. The  $T_1$  curve of Figure 2 indicates that such a small change does not necessarily represent a small change in effective rotational correlation time. In fact, an examination of the  $\alpha$ -carbon NOE values (Table II) and line widths (Tables III-IV) clearly shows that the effective correlation times of  $C^{\alpha}$  in the helix and the random coil are on different sides of the  $T_1$  curve. It should be noted that Figures 2 and 3 are only approximately valid here. Because of their rod-like shape (Doty *et al.*, 1956), helical (BzlGlu)<sub>n</sub> molecules may rotate anisotropically. Moreover, the equations used here can only be approximately valid for the  $\tau_{eff}$  of the segmental motions of the random-coil backbone.

We believe that our observation of a negligible NOE for the  $\alpha$ -carbons of helical (BzlGlu)<sub>n</sub> is the first experimental verification of the theoretical prediction (Kuhlmann *et al.*,



FIGURE 6: Saturated carbon region in the proton-decoupled naturalabundance <sup>13</sup>C PRFT nmr spectra of helical (BzlGln)<sub>n</sub> of mol wt 17,000 at 40°, recorded at 14.2 kG using 512 accumulations with a recycle time of 1.1 sec. Solvent and concentration are the same as in Figure 4A. Number next to each spectrum is the interval between each 180° radiofrequency pulse and the following 90° pulse, in msec. The normal Fourier transform spectrum (NFT) shown on top is one obtained in the absence of 180° pulses.

TABLE III: Comparison of Experimental Line Widths and NOE Values of the  $\alpha$ -Carbons of Helical Poly( $\gamma$ -benzyl L-glutamate) with the Theoretical Values Consistent with Measured Spin-Lattice Relaxation times.<sup>*a*</sup>

	7000°	17,000°	46,000°
Measured $T_i$ (msec) <sup>c</sup>	35.5	39	44
Computed $\tau_{\rm R}$ (nsec) <sup>d</sup>	24 (1.8)	27 (1.6)	32 (1.3)
Calculated NOE <sup>e</sup>	1.2(2.5)	1.2 (2.6)	1.2(2.7)
Experimental NOE <sup>f</sup>	1.0	1.2	1.1
Calculated $W$ (Hz) <sup>g</sup>	38 (9.5)	42 (8.7)	48 (7.6)
Experimental $W(Hz)^{\hbar}$	28	37	48

<sup>*a*</sup> Calculated values inconsistent with experimental observations are given in parentheses. See text. <sup>*b*</sup> Molecular weight. <sup>*c*</sup> From Table I. <sup>*d*</sup> Computed from the measured  $T_1$  values using the theoretical  $T_1$  curve of Figure 2. Each  $T_1$  yields two possible  $\tau_{\rm R}$  values. <sup>*e*</sup> Computed from Figure 3 for each  $\tau_{\rm R}$ consistent with the experimental  $T_1$ . <sup>*f*</sup> From Table II. <sup>*g*</sup> Computed line width, from the theoretical  $T_2$  curve of Figure 2 ( $W = 1/\pi T_2$ ), for each  $\tau_{\rm R}$  consistent with the experimental  $T_1$ . <sup>*h*</sup> Experimental full width at half-height. Estimated accuracy is  $\pm 15\%$ .

TABLE IV: Comparison of Experimental Line Widths and NOE Values of the  $\alpha$ -Carbons of Random-Coil Poly( $\gamma$ -benzyl L-glutamate) with the Theoretical Values Consistent with Measured Spin-Lattice Relaxation Times.<sup>*a*</sup>

	7000 <sup><i>b</i></sup>	17,000	46,000 <sup><i>b</i></sup>
Measured $T_1$ (msec) <sup>c</sup>	52	70	64
Computed $\tau_{\rm R}$ (nsec) <sup>d</sup>	1.0 (39)	0.71 (53)	0.79 (49)
Calculated NOE <sup>e</sup>	2.8(1.2)	2.9(1.2)	2.9(1.2)
Experimental NOE <sup>f</sup>	2.3	2.6	2.2
Calculated $W(Hz)^g$	6.2 (56)	4.6 (75)	5.0 (69)
Experimental W (Hz)	$\lesssim 8$	<b>≲</b> 8	$\lesssim 12$

