2D NMR Analysis of the Conformational and Dynamic Properties of α-Helical Poly(γ-benzyl L-glutamate)

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Abstract: Two-dimensional nuclear Overhauser experiments (2D NOE) have been used to study the conformational and dynamic properties of a 20 amino acid α-helix of poly(γ-benzyl L-glutamate) (PBLG). The diagonal and cross-peak volumes have been measured for ten different mixing times and have been used to determine the selective spin-lattice relaxation times and the cross-relaxation rates between those protons which are in close proximity. Together with the nonselective relaxation rates, these values can be used to determine the effective correlation times. The results show that the correlation times for NH and αH are equal and the same as expected for a rigid 20 amino acid helix, while βH and γH show internal motion relative to the overall tumbling. Following determination of the effective correlation time, the proton-proton distances can be calculated from the cross-relaxation rates obtained from the initial buildup of the cross-peak volumes. The proton-proton distances determined by this approach fall within the range expected for an α-helix and provide sufficient information to define the conformation.

Determination of the physical properties of natural and synthetic polymers and biopolymers is an area of intense interest and investigation.[1,2] Interest in this area arises in part from the possible relationship between the structure and dynamics on a microscopic level and the functional properties on the macroscopic level. Proton NMR in solution is a powerful tool for such studies because the NMR parameters (chemical shift, line widths, relaxation rates, and coupling constants) depend on both the average conformation and the dynamics.[2,3] While much information is contained in a proton NMR spectrum, interpretation of the results is often complicated by the overlap of resonances and the difficulties in separating the conformational and dynamic contributions to the relaxation behavior. A possible way to avoid such complexities is to use 2D NMR, where the spectra are better resolved because they are spread into two dimensions.[4,5]

Nuclear Overhauser effects (NOE) are a powerful means for measuring the interaction between two protons which are close in space, since the strength and time dependence of the interaction depend on the sixth power of the proton separation and on the molecular tumbling time.[6,7] In 1D measurements, the cross-relaxation rates can be obtained from the initial buildup of the NOE,[8] but this approach is limited by the ability to apply long selective pulses to crowded regions of the spectra, and in larger molecules, by spin diffusion.[9] This same information can be obtained from two-dimensional NMR studies. The advantage of this approach is that the interacting spins are labeled by their precessional frequencies rather than by selective pulses.[4,5] Since 2D experiments are more time consuming, they have thus far been mostly used for setting limits on the distances between protons. This has proved sufficient to determine the structure of some small proteins,[1] but it is not suitable for problems where distance determinations with an accuracy of better than 0.5 Å are required. For these studies it is necessary to measure the peak intensities in several 2D experiments as a function of the time allowed for the spins to interact.

In these experiments we have used 2D NMR to study the conformation and dynamics of a small fragment (MW = 4100) of poly(γ-benzyl L-glutamate) (PBLG) under conditions where it assumes an α-helical conformation.[11] The PBLG has a molecular weight similar to the small proteins which have been extensively studied by 2D NMR.[1] We have measured the intensity of the diagonal and cross peaks in the 2D NOE spectrum as a function of mixing time and extracted the relaxation rates for the various interactions.[4,5,12] By monitoring both the decay of the diagonal and the rise of the cross peaks, it is possible to extract the selective spin-lattice relaxation and cross-relaxation rates.[12,13] From the ratio of the nonselective and the selective rates and the cross-relaxation rates we can determine both the correlation times[14,15] and the proton distances.[1] We consider the strengths and weaknesses of this approach and examine how the interproton distances depend on the φ and ψ angles in an α-helix and the possible implications for the determination of the conformational properties of natural and synthetic polymers.

Materials and Methods

Poly(γ-benzyl L-glutamate) (DP = 20) was obtained from Pilot Chemical Co., and deuterated chloroform and trifluoroacetic acid (TFA) were obtained from Aldrich. PBLG was dissolved in 95:5 chloroform: trifluoroacetic acid and sealed in NMR tubes to prevent solvent evaporation. Identical results were obtained on two samples which differed in concentration by a factor of 4 (6 and 22 mg/mL).

