Characterization of ^{15}N Chemical Shift and $^{1}H-^{15}N$ Dipolar Coupling Interactions in a Peptide Bond of Uniaxially Oriented and Polycrystalline Samples by One-Dimensional Dipolar Chemical Shift Solid-State NMR Spectroscopy

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Abstract: The magnitudes and orientations of the principal elements of the ^{15}N chemical shift and $^{1}H-^{15}N$ dipolar coupling interaction tensors pertaining to the glycine residue in ^{15}N-acetyl glycine (NAG) and [^{15}N-Gly]collagen were determined by the analysis of one-dimensional dipolar chemical shift powder patterns. A one-dimensional $^{1}H-^{15}N$ dipolar ^{15}N chemical shift spectrum was obtained on a [^{15}N-Gly]collagen fiber sample with the fiber axis oriented parallel to the external magnetic field. The dipolar chemical shift spectrum enabled the orientation of the peptide plane to be determined relative to the direction of the applied magnetic field or the triple-helix axis of the collagen fiber. The magnitudes of the principal elements of the tensors and their orientations in the molecular frame for these two sites are quite different. The magnitudes of the chemical shift tensors are 42.3, 67, and 223.4 ppm for [^{15}N-Gly]collagen and 37, 82.8, and 220.4 ppm for NAG. The angle (β_N) between the least shielded ^{15}N chemical shift tensor element, σ_{33N}, and the N−H bond is 24.5° for [^{15}N-Gly]collagen and 25.5° (or 154.5°) for NAG. The angle (α_N) between the most shielded ^{15}N chemical shift tensor element, σ_{11N}, and the projection of the N−H bond on the $\sigma_{11N}-\sigma_{22N}$ plane is 145° for [^{15}N-Gly]-collagen and 25° (or 155°, 205°, or 335°) for NAG. Because of the identical dipolar chemical shift powder patterns for four different α_N values (35°, 145°, 215°, and 325°) the correct value of the α_N angle was determined as 145° using the dipolar chemical shift spectrum of the oriented [^{15}N-Gly]collagen sample.

Introduction

Over the years, solid-state NMR spectroscopy has developed into a powerful analytical tool, not only in the sense of molecular structure elucidation but also as a means of determining the magnitude and sign of many nuclear and internuclear interactions. The magnitudes and orientations of nuclear spin interaction tensors provide very powerful information for characterizing rapid, large-amplitude motions in rigid solids and also for interpreting the relaxation rates measured through solution NMR spectroscopy.[1−10] Variations in molecular structure, conformation, and intra- and intermolecular hydrogen bonding are some of the properties that are responsible for the observed variations in chemical shift tensors and dipolar couplings. We have recently developed a method to characterize these tensors in powder samples using one-dimensional dipolar chemical shift spectroscopy.[11] Previously, we demonstrated this approach on *N*-acetyl D,L-valine powder sample to characterize the $^{1}H-^{15}N$ dipolar and ^{15}N chemical shift tensors of a peptide bond.[11] In this paper, we describe its application to compare the spin interaction tensors of a glycine residue in a model peptide (*N*-acetyl glycine) and in collagen (Figure 1). We also illustrate the application of the one-dimensional method to study the conformation of uniaxially oriented systems. Oriented [^{15}N-Gly]collagen fibers were used to determine the conformation of the peptide plane relative to the direction of the external magnetic field.

Much valuable information about the spin interaction tensors has been obtained accurately using studies on single-crystal samples.[12−17] However, because of the difficulties in obtaining large, high-quality single crystals of biological molecules, such

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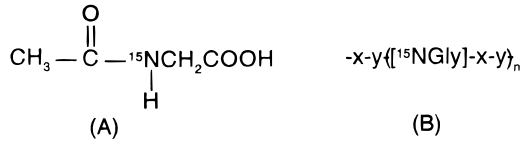

(A) (B)

Figure 1. (A) Chemical structure of *N*-acetyl glycine (NAG) showing the ^{15}N site. (B) Amino acid sequence of type I collagen showing the ^{15}N site. Often the residues x and y in collagen are found to be Pro and hydroxy-Pro.

studies are limited to selected examples. An alternative approach to the single-crystal method is the use of a powder sample of a molecule in which there is a single nucleus dipolar-coupled with the nucleus of interest (for example, ^{15}N or ^{13}C).[18−38] In the recent past, solid-state NMR experiments that correlate chemical shift and dipolar interactions in one, two, or three dimensions have been used to characterize a number of tensors in polycrystalline samples.[18,19,24,35,36,39−41] The tensors from multiple sites can be characterized using the multidimensional experiments.[42,43] However, these measurements are time-consuming, and they require a large amount of the sample. Often, it is difficult to obtain a large amount of the most interesting biological system. Further, the stability of these samples is sensitive to the high rf power needed for time-consuming multidimensional experiments. On the other hand, such systems specifically labeled with ^{15}N or ^{13}C can be studied using the one-dimensional dipolar chemical shift method.[11]

