Amiconi, G., Antonini, E., Brunori, M., Formanek, H., and Huber, H. (1972), *Eur. J. Biochem. 31*, 52.

Antonini, E., and Brunori, M. (1971), Hemoglobin and Myoglobin in Their Reactions with Ligands, Amsterdam, North-Holland Publishing Co.

Brunori, M., Bonaventura, J., Bonaventura, C., Antonini, E., and Wyman, J. (1972), *Proc. Nat. Acad. Sci. U. S. 69*, 868.

Bucci, E., and Fronticelli, E. (1965), *J. Biol. Chem. 240*, PC551.

Bucher, T., and Kaspers, J. (1947), *Biochim. Biophys. Acta 1*, 21.

De Renzo, E. C., Ioppolo, C., Amiconi, G., Antonini, E., and Wyman, J. (1967), *J. Biol. Chem. 242*, 4850.

Eigen, M., and De Maeyer, L. (1963), *Tech. Org. Chem. 8*, 895.

Gibson, Q. H. (1956), *J. Physiol. 134*, 112.

Haldane, J., and Lorrain-Smith, J. (1895), *J. Physiol. 20*, 497.

Noble, R. W., Brunori, M., Wyman, J., and Antonini, E. (1967), *Biochemistry 6*, 1216.

Rossi-Fanelli, A., and Antonini, E. (1957), *Biochimica 22*, 335.

Warburg, O. (1949), Heavy Metal Prosthetic Groups and Enzyme Action, Oxford, Clarendon Press.

Determination of Rotational Mobilities of Backbone and Side-Chain Carbons of Poly(γ-benzyl L-glutamate) in the Helical and Random-Coil States from Measurements of Carbon-13 Relaxation Times and Nuclear Overhauser Enhancements†

Adam Allerhand* and Eric Oldfield

ABSTRACT: We have determined the ^{13}C spin–lattice relaxation times (T_1) of the various types of carbons in helical and random-coil poly(γ-benzyl L-glutamate) ((BzlGlu)$_n$) samples of molecular weight 7,000, 17,000, and 46,000 in $CDCl_3$–CF_3COOH mixtures, by means of proton-decoupled natural-abundance ^{13}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 ^{13}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 α-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 (τ_{eff}) for C$^\alpha$ of 24–32 nsec for the helix and only about 0.8 nsec for the random coil. These results suggest that τ_{eff} of C$^\alpha$ of the helical species is dominated by overall rotation with little or no contribution from segmental motion. When going to the random-coil species, τ_{eff} of C$^\alpha$ decreases by a factor of about 30 as a result of the onset of rapid segmental motion. The α-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 τ_{eff} acquires contributions from internal motions with rates that satisfy the extreme narrowing condition. In contrast, in random-coil (BzlGlu)$_n$ τ_{eff} of C$^\alpha$ is already in the extreme narrowing limit, and changes in NOE along the side chains are small.

Proton nuclear magnetic resonance (nmr) has been used extensively for studying the helix–coil transition of homopolymeric polypeptides (Bovey, 1972; Bradbury *et al.*, 1973). Recently, ^{13}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 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 ^{13}C spin–lattice relaxation times (T_1), spin–spin relaxation times (T_2), and nuclear Overhauser enhancements (NOE) of α-carbons provide a probe for studying transitions from rigid to flexible forms of a polymer without *a priori* knowledge about conformations. The ^{13}C relaxation and NOE measurements should distinguish the relatively immobile C$^\alpha$–H$^\alpha$ groups of a helical polypeptide from the mobile C$^\alpha$–H$^\alpha$ groups of a random-coil polymer undergoing fast segmental motion. We present ^{13}C T_1, T_2, and NOE measurements for the α-carbons of poly(γ-benzyl L-glutamate) ((BzlGlu)$_n$ Figure 1) of various molecular

---

† Contribution No. 2209 from the Department of Chemistry, Indiana University, Bloomington, Indiana 47401. *Received April 25, 1973.* This research was supported by the National Institutes of Health (Grant NS-10977-01), the National Science Foundation (Grant GP-17966), the donors of the Petroleum Research Fund, administered by the American Chemical Society, and by Eli Lilly and Co. One of us (E. O.) thanks the European Molecular Biology Organization and the Gilbert Foyle Trust of Great Britian for partial support.