2D NOE experiments were performed on a JEOL GX-500 spectrometer at 500 MHz with use of the $(\pi/2)$-t_1-$(\pi/2)$-τ_m-$(\pi/2)$-t_2 pulse sequence.[4,5] Typically, 256 1 K spectra were acquired with a sweep width of 5 kHz in each dimension. Phase sensitive 2D NOE spectra were obtained with the procedure of States et al.,[12] and the diagonal and cross-peak volumes were obtained by either integrating the peaks in the five most intense cross sections or summing the peak heights over the cross sections. This procedure was required because of the relatively low digital resolution in the t_1 domain and the difference in line widths for some of the peaks. Control experiments with a π pulse inserted into the mixing period showed that none of the cross-peak intensities arose from modulation of the zero-quantum coherence due to scalar coupling.[12] Nonselective spin-lattice relaxation rates were obtained by inversion recovery.

Theory

Spin-Lattice Relaxation. The return to equilibrium of a proton following perturbation of the spin system depends on the strength

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of its interactions with its neighbors. Among the important factors which determine the relaxation rate are the interproton distances, the correlation times, and the populations of the other interacting spins.[14] For a two-spin system (i and s)

$$d\mathbf{m}_i/dt = -\rho_{is}\mathbf{m}_i - \sigma_{is}\mathbf{m}_s \quad (1)$$

where $\mathbf{m}_i$ and $\mathbf{m}_s$ are the populations of spins i and s (i.e., $\mathbf{m}_i = (M^i_{z,t} - M^i_{z,0})/M^i_{z,0}$, where $M^i_{z,t}$ is the longitudinal magnetization at time t and $M^i_{z,0}$ is the equilibrium magnetization), and the quantities ρ_{is} and σ_{is} are the rate constants, which depend on the strength of the through-space dipolar interactions. Equation 1 can be rewritten to show more explicitly the dependence of the relaxation rate on the spin populations and the spectral densities[18]

$$\frac{d\mathbf{m}_i}{dt} = -K\mathbf{m}_i\left\{J_0(0)\left[1 - \frac{\mathbf{m}_s}{\mathbf{m}_i}\right] + 3J_1(\omega) + 6J_2(2\omega)\left[1 + \frac{\mathbf{m}_s}{\mathbf{m}_i}\right]\right\} \quad (2)$$

where

$$K = {}^1/_{10}\gamma^4\hbar^2 r_{is}^{-6} \quad (3)$$

for two spin-$^1/_2$ nuclei. The motional information is contained in the spectral densities ($J_0(0)$, $J_1(\omega)$, and $J_2(2\omega)$) and depends on the molecular correlation times τ_c. For isotropic motion the spectral densities are

$$J_n(n\omega) = \frac{\tau_c}{1 + (n\omega)^2\tau_c^2} \quad (4)$$

The measured relaxation rates depend on the initial populations of $\mathbf{m}_i$ and $\mathbf{m}_s$, and a number of different relaxation rates can be envisioned.[14,15] The nonselective rate (R_1^{ns}), measured following equal excitation of $\mathbf{m}_i$ and $\mathbf{m}_s$ ($\mathbf{m}_s/\mathbf{m}_i = 1$), is

$$R_1^{ns} = K\{3J_1(\omega) + 12J_2(2\omega)\} \quad (5)$$

The initial rate measured following selective excitation of $\mathbf{m}_i$ ($\mathbf{m}_s/\mathbf{m}_i = 0$) is

$$R_1^s = K\{J_0(0) + 3J_1(\omega) + 6J_2(2\omega)\} \quad (6)$$

The important feature to note is that R_1^{ns} and R_1^s have a different dependence on the spectral density terms and the same dependence on the proton–proton distances. The *ratio* of these relaxation rates depends only on the correlation time.

Determination of Correlation Times. In the regime of $0.1 > \omega\tau_c > 10$ the ratio of the nonselective and selective relaxation rates changes from 1.5 to 0.01.[14,15] The dependence of the ratio on the correlation time (assuming isotropic motion) at 500 MHz is shown in Figure 1. In this study we extract the R_1^s from the 2D NOE analysis and use this rate, along with R_1^{ns} measured by inversion recovery, to calculate the correlation time for the 20 amino acid α-helix.