Investigation of the structure and dynamics of a protein has often been carried out using the spin interaction tensors determined from model systems since it has been difficult to characterize the tensors of a relevant site in the protein. Solid-state NMR studies have been reported wherein the tensors vary not only with the lattice environment but also with the secondary structure of the protein. In this paper, we report the variations in the ^{15}N chemical shift and the $^1H-^{15}N$ dipolar coupling tensors of *N*-acetyl-^{15}N-glycine and [^{15}N-Gly]collagen samples.

Experimental Section

[^{15}N-Gly]collagen was prepared from rats that were subcutaneously injected with the commercially purchased (Cambridge Isotope Laboratories) L-glycine amino acid labeled with ^{15}N isotope. Details of the sample preparation are given elsewhere.[44] Gas chromatography and mass spectrometry analysis showed that the level of [^{15}N]Gly incorporation was 7%, and no scrambling of the isotope label to other amino acid residues was detected. Oriented fibrous collagen sample was prepared by pulling the collagen through a 2-mm outer diameter glass capillary with a fine copper wire after folding the fibers at their center over the wire. The capillary tubes were sealed at both the ends with Parafilm and Teflon tapes to prevent the sample from drying.

All of the experiments were performed on a Chemagnetics Infinity 400-MHz solid-state NMR spectrometer operating at a field of 9.4 T with resonance frequencies of 400.14 and 40.54 MHz for ^1H and ^{15}N, respectively. The powder samples in sealed glass tubes were placed within a 5-mm solenoid coil double-tuned to the ^1H and ^{15}N resonance frequencies in a home-built probe for all the experiments performed at room temperature. Low-temperature and magic angle spinning (MAS) experiments were performed using a Chemagnetics double resonance MAS probe. Collagen powder spectra were obtained at 233 K (-40 ± 1 °C) in order to freeze out the inherent large amplitude backbone motion present in the collagen fibrils.[45,46]

Typical 90°-pulse lengths were 3.0 to 3.7 μs and 3.5 to 4.8 μs for ^1H and ^{15}N, respectively. All spectra were obtained using the cross-polarization sequence with a contact time of 2 ms. After the cross-polarization, the ^{15}N magnetization was refocused by a 180° pulse to overcome the difficulties due to the receiver dead time, and the second-half of the spin−echo signal was acquired. For experiments under MAS conditions, a two-pulse phase modulated (TPPM) decoupling sequence[47] was used to decouple protons during the ^{15}N signal acquisition. The recycle delay was 3 to 5 s which was adequate to allow full relaxation for collagen samples. For the ^{15}NAG sample, the recycle delay of 10 s was used due to the long relaxation time. For the one-dimensional dipolar chemical shift experiment,[11] the frequency of proton decoupling was shifted by an offset in order to establish an effective field at the magic angle.[48−50] This magic angle rf irradiation of protons suppresses the $^1H-^1H$ homonuclear dipolar interactions and thus the resultant one-

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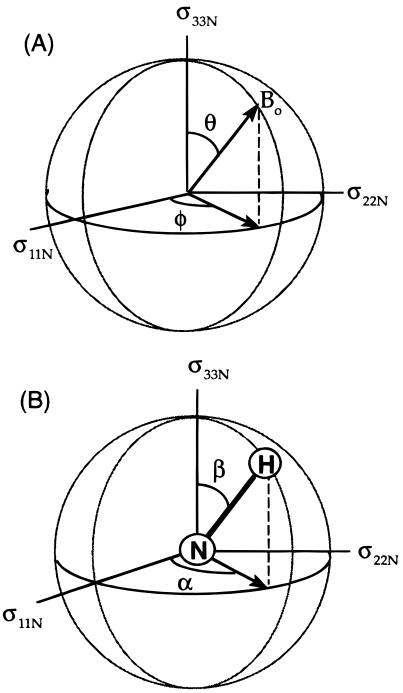