<sup>*a*</sup> Calculated values inconsistent with experimental observations are given in parentheses. See text. <sup>*b*</sup> Molecular weight. <sup>*c*</sup> From Table I. <sup>*d*</sup> Computed from the measured  $T_1$  values using the theoretical  $T_1$  curve of Figure 2. Each  $T_1$  yields two possible  $\tau_{\rm R}$  values. <sup>*e*</sup> Computed from Figure 3 for each  $\tau_{\rm R}$ consistent with the experimental  $T_1$ . <sup>*f*</sup> From Table II. <sup>*g*</sup> Computed line width, from the theoretical  $T_2$  curve of Figure 2 ( $W = 1/\pi T_2$ ), for each  $\tau_{\rm R}$  consistent with the experimental  $T_1$ .

1970) of this behavior when molecular motion is sufficiently slow to satisfy eq 2.

In Table III we show the two values of  $\tau_{\rm R}$  consistent with the  $\alpha$ -carbon  $T_1$  value of the helical (BzlGlu)<sub>n</sub> samples. Only the longer  $\tau_{\rm R}$  (24-32 nsec) predicts a NOE value (1.2) and line width (38-48 Hz) in reasonable agreement with the experimental NOE (about 1.1) and line width (about 28-48 Hz). Correlation times of 8-16 nsec have been reported for the overall rotation of some aqueous proteins of mol wt 14,000-25,000 (Irwin and Churchich, 1971; Dubin et al., 1971; Rawitch, 1972; Norris and Crespi, 1972; Haugland and Stryer, 1967; Shimshick and McConnell, 1972). If we assume that similar overall rotational correlation times apply here, our results suggest that  $\tau_{eff}$  for  $C^{\alpha}$  of helical (BzlGlu)<sub>n</sub> is determined by the overall rotational motion, with little or no contribution from segmental motion. In contrast  $\tau_{eff}$  for C<sup> $\alpha$ </sup> of random-coil  $(BzlGlu)_n$  is dominated by segmental motions that are faster than the overall reorientation (see below).

When going from the helical to the random-coil conformation, the change in  $T_1$  of the  $\alpha$ -carbons is small, while the NOE increases from about 1.1 to about 2.5 (Table II), and the linewidths decrease from about 28–48 Hz to less than 12 Hz (Figure 5 and Tables III–IV). Moreover,  $\tau_{eff}$  for the  $C^{\alpha}$ –H<sup> $\alpha$ </sup> groups of random-coil (BzlGlu)<sub>n</sub> is about 0.8 nsec (Table IV) independent of molecular weight. All these observations are only consistent with a drastic decrease in  $\tau_{eff}$ , when going from helix to random coil, as a result of the onset of fast segmental motion.

It appears that the  $\alpha$ -carbons of random-coil (BzlGlu)<sub>n</sub> do not have the maximum NOE of 2.988 (Table II), even though a  $\tau_{eff}$  of 0.8 nsec satisfies the extreme narrowing condition (eq 1). Such a result has been reported for other random-coil polymers (Schaefer and Natusch, 1972). It has been explained in terms of  ${}^{13}C{}^{-1}H$  dipolar relaxation dominated by segmental motions, the correlation times of some of which do not satisfy eq 1. However, our NOE values are not sufficiently accurate (see Experimental Section) to *unambiguously* establish that the  $\alpha$ -carbons of random-coil (BzlGlu)<sub>n</sub> indeed have significantly less than the maximum NOE of 2.988. It should be noted that measurements of  $\alpha$ -carbon  $T_1$ ,  $T_2$ , and NOE can be used, as we have done above, to detect the transition from a rigid to a flexible polymer backbone. These measurements *do not* yield information about the actual conformations.