Analysis of 2D NOE Spectra. In the 2D experiments, the spins are labeled via their precessional frequencies during the evolution period (t_1) and are allowed to interact during the mixing time (τ_m).[4,5,10,12,13] The frequency labeling results in a difference in the z magnetization of the spins, and relaxation during the mixing time returns the spin system toward equilibrium. The differences in the z magnetization are those expected following selective excitation. Relaxation (given by eq 6) may occur either through relaxation to the lattice or through cross relaxation. During the detection period (t_2) the frequency labeled peaks are detected as diagonal peaks and the off-diagonal peaks arise from cross relaxation.

For a multispin system, the relaxation can be written in matrix form as

$$\dot{\mathbf{M}}_z = \mathbf{R}\mathbf{M}_z \quad (7)$$

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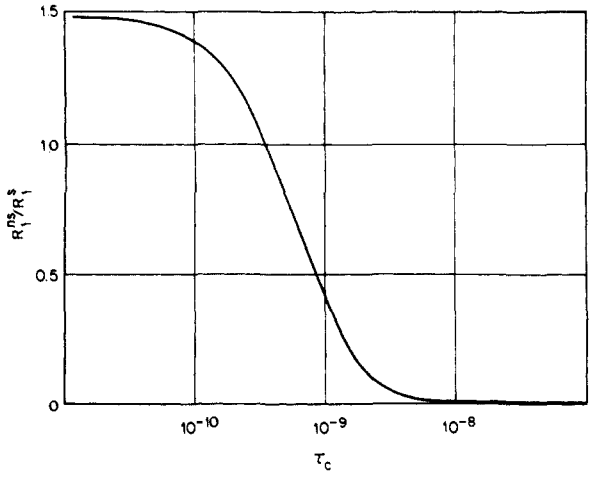

Figure 1. The dependence of the ratio of R_1^{ns}/R_1^s on τ_c at 500 MHz assuming isotropic motion.

where $\dot{\mathbf{M}}_z$ is the deviation of the z magnetization from thermal equilibrium and the relaxation matrix $\mathbf{R}$ is

$$\mathbf{R} = \begin{bmatrix} R_{ii} & R_{ij} & \cdot & \cdot \\ R_{ji} & R_{jj} & \cdot & \cdot \\ \cdot & \cdot & \cdot & \cdot \\ \cdot & \cdot & \cdot & \cdot \end{bmatrix} \quad (8)$$

The diagonal elements ($i = j$) describe the relaxation of the diagonal peaks; this rate corresponds to the selective spin–lattice relaxation from all sources and is given by eq 6. The off-diagonal terms describe the cross relaxation (R_1^c) and are given by

$$R_1^c = K\{6J_2(2\omega) - J_0(0)\} \quad (9)$$

The volumes of the peaks in the 2D NOE spectrum are related to relaxation rates by the mixing coefficients (a_{lk})

$$a_{lk} = \exp\{\mathbf{R}\tau_m\}\mathbf{M}_z \quad (10)$$

A rigorous analysis of the peak volumes at all mixing times is possible for simple spin systems but becomes increasingly complex as the number of interacting spin increases. If only short mixing times are considered,

$$\exp\{\mathbf{R}\tau_m\} = 1 - \mathbf{R}\tau_m + {}^1/_2\mathbf{R}^2\tau_m^2 + \ldots \quad (11)$$

In the limit of $\tau \to 0$ the higher order terms are small and the mixing coefficient which governs the intensity of the peaks is given by

$$a_{lk} \approx (\delta_{lk} - \mathbf{R}_{lk}\tau_m)\mathbf{M}_z \quad (12)$$

The diagonal peaks ($l = k$) are relatively intense for short mixing times and decay as the mixing time increases. The cross peaks ($l \neq k$) have no intensity at zero mixing time but increase in intensity due to cross relaxation and finally decay to equilibrium due to both cross and lattice relaxation.