Figure 2. Orientations of the principal axes of the ^{15}N chemical shift tensor relative to the direction of the external magnetic field, B_0 (A) and to the N−H bond (B). θ is the angle between σ_{33N} and the magnetic field axis (z or B_0 axis). ϕ is the angle between the σ_{11N} and the projection of B_0 on the $\sigma_{11N}-\sigma_{22N}$ plane. α_N is the angle between σ_{11N} and the projection of the N−H bond on the $\sigma_{11N}-\sigma_{22N}$ plane. β_N is the angle between σ_{33N} and the N−H bond.

dimensional spectrum consists of the ^{15}N chemical shift and ^{1}H−^{15}N dipolar interactions. All the ^{15}N spectra are referenced relative to NH_3 (liquid, 25 °C) by setting the observed ^{15}N signal of the saturated aqueous NH_4Cl solution to 27.3 ppm.[51]

Calculations of the one-dimensional chemical shift and dipolar chemical shift powder patterns and the oriented collagen spectra were carried out using a FORTRAN-77 program in a Macintosh computer. The magnitudes of the principal values (σ_{11N}, σ_{22N}, and σ_{33N}) of the ^{15}N chemical shift tensor were obtained from the frequencies of the discontinuities of a powder pattern. Experimentally measured principal values were used as starting parameters to simulate the chemical shift pattern. The resultant simulated powder patterns were varied on the basis of the direct comparison with the respective experimental spectra in order to obtain the best-fitting results. The principal elements of the chemical shift tensors are represented according to the convention $|\sigma_{33N}| > |\sigma_{22N}| > |\sigma_{11N}|$. Coordinates of the chemical shift and dipolar tensors are defined in Figure 2. Experiments under MAS displayed only a single sharp line for each sample under study, ruling out the presence of multiple crystal forms. The isotropic ^{15}N chemical shift frequencies obtained from MAS experiments were identical to the values determined from the average of the principal values of the chemical shift tensors. This confirms the accuracy of the magnitudes of the chemical shift tensors.

The dipolar chemical shift powder pattern contains contributions from both the ^{15}N chemical shift anisotropy as well as the scaled dipolar coupling, therefore it is the sum frequency components from two sources[11]

$$\delta_S = \sigma_{11N}\cos^2\phi\sin^2\theta + \sigma_{22N}\sin^2\phi\sin^2\theta + \sigma_{33N}\cos^2\theta \quad (1)$$

$$\omega_{IS} = \frac{0.58}{2} D_{IS}[1 - 3\{\sin\theta\sin\beta_N\cos(\phi - \alpha_N) + \cos\theta\cos\beta_N)\}^2] \quad (2)$$

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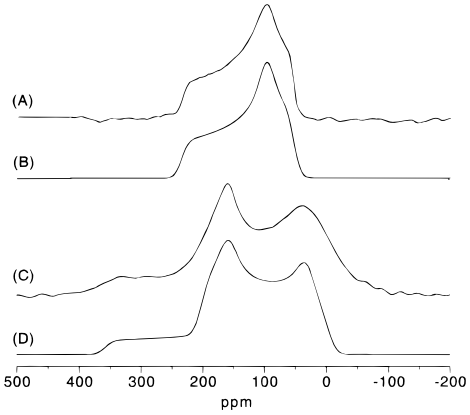

Figure 3. One-dimensional NMR spectra of a polycrystalline sample of *N*-acetyl-^{15}N−D-glycine. Experimental (A) and simulated (B) ^{15}N chemical shift spectra. Experimental (C) and simulated (D) ^{1}H−^{15}N dipolar ^{15}N chemical shift spectra. Spectra (A) and (C) are the results of 16 and 512 scans, respectively.

where δ_S is the chemical shift, ω_{IS} is the dipolar coupling between I and S nuclei and is given as $\mu_0\hbar\gamma_I\gamma_S/4\pi r_{IS}^3$. Dipolar chemical shift powder patterns were simulated using the principal values of the ^{15}N chemical shift tensor and varying the angles α_N and β_N and the ^{1}H−^{15}N dipolar coupling. The best-fitting simulated spectra were obtained by comparing the ratios of the intensities of the shoulders and their frequency separations with those of the experimental powder pattern spectra.