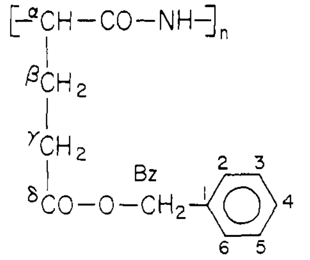

FIGURE 1: Structure of poly(γ-benzyl L-glutamate).

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 ^{13}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% ^{2}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 ^{13}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 ± 2°. For studies of the helical species, samples were 0.8 M γ-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 ±10% for narrow resonances and about ±15% for broad ones. Spin–spin relaxation times were determined from measured line widths, corrected for instrumental broadening. The estimated accuracy is ±25% for C$^\beta$ and C$^\gamma$ in helical (BzlGlu)$_n$ 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 ±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 single-carbon intensities are given in the footnotes to Table II. The estimated errors are ±10–20%.

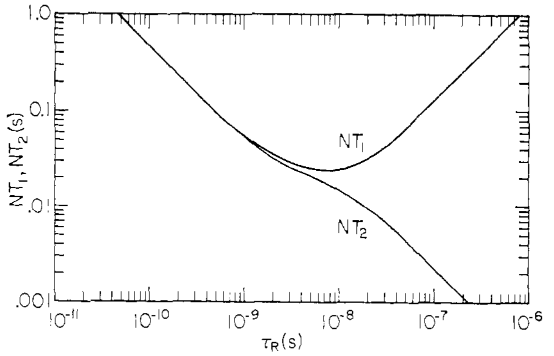

FIGURE 2: Log–log plot of NT_1 and NT_2 *vs.* τ_R, at 14.2 kG, for a ^{13}C nucleus relaxing by a dipolar interaction with N protons 1.09 Å away (typical C–H bond length), in the case of isotropic rotational reorientation, and under conditions of complete proton decoupling.

Theoretical Considerations

It has been shown (Allerhand *et al.*, 1971b) that because of the very short C–H bond length, the ^{13}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 ^{13}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 ^{13}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 ^{13}C NT_1 and NT_2 values (at 14.2 kG) of a carbon with N directly-attached hydrogens, which is part of a rigid molecule rotating isotropically with a correlation time τ_R. From the measured ^{13}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, τ_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 τ_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.

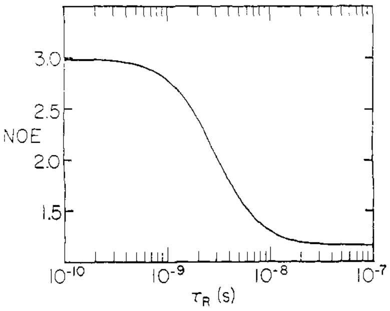

FIGURE 3: Semilog plot of the NOE *vs.* τ_R, at 14.2 kG, for a ^{13}C nucleus relaxing exclusively by dipolar interactions with one or more protons, in the case of isotropic rotational reorientation.