Results

Proton Spectra. Figure 2 shows the 500-MHz proton spectrum of the poly(γ-benzyl L-glutamate) (PBLG) 20-mer in chloroform with 5% TFA. PBLG has been extensively studied as a model for the helix-to-coil transition in polypeptides, and the assignments for the proton resonances in both the helix and the coil form have been established.[11] The effect of TFA on the helix-to-coil transition has also been studied, and it has been determined that PBLG exists as a helix in chloroform solution and is denatured with increasing amounts of TFA. The concentration of TFA required to denature the helix depends on the helix length and temperature, and for the 20-mer at 20 °C, the denaturation takes place at 9% TFA (not shown). In the absence of TFA the molecules tend to associate into bundles of high molecular weight and are not easily studied by NMR. Under the conditions of these studies, the PBLG is helical but enough TFA has been added to prevent aggregation.[11]

Figure 2 shows the spectral features which are expected for a 20-mer helix. The line widths are greater than those observed

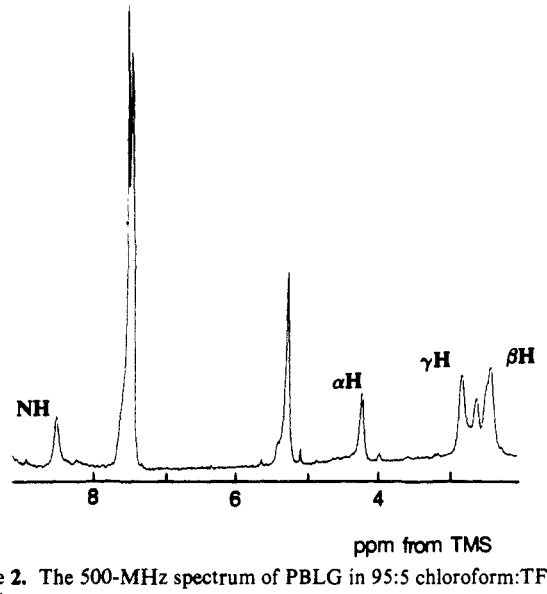

Figure 2. The 500-MHz spectrum of PBLG in 95:5 chloroform:TFA at 20 °C.

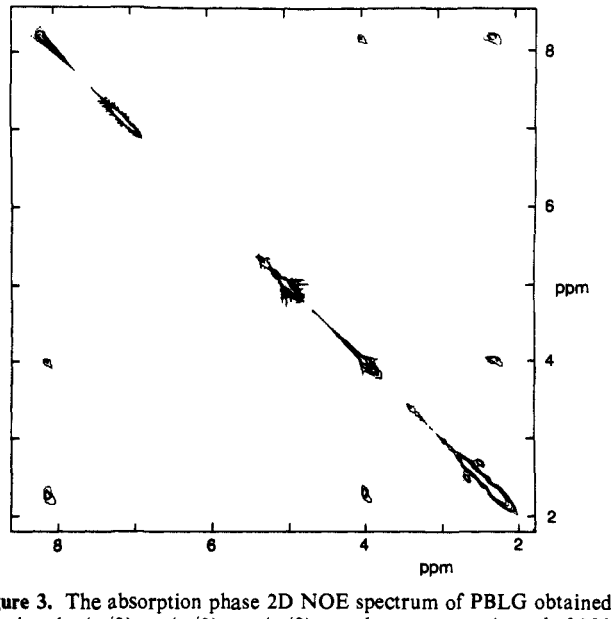

Figure 3. The absorption phase 2D NOE spectrum of PBLG obtained by using the $(\pi/2)$–t_1–$(\pi/2)$–τ_m–$(\pi/2)$–t_2 pulse sequence. A total of 256 1 K spectra were acquired with a t_1 increment of 0.2 ms to give a sweep width in both dimensions of 5 kHz. The data matrix was zero filled to 512 × 1 K and 2 Hz line broadening was applied prior to Fourier transformation. The $\pi/2$ pulse width was 9 μs, τ_m was 76 ms, and the delay between acquisitions was 5 s.

for high molecular weight PBLG[11,12] in the denatured form where rapid internal motions lead to line narrowing. Studies on proteins[19] and synthetic polypeptides[20-22] reveal that the α-carbon backbone experiences little motion relative to the overall tumbling of the helix, while the side chains show additional mobility. The degree of mobility in the side chains increases with the distance from the polymer backbone. This behavior is observed in the PBLG 20-mer; the protons furthest from the main chain (aromatic and benzyl methylene) are sharper than the NH and αH protons.