Carbons of the Side Chain. In both the helical and randomcoil molecules, as one goes away from the polymer backbone. there is a progressive increase in  $T_1$  (Table I). In the helical molecules, there is also a large increase in NOE when going from  $C^{\alpha}$  to  $C^{\beta}$ , and additional increases when going to  $C^{\gamma}$ and beyond (Table II). This is because in the helical species,  $\tau_{\rm eff}$  of C<sup> $\alpha$ </sup> satisfies eq 2, but contributions from internal rotations shorten  $\tau_{\rm eff}$  of side-chain carbons in the direction of the extreme narrowing limit (eq 1). Peak overlaps in the spectra of helical  $(BzlGlu)_n$  limited the accuracy of our NOE values (see Experimental Section) and prevented the observation of details of the change in NOE along the side chains. Nevertheless, there is an unambiguous change from practically no NOE at  $C^{\alpha}$  to approximately the theoretical maximum at the protonated aromatic carbons. In contrast, the change in NOE along the side chains of the random-coil species is small (Table II) because in this case  $\tau_{eff}$  of  $C^{\alpha}$  is already close to the extreme narrowing limit.

The resonances get progressively narrower, and the broadening effect of increased molecular weight gets less significant, as one goes further away from the polymer backbone of the helical species (Figure 5). Note that  $C^{\beta}$ ,  $C^{\gamma}$ , and  $C^{B_{Z}1}$  are methylene carbons while  $C^{\alpha}$  is a methine carbon, and thus the line widths of  $C^{\beta}$ ,  $C^{\gamma}$ , and  $C^{B_{Z}1}$  should be halved before comparing with the line width of  $C^{\alpha}$  (Allerhand *et al.*, 1971b). The resonances of random-coil (BzlGlu)<sub>n</sub> are considerably narrower than those of the helical species. The difference becomes progressively smaller as one goes away from the backbone. The resonances of the random-coil species are not greatly broadened by increases in the molecular weight (Figure 5).

The above results can be used to reach the following conclusions. (1) For helical (BzlGlu)<sub>n</sub>, the correlation time of the  $\alpha$ -carbons satisfies eq 2, while  $\tau_{eff}$  of the aromatic carbons satisfies eq 1. (2) Internal rotations of the C–H vectors of the aliphatic side-chain carbons are sufficiently fast to contribute to  $\tau_{eff}$ , which gets progressively shorter as one goes away from the backbone in both the helical and random-coil species. (3) The effective correlation times of all carbons of random-coil (BzlGlu)<sub>n</sub> approach values that satisfy eq 1.

If we assume that overall rotation of helical  $(BzIGlu)_n$  is isotropic (with  $\tau_R$  given in Table III), then the  $T_1$  value of the  $\beta$ -carbons can be used to compute the correlation time for internal rotation ( $\tau_G$ ) about the  $C^{\alpha}-C^{\beta}$  bond. Details have been given elsewhere (Allerhand *et al.*, 1971b; Doddrell *et al.*, 1972). The resulting value of  $\tau_G$  is about 0.25 nsec, independent of molecular weight. The model for internal rotation and definition of  $\tau_G$  used here are those used by Doddrell *et al.* (1972).

Implications for <sup>13</sup>C Nmr of Proteins. The trends in  $T_1$ , the line widths, and the NOE of helical (BzlGlu)<sub>n</sub> that we have presented here are helpful in making *approximate* estimates of the corresponding values of similar carbons in native proteins. One should bear in mind, however, that there are structural differences between the system we have studied and naturally occurring residues of proteins in water. One generalization that can be made with confidence is that when recording proton-decoupled <sup>13</sup>C spectra of proteins in their native conformation, one should expect a negligible NOE for  $\alpha$ -carbons and other carbons which have a value of  $\tau_{eff}$  that

satisfies eq 2. On the other hand, side-chain carbons with several degrees of *fast* internal rotation may have a significant NOE. It is important to consider these variations in the NOE when estimating the minimum concentration of protein required to observe single-carbon resonances in natural-abundance  ${}^{13}$ C spectra (Allerhand *et al.*, 1973).

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