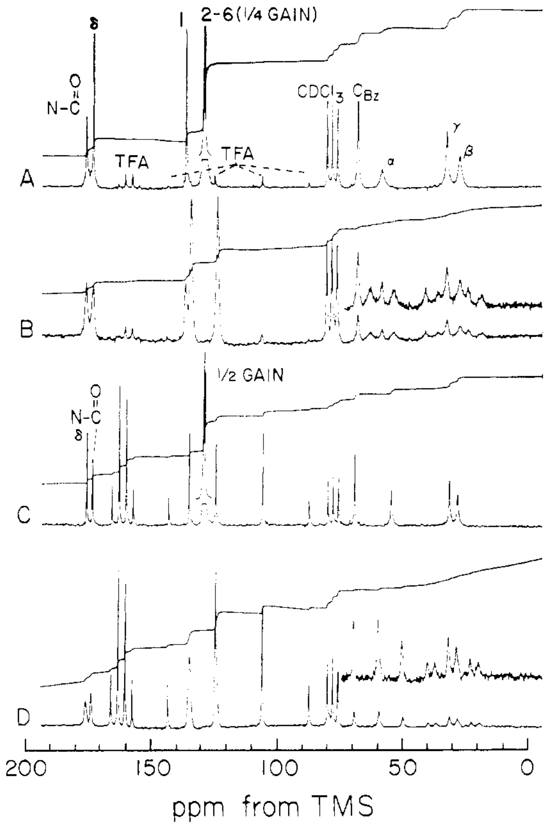

FIGURE 4: Natural-abundance ^{13}C Fourier transform nmr spectra of poly(γ-benzyl L-glutamate) of molecular weight 7,000 in $CDCl_3$–CF_3COOH solvent mixtures at 40°, recorded at 15.18 MHz with 4096 points in the time domain and 250-ppm spectral widths. Vertical presentations of spectra and their integrals 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 M (in residue) $(BzlGln)_n$ in 97% $CDCl_3$–3% CF_3COOH (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% $CDCl_3$–29% CF_3COOH (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.

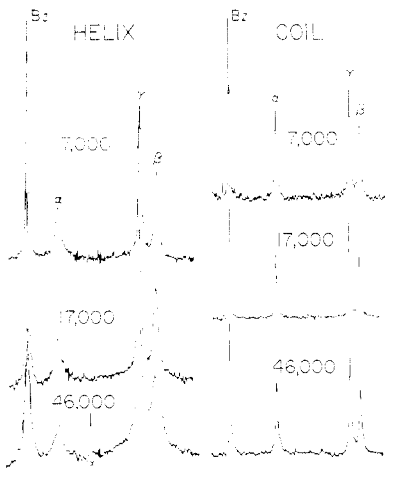

FIGURE 5: Effect of molecular weight on the saturated carbon region in the proton-decoupled natural-abundance ^{13}C spectra of helical and random-coil $(BzlGln)_n$ at 40° and 14.2 kG. Solvents and concentrations are the same as in Figure 4A, C, respectively, for helical and random-coil $(BzlGln)_n$. 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.

The nuclear Overhauser enhancement (NOE) may also be useful for determining rotational mobility of C–H groups. The NOE in proton-decoupled ^{13}C spectra is the increase in integrated intensity of the ^{13}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 τ_R for a carbon undergoing ^{13}C–^1H 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 ^{13}C–^1H 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 ^{13}C relaxation is purely dipolar *and* when the rotational motion is sufficiently fast to satisfy the "extreme narrowing" condition (Kuhlmann *et al.*, 1970)

$$4\pi^2(\nu_H + \nu_C)^2\tau_R^2 \ll 1 \qquad (1)$$

Here ν_H and ν_C are the resonance frequencies, in hertz, of ^1H and ^{13}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

$$4\pi^2(\nu_H + \nu_C)^2\tau_R^2 \gg 1 \qquad (2)$$

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

Experimental Results and Discussion

In Figure 4 we show proton-decoupled and undecoupled ^{13}C spectra and the corresponding integrals for helical (in

TABLE I: ^{13}C Spin–Lattice Relaxation Times of Helical (in $CDCl_3$ with 3% CF_3COOH) and Random Coil (in $CDCl_3$ with 29% CF_3COOH) Poly(γ-benzyl L-glutamate) at 40°.

	NT_1 (msec)[b]					
	7000[c]		17,000[c]		46,000[c]	
Carbon[a]	Helix	Coil	Helix	Coil	Helix	Coil
α	35.5	52	39	70	44	64
β	65	85	71	107	72	91
γ	108	139	127	159	109	143
Bzl	264	419	279	480	281	397

[a] Carbon designations of Figure 1. [b] N is the number of directly attached hydrogens. Estimated accuracy of T_1 values is ±10 to ±15% (see Experimental Section). [c] Molecular weight.

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

	NOE[b]					
	7000[c]		17,000[c]		46,000[c]	
Carbon[a]	Helix[d]	Coil[d]	Helix[e]	Coil[e]	Helix[d]	Coil[f]
α	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	

[a] Carbon designations of Figure 1. [b] Estimated accuracy ranges from ±10 to ±20%, depending on the extent of peak overlap (see Figures 4 and 5). [c] Molecular weight. [d] Average of all measurable integrated intensities in the undecoupled spectrum was used as the single-carbon undecoupled intensity. [e] Average of saturated-carbon integrated intensities in the undecoupled spectrum was used as the single-carbon undecoupled intensity. [f] Average of integrated intensities of C^{α}, C^{β}, and C^{γ} in the undecoupled spectrum was used as the single-carbon undecoupled intensity.