Results

The experimental ^{15}N chemical shift spectrum of the ^{15}*N*-acetyl glycine powder sample presented in Figure 3A was obtained using the conventional cross-polarization sequence at room temperature. The anisotropic chemical shielding tensor values were determined from the best-fitting calculated spectrum shown in Figure 3B and are listed in the Table 1. The principal elements of the ^{15}N chemical shift tensor are $\sigma_{11N} = 37 \pm 2$ ppm, $\sigma_{22N} = 82.8 \pm 2$ ppm, and $\sigma_{33N} = 220.4 \pm 2$ ppm. Since the isotropic chemical shift value (σ_{iso}) should be equal to the average of the principal values, σ_{iso} was confirmed by the ^{15}N resonance frequency value of the CPMAS spectrum. The dipolar chemical shift experimental spectrum of the polycrystalline ^{15}NAG sample is shown in Figure 3C. The effect of the ^{1}H−^{15}N dipole−dipole interaction on the ^{15}N chemical shift powder pattern was calculated as described in our recent publication.[11] In the numerical evaluation of the spectra of randomly oriented samples, θ and ϕ were averaged over the range 0° to 180° and 0° to 360°, respectively. The angles α_N and β_N are unique values for a given sample as defined in Figure 2. To illustrate the effect of these two angles on the dipolar chemical shift spectrum of the ^{15}NAG powder sample, spectra were calculated for various values of α_N and β_N angles. As shown in Figures 4 and 5, both the angles affect the powder line shapes. A change of 1° in the angle β_N can be estimated from the changes in the powder pattern spectra. Even though the powder pattern is less sensitive to the α_N angle, a change of 5° induces notable variations in the frequency separation of the shoulders in the spectra (Figure 5). By adjusting these angles, the optimum α_N and β_N values for the ^{15}NAG sample were uniquely determined from the best-fitting calculated dipolar chemical shift spectrum (Figure 3D). The experimental data in Figure 3C are best simulated by the calculated powder pattern spectra in Figure 3D with $\beta_N = (25.5°$ or $154.5°) \pm 1°$, $\alpha_N = 25°$, 155°, 205°, or 335°) ± 5° and D_{NH} (the ^{1}H−^{15}N dipole coupling constant) = 9.8 ± 0.5 kHz. The N−H bond length

Table 1. Summary of the ^{15}N Chemical Shift Tensors for the Glycine Residue Obtained from Various Peptides[a]

sample	σ_{11N}	σ_{22N}	σ_{33N}	σ_{iso}	α_N	β_N	$R/10^{+4}$	$\Delta\sigma$	ref
^{15}NAG	37.0	82.8	220.4	113.4	(25 or 155 or 205 or 335) ± 5°	(25.5 or 154.5) ± 1°	1.08	160.50	*b*
[^{15}N-Gly] collagen powder	42.3	67.0	223.4	110.9	(35 or 145 or 215 or 325) ± 10°	(24.5 or 155.5) ± 1°	1.25	168.75	*b*
[^{15}N-Gly] collagen oriented	42.3	67.0	223.4	110.9	145 ± 10°	24.5 ± 2°	1.25	168.75	*b*
[^{2}H-^{15}N-Gly] collagen	45.6	67.6	216.8	110.0	0°	23°	1.12	160.20	45
[^{15}N-Gly] maganine	45.0	75.4	218.0	112.8	(30 or 150 or 210 or 330) ± 10°	22 or 158 ± 1°	1.08	157.80	*c*
Boc-(Gly)$_2$15NGly-OBz	55.1	62.1	223.0	113.4		22 ± 1°	1.20	164.40	25
Boc-(Gly)$_2$15NGly-OBz	36.4	83.4	220.4	113.4	0 ± 10°	24 ± 1°	1.08	160.50	25
AcGly[d]AlaNH$_2$	44.6	85.1	229.4	119.7		17.6 ± 2°	1.15	164.55	22
AcGly[d]TyrNH$_2$	52.1	77.1	209.3	112.8		19.6 ± 2°	0.91	144.70	22
Gly^{15}NGly	46.8	79.7	220.8	115.8			1.07	157.55	53
GlyGly·HCl	57.3	59.8	210.0	109.0		18.6 ± 2°	1.02	151.45	22
AcGlyGlyNH$_2$	40.7	64.2	210.0	105.0		17.6 ± 2°	1.09	157.55	22
GlyGly·HCl·H$_2$O (powder)	58.5	64.1	209.5	110.7		25 ± 5°	0.98	148.20	24, 52
GlyGly·HCl·H$_2$O (crystal)	60.3	70.9	215.9	115.7		21.3°	1.00	150.30	17
[Gly[d]]$_n$ (β-sheet)	45.7	61.4	205.7	104.3			1.02	152.15	32, 38
[Gly[d]]$_n$(3_1-helix)	49.7	62.8	214.7	109.1			1.11	158.45	32, 38
[Gly[d],Ala]$_n$(α−helix)	44.7	57.6	212.7	105.0			1.15	161.55	32, 38
[Gly[d],Ala]$_n$(β-sheet)	39.7	66.0	206.7	104.1			1.03	153.85	32, 38
[Gly[d],Leu]$_n$(α-helix)	45.7	61.7	210.7	106.0			1.09	157.00	32, 38
[Gly[d],Leu]$_n$(β-sheet)	40.7	66.2	206.7	104.5			1.02	153.25	32, 38
[Gly[d],Val]$_n$(β-sheet)	39.7	74.6	203.7	106.0			0.92	146.55	32, 38
[Gly[d],Ile]$_n$(β-sheet)	35.7	68.3	209.7	104.6			1.07	157.70	32, 38
[Gly[d],Lys(Z)]$_n$(α-helix)	40.7	69.2	208.7	106.2			1.02	153.75	32, 38
[Gly[d],Glu(OBzl)]$_n$(α-helix)	47.7	61.2	210.7	106.5			1.08	156.25	32, 38
[Gly,Sar]$_n$	38.7	65.8	204.7	103.1			1.01	152.45	32, 38