2D NOE Spectra. Figure 3 shows the phase-sensitive 2D NOE spectrum for PBLG at 21 °C obtained with a mixing time of 76 ms. Cross peaks are observed connecting several of the resonances and show which protons are in close contact. Strong cross peaks

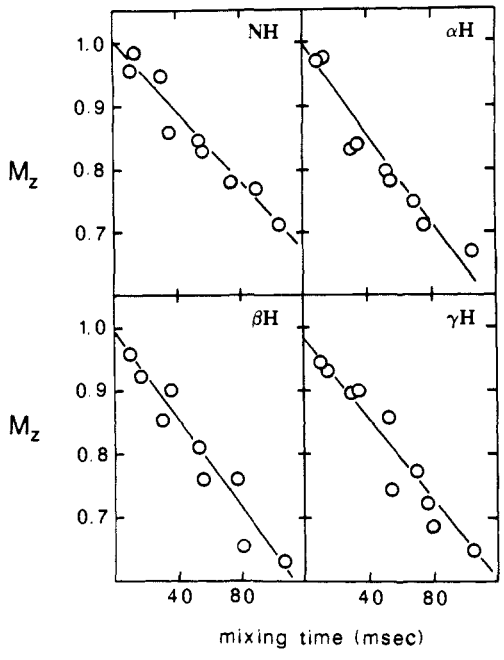

Figure 4. The decay of the diagonal peak intensity as a function of mixing time for several 2D NOE experiments. For comparison, the peak intensities have been normalized to 1 and the intensity at a given mixing time was obtained by integration of the 5 most intense cross sections of the 2D spectra, so M_z is the peak volume for the NH, αH, βH, and γH peaks.

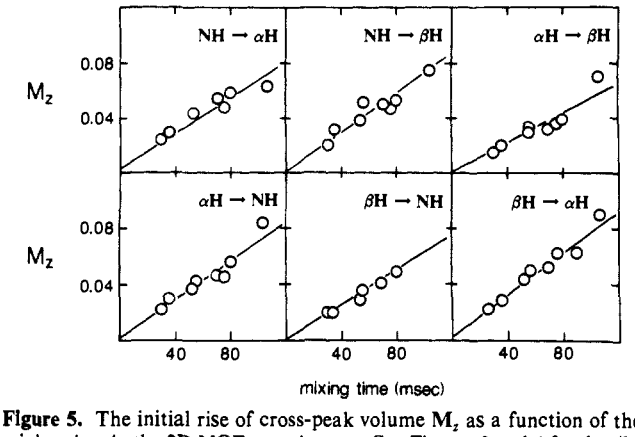

Figure 5. The initial rise of cross-peak volume M_z as a function of the mixing time in the 2D NOE experiments. See Figures 3 and 4 for details. The intensities are normalized to the intensity of the diagonal peaks detected in t_2.

are observed between the NH–αH, NH–βH, and αH–βH resonances, and (at mixing times longer than 50 ms) weaker ones are observed between the NH–γH peaks. Strong cross peaks are also observed between the βH–γH protons, but they are too close to the intense diagonal peaks to study. No cross peaks are observed for the aromatic or benzylic methylene protons. This may be due either to the faster motions of these protons or their greater distance from the main chain atoms. The diagonal and cross peaks for the NH, αH, and βH protons are of the same relative phase (i.e., positive), indicating that the correlation time of the PBLG 20-mer lies within the slow motion limit.[5]

Peak Intensities vs. Mixing Time. In order to quantitate the relaxation of the protons in PBLG, we measured the 2D NOE spectra for ten mixing times between 10 and 106 ms. The diagonal and cross-peak intensities were obtained by integration of the five largest cross sections and are normalized relative to the intensity of the diagonal peak at zero mixing time. Figure 4 shows the initial decay of the diagonal peak intensity for the NH, αH, βH, and γH resonances while Figure 5 shows the corresponding increase in the cross-peak volumes. Within the limits of our precision, the data in Figures 4 and 5 fall within the linear region of the relaxation behavior. This suggests that the assumption implicit in