$CDCl_3$ with 3% CF_3COOH) and random-coil (in $CDCl_3$ with 29% CF_3COOH) poly(γ-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_3COOH is not sufficient to destroy the helical form of $(BzlGlu)_n$ (Marlborough *et al.*, 1965). When the concentration of CF_3COOH 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^{α}, C^{β}, and C^{γ} 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 ^{13}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.

α-Carbons. The T_1 values of the α-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 α-carbon NOE values (Table II) and line widths (Tables III–IV) clearly shows that the effective correlation times of C^{α} 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 $(BzlGlu)_n$ molecules may rotate anisotropically. Moreover, the equations used here can only be approximately valid for the τ_{eff} of the segmental motions of the random-coil backbone.

We believe that our observation of a negligible NOE for the α-carbons of helical $(BzlGlu)_n$ is the first experimental verification of the theoretical prediction (Kuhlmann *et al.*,

FIGURE 6: Saturated carbon region in the proton-decoupled natural-abundance ^{13}C PRFT nmr spectra of helical $(BzlGln)_n$ 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 α-Carbons of Helical Poly(γ-benzyl L-glutamate) with the Theoretical Values Consistent with Measured Spin–Lattice Relaxation times.[a]

	7000[b]	17,000[b]	46,000[b]
Measured T_1 (msec)[c]	35.5	39	44
Computed τ_R (nsec)[d]	24 (1.8)	27 (1.6)	32 (1.3)
Calculated NOE[e]	1.2 (2.5)	1.2 (2.6)	1.2 (2.7)
Experimental NOE[f]	1.0	1.2	1.1
Calculated W (Hz)[g]	38 (9.5)	42 (8.7)	48 (7.6)
Experimental W (Hz)[h]	28	37	48

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

TABLE IV: Comparison of Experimental Line Widths and NOE Values of the α-Carbons of Random-Coil Poly(γ-benzyl L-glutamate) with the Theoretical Values Consistent with Measured Spin–Lattice Relaxation Times.[a]

	7000[b]	17,000[b]	46,000[b]
Measured T_1 (msec)[c]	52	70	64
Computed τ_R (nsec)[d]	1.0 (39)	0.71 (53)	0.79 (49)
Calculated NOE[e]	2.8 (1.2)	2.9 (1.2)	2.9 (1.2)
Experimental NOE[f]	2.3	2.6	2.2
Calculated W (Hz)[g]	6.2 (56)	4.6 (75)	5.0 (69)
Experimental W (Hz)	$\lesssim 8$	$\lesssim 8$	$\lesssim 12$

[a] Calculated values inconsistent with experimental observations are given in parentheses. See text. [b] Molecular weight. [c] From Table I. [d] Computed from the measured T_1 values using the theoretical T_1 curve of Figure 2. Each T_1 yields two possible τ_R values. [e] Computed from Figure 3 for each τ_R consistent with the experimental T_1. [f] From Table II. [g] Computed line width, from the theoretical T_2 curve of Figure 2 ($W = 1/\pi T_2$), for each τ_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 τ_R consistent with the α-carbon T_1 value of the helical $(BzlGlu)_n$ samples. Only the longer τ_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 τ_{eff} for C^α of helical $(BzlGlu)_n$ is determined by the overall rotational motion, with little or no contribution from segmental motion. In contrast τ_{eff} for C^α 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 α-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, τ_{eff} for the C^α–H^α groups of random-coil $(BzlGlu)_n$ is about 0.8 nsec (Table IV) independent of molecular weight. All these observations are only consistent with a drastic decrease in τ_{eff}, when going from helix to random coil, as a result of the onset of fast segmental motion.

It appears that the α-carbons of random-coil $(BzlGlu)_n$ do not have the maximum NOE of 2.988 (Table II), even though a τ_{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–^1H 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 α-carbons of random-coil $(BzlGlu)_n$ indeed have significantly less than the maximum NOE of 2.988.

It should be noted that measurements of α-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 random-coil 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^α to C^β, and additional increases when going to C^γ and beyond (Table II). This is because in the helical species, τ_{eff} of C^α satisfies eq 2, but contributions from internal rotations shorten τ_{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^α 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 τ_{eff} of C^α 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^β, C^γ, and C^{Bzl} are methylene carbons while C^α is a methine carbon, and thus the line widths of C^β, C^γ, and C^{Bzl} should be halved before comparing with the line width of C^α (Allerhand *et al.*, 1971b). The resonances of random-coil $(BzlGlu)_n$ 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 $(BzlGlu)_n$, the correlation time of the α-carbons satisfies eq 2, while τ_{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 τ_{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 $(BzlGlu)_n$ approach values that satisfy eq 1.

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

Implications for ^{13}C Nmr of Proteins. The trends in T_1, the line widths, and the NOE of helical $(BzlGlu)_n$ 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 ^{13}C spectra of proteins in their native conformation, one should expect a negligible NOE for α-carbons and other carbons which have a value of τ_{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).

References

Allerhand, A., Childers, R. F., Goodman, R. A., Oldfield, E., and Ysern, X. (1972), *Amer. Lab. 4* (*11*), 19.

Allerhand, A., Childers, R. F., and Oldfield, E. (1973), *Biochemistry 12*, 1335.

Allerhand, A., Cochran, D. W., and Doddrell, D. (1970), *Proc. Nat. Acad. Sci. U. S. 67*, 1093.

Allerhand, A., Doddrell, D., Glushko, V., Cochran, D. W., Wenkert, E., Lawson, P. J., and Gurd, F. R. N. (1971a), *J. Amer. Chem. Soc. 93*, 544.

Allerhand, A., Doddrell, D., and Komoroski, R. (1971b), *J. Chem. Phys. 55*, 189.

Boccalon, G., Verdini, A. S., and Giacometti, G. (1972), *J. Amer. Chem. Soc. 94*, 3639.

Bovey, F. A. (1972), High Resolution NMR of Macromolecules, New York, N. Y., Academic Press.

Bradbury, E. M., Crane-Robinson, C., Paolillo, L., and Temussi, P. (1973), *J. Amer. Chem. Soc. 95*, 1683.

Doddrell, D., Glushko, V., and Allerhand, A. (1972), *J. Chem. Phys. 56*, 3683.

Doty, P., Bradbury, J. H., and Holtzer, A. M. (1956), *J. Amer. Chem. Soc. 78*, 947.

Dubin, S. B., Clark, N. A., and Benedek, G. B. (1971), *J. Chem. Phys. 54*, 5158.

Glushko, V., Lawson, P. J., and Gurd, F. R. N. (1972), *J. Biol. Chem. 247*, 3176.

Haugland, R. P., and Stryer, L. (1967), *in* Conformation of Biopolymers, Ramachandran, G. N., Ed., Vol. 1, New York, N. Y., Academic Press, p 321.

Irwin, R., and Churchich, J. E. (1971), *J. Biol. Chem. 246*, 5329.

Kuhlmann, K. F., and Grant, D. M. (1968), *J. Amer. Chem. Soc. 90*, 7355.

Kuhlmann, K. F., Grant, D. M., and Harris, R. K. (1970), *J. Chem. Phys. 52*, 3439.

Lyerla, J. R., and Grant, D. M. (1972), *in* Magnetic Resonance, McDowell, C. A., Ed., MPT Review of Physical Chemistry (Series One, Vol. 4), Baltimore, Md., University Park Press, p 155.

Marlborough, D. I., Orrell, K. G., and Rydon, H. N. (1965), *Chem. Commun.*, 518.

Moon, R. B., and Richards, J. H. (1972), *Proc. Nat. Acad. Sci. U. S. 69*, 2193.

Norris, J. R., and Crespi, H. L. (1972), *Chem. Phys. Lett. 16*, 542.

Paolillo, L., Tancredi, T., Temussi, P. A., Trivellone, E., Bradbury, E. M., and Crane-Robinson, C. (1972), *Chem. Commun.*, 335.

Rawitch, A. B. (1972), *Arch. Biochem. Biophys. 151*, 22.

Schaefer, J., and Natusch, D. F. S. (1972), *Macromolecules 5*, 416.

Shimshick, E. J., and McConnell, H. M. (1972), *Biochem. Biophys. Res. Commun. 46*, 321.

Vold, R. L., Waugh, J. S., Klein, M. P., and Phelps, D. E. (1968), *J. Chem. Phys. 48*, 3831.