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Table I. PBLG Relaxation Rates Obtained from Analysis of the Time Dependence of the 2D NOE Spectra as a Function of Mixing Time[a]

	NH	αH	βH	γH
NH	2.91	0.53	0.52	<0.1
αH	0.56	3.16	0.65	<0.1
βH	0.57	0.80	3.48	
γH	<0.1	<0.1		3.36

[a] All rates are in s^{-1}. The values along the diagonal are the selective spin–lattice relaxation rates obtained from the decay of the diagonal peak volumes as a function of mixing time. The off-diagonal values are the cross-relaxation rates obtained from the buildup of cross-peak intensity.

Table II. The Nonselective Relaxation Rate,[a] the Ratio of Selective to Nonselective Relaxation Rates, and the Correlation Times[b] for the Protons in PBLG

proton	R_1^{ns} (s^{-1})	R_1^{ns}/R_1^{s}	τ_c (ns)
NH	1.20	0.41	1.00
αH	1.20	0.38	1.02
βH	1.77	0.51	0.85
γH	2.04	0.61	0.70

[a] The nonselective spin–lattice relaxation rate was obtained by inversion recovery. [b] The correlation times were obtained from Figure 1.

eq 11 are valid, as was observed for denatured PBLG in an earlier study.[12] Initial relaxation rates were obtained from a linear least-squares fit of the peak volumes vs. τ_m and are compiled in Table I.

The Dynamic Properties of PBLG. The ratio of R_1^{ns}/R_1^{s} and the relative sign of the diagonal and cross peaks depend on the overall motion and any internal motions which might occur in PBLG. At $\omega\tau_c > 1$, the sign of the NOE changes from positive to negative. In the 2D NOE experiment this results in the diagonal and cross peaks having the same phase. Inspection of the phase-sensitive 2D NOE spectra of PBLG shows that diagonal and cross peaks have the same phase, so the effective correlation time for the NH, αH, βH, and γH protons must be greater than $1/\omega$ (0.31 ns). A more precise estimate of τ_c can be obtained by taking the ratio of R_1^{ns}/R_1^{s} as shown in Figure 1. The values for R_1^{ns}, the ratio of R_1^{ns}/R_1^{s}, and τ_c of the backbone and side chain atoms are compiled in Table II. The correlation times calculated by this approach fall within the range of 0.7 to 1.0 ns, and the correlation time decreases with increasing distance from the polymer backbone. This confirms the conclusions discussed above on the basis of the observed line widths.

The observed values of τ_c may be compared with those expected from simple hydrodynamic theory. Assuming standard values for the dimensions of an α-helix (3.6 residues/turn, 5.5 Å/turn), the calculated length and radius of the PBLG 20-mer are 30 and 10 Å. With use of the Broersma[23] formula for prolate ellipsoids, this would result in correlation times for tumbling about the long and short axis of 0.4 and 0.9 ns. Since these values are not very different from each other, the value measured by NMR relaxation would appear isotropic. These calculated values compare favorably with those calculated from the 2D NOE data. The slightly longer experimental values may reflect the solvent shell which was not included in these calculations.

Determination of Interproton Distances. The cross-relaxation rates obtained from the rise of the cross peaks and the decay of the diagonal peaks depend on both τ_c and the separation between the protons. The above analysis shows that the correlation times for the NH and αH are identical with those expected for the rigid 20-mer. Thus, R_1^c obtained from the initial increase in cross-peak volumes can be used to determine the proton distances (eq 9). By using the values of τ_c listed in Table II, the calculated interproton NH–αH, NH–βH, and αH–βH distances are compiled in Table III. These distances depend on peptide secondary structure and side chain conformation. For comparison, the distances expected for a standard α-helix are also listed in Table III.[27] All of the distances fall within the range expected for the α-helix. The proton–proton distances depend not only on the Φ and Ψ angles but also on the χ angle, for the NH–βH. The exact value of the χ angle will depend on the nature of the side chain.

Table III. The Cross-Relaxation Rates, Calculated Distances, and Expected Range of Distances for α-Helical PBLG

interaction	R_1^c (s^{-1})	r (Å)	range[a] (Å)
NH–αH	0.54	2.20	2.2–2.8
NH–βH	0.55	2.20	2.0–4.1
αH–βH	0.72	2.1	2.0–3.5

[a] The expected range of values in an α-helix.[27]

Discussion

Determination of the structural and dynamic properties of proteins, nucleic acids, and synthetic polymers is an important part of understanding their functional and macroscopic properties. The information is contained in the time dependence of the interaction between the nuclei and can be measured via the NMR relaxation behavior. The relaxation rates depend on both the internuclear distances and the molecular correlation times, and for complex molecules, with many degrees of freedom, both of these quantities may be unknown. For this reason, ^{13}C NMR has been particularly useful in the analysis of polymer dynamics.[2] Since the proton–carbon distances are known with great precision, the relaxation rates can be interpreted directly in terms of the correlation times. Analysis of the proton relaxation rates depends on both the conformational and dynamic properties, and the two contributions are not easy to resolve in relaxation measurements using nonselective pulses. The selective and cross relaxation rates must be measured so that the rate between the spin pairs can be quantitated.[14,15] These studies are limited by the difficulty in applying selective pulses to crowded regions of the spectra and can only be used when the relaxation rates are slow compared to the length of the pulse. This information can also be obtained from the analysis of the peak intensities in the 2D NOE experiments as a function of mixing time.

The ratio of the nonselective spin–lattice relaxation rate obtained from 1D measurements and the selective rate obtained from the decay of the diagonal peak intensity as a function of mixing time can be used to characterize the dynamics of the spin system because this ratio depends only on the correlation time (Figure 1). The correlation times for the NH and αH protons determined by this approach (1 ns) agree very well with the calculated value of the overall tumbling time of the 20-mer α-helix (0.9 ns). Thus, these protons do not experience any motion relative to the overall motions of the helix. This conclusion is based on the assumption that the tumbling in solution is isotropic, a reasonable assumption in view of the fact that a 20 amino acid helix is not much longer than it is wide. For a longer α-helix the tumbling would be anisotropic and would depend on the correlation times for tumbling about the long and short axes of the helix.[19–21] The correlation times for the side chain protons (βH and γH) are shorter than for the main chain protons and the ratio of R_1^{ns}/R_1^{s} increases with increasing distance from the peptide backbone. We attribute this additional motion to the relatively free rotation about the bonds in the side chain. This behavior has been analyzed in detail for several systems.[19–21]

Our 2D NMR analysis of the dynamic properties compares favorably with the reported ^{13}C NMR data on different sized α-helical polyglutamates and small globular proteins.[19–21] These studies have consistently shown that the backbone carbons are relatively rigid and show the same correlation time as expected

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for overall tumbling of the molecule. In larger proteins, it has been suggested that the backbone undergoes some librational motion (±10°) on the nanosecond time scale.[19] Since the frequency of this rather low amplitude motion is as slow as the overall tumbling, it will not make a significant contribution to the relaxation of the 20-mer. The ^{13}C NMR relaxation of α-helical polylysine and poly(L-glutamate) (400 residues)[20,21] has been interpreted in terms of a rigid backbone and side chain motion which increases with increasing distance from the backbone.

Once the correlation times have been determined, the initial cross-relaxation rates determined from the buildup of the cross-peak intensities can be interpreted in terms of the interproton distances. The data compiled in Table III show the close correspondence between the values expected for an α-helix and the observed values.[27] The values measured for PBLG are also consistent with the X-ray fiber diffraction data[24] which indicated that in the solid state PBLG forms a canonical α-helix with 5.6 residues per turn and a rise per residue of 1.5 Å.

The approach taken in these studies can be applied to arbitrarily complex molecules, including small proteins, nucleic acids, and synthetic polymers. The strength of this approach is that the correlation times and proton–proton distances can be determined independently from the 2D NOE data. The limitations are mostly ones of resolution and signal-to-noise. The peaks need to be resolved on the diagonal, and the signal-to-noise must be sufficient to quantitate the peak intensities at short mixing times. In other 2D NOE studies, the correlation times have been determined from the buildup of cross-peak intensity between protons which are a fixed distance apart. Since this occurs rather infrequently, it is difficult to determine if all of the protons are characterized by this same correlation time.

It is instructive to consider the possible sources of error in the NMR measured distances and the possible problems which might complicate the analysis of the conformational and dynamic properties of repeating sequence polymers. Central to this problem is the accurate determination of the correlation time and the interproton distances through the relaxation rates R_1^s and R_1^c from the 2D NOE spectra and R_1^{ns} from the 1D spectra. Since R_1^s and R_1^c were obtained from ten different 2D NOE experiments, it is not feasible to repeat the experiments a number of times to estimate the experimental uncertainties. From consideration of the scatter in the data plotted in Figures 4 and 5, we estimate that the rates are within 10–15% of the true values. In a study of the conformation of a small peptide in solution, Bruch et al.[25] found identical cross-relaxation rates by both 1D and 2D methods, and other studies have found good agreement between the cross-relaxation rates measured by 2D NMR and other techniques.[26] On the other hand, R_1^{ns} can be measured with great precision. The ratio of R_1^{ns}/R_1^s is sensitive to τ_c within the range of 10^{-10}–10^{-8} s. The τ_c values for PBLG lie on the steep part of the curve and so are relatively insensitive to experimental errors in the determination of the correlation time. Therefore, we esimate that τ_c is accurate to ±20%. This ratio procedure is less useful for molecules which lie outside this range. For τ_c less than 10^{-10} the ratio is insensitive and the R_1^{ns} cannot be accurately measured for molecules which tumble slower that 10^{-8}, where the relaxation is dominated by spin diffusion.[9] The cross-relaxation rate depends linearly on τ_c but on the sixth power of the proton–proton distances. Therefore, errors in the measurement of τ_c will have only a minor effect on the calculated distances. This analysis also assumes that the initial rates measured as a function of mixing time fall within the initial linear part of the buildup curves.[12] The relaxation is exponential in nature, and the number of interacting spins and the selective relaxation rate determine how large the initial linear portion of the buildup curve is.[5] The plots in Figures 4 and 5 appear to be linear up to 100 ms mixing times.

Determination of proton–proton distances in repeating sequence polymers is a particularly difficult problem because the protons on the neighboring units have the same chemical shift position, and it is difficult to distinguish intra- from interresidue interactions. Protons with the same chemical shift (like spins[17]) are of course equally excited by the frequency labeling of the 2D NOE experiment and cannot cross relax with each other. This may lead to an underestimation of R_1^s and τ_c. As discussed above, the distances calculated for other interactions are not particularly sensitive to small errors in the calculation of τ_c. The separation of the intra- and interresidue interactions is more difficult to determine, and the distances in Table III have to be considered a lower limit on the average proton separation. For example, we have assumed that the NH–αH interaction is due only to the intraresidue interaction. The interaction between the NH protons and of the αH on the neighboring residue would give rise to a cross peak at the same frequency, and the measured R_1^c could be interpreted in terms of 2.3 Å intraresidue interactions and a 2.7 Å interresidue one. To some extent, these problems have been minimized in this study because the correlation time is 1 ns. At 500 MHz, this is not very far into the spin diffusion limit and is insensitive to interactions greater than 2.8 Å away; the R_1^c for a 2.8 Å interaction is only 0.1 s^{-1}, which is less than the experimental accuracy of our measurements.

In summary, we have shown how 2D NMR can be used to probe both the solution conformation and dynamics of a synthetic polypeptide helix. Analysis of the rise and fall of the peaks in the 2D NOE spectrum as a function of mixing time yields a number of different relaxation rates which can be interpreted in terms of a correlation time and a set of proton–proton distances. This approach can be used to study the properties of more complex molecules and is not limited by the ability to apply selective pulses to crowded regions of the spectrum. The conformation of PBLG in solution is found to closely resemble that of the canonical α-helix.

Registry No. Poly(γ-benzyl L-glutamate) (homopolymer), 25014-27-1; poly(γ-benzyl L-glutamate) (SRU), 25038-53-3.