[a] The range of the variation of σ_{11N}, σ_{22N} and σ_{33N} values are 25, 27 and 26 ppm, respectively. All of the σ_{iso} values are the average of the principal values of the ^{15}N chemical shift tensor $R = (\sigma_{33N} - \sigma_{iso})^2/(1 + \eta^2/3)$ with $\eta = (\sigma_{22N} - \sigma_{11N})/(\sigma_{33N} - \sigma_{iso})$; $\Delta\sigma = \sigma_{33N} - 0.5(\sigma_{22N} + \sigma_{11N})$. [b] Present work. [c] Manuscript submitted for publication. [d] Glycine residue labeled with ^{15}N.

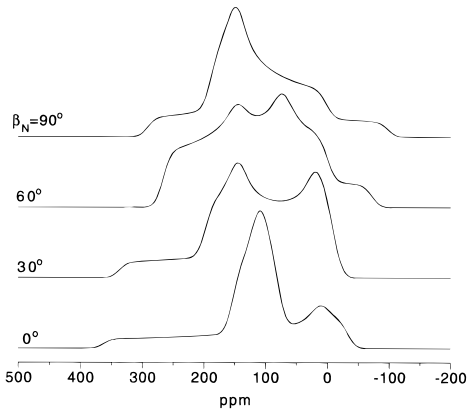

Figure 4. Simulated one-dimensional $^1H-^{15}N$ dipolar ^{15}N chemical shift spectra of ^{15}N-acetyl glycine for various values of β_N with α_N = 25°, 155°, 205°, or 335°.

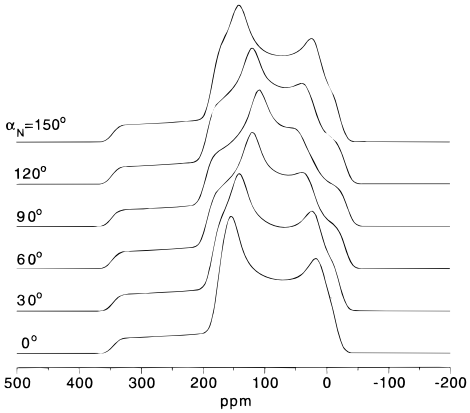

Figure 5. Simulated one-dimensional $^1H-^{15}N$ dipolar ^{15}N chemical shift spectra of ^{15}N-acetyl glycine for various values of α_N with β_N = 25.5° or 154.5°.

determined from the best-fitting simulations is 1.07 ± 0.02 Å. This value is in good agreement with previous solid-state NMR measurements on the length of the amide N−H bond in the literature.[24,35,52]

The analysis of the experimental results in terms of orientations of the tensors in the molecular frame starts with the usual assumption that the axially symmetric $^1H-^{15}N$ dipolar interaction is collinear with the N−H bond. This assumption allows the relative orientations of the principal components of the ^{15}N chemical shift tensor to be placed in the molecular frame of reference. From our results, we predict that the σ_{33N} axis is tilted by (25.5° or 154.5°) ± 1° away from the N−H bond in NAG. The projection of the N−H bond on the $\sigma_{11N}-\sigma_{22N}$ plane makes an angle (25°, 155°, 205°, or 335° ± 5° with the σ_{11N} axis. Since these angles are given only with reference to the N−H bond, the complete description of the orientation of the tensor axes in the molecular frame requires the results from a single-crystal study.[17] Accordingly, we have adapted this information in the tensor characterization, and the final results are presented in Figure 6. Based on the single-crystal study,[17] σ_{33N} is assumed to be in the peptide plane. Our results predict that σ_{11N} and σ_{22N} make an angle of (25°, 155°, 205°, or 335°) ± 5° with the peptide plane and the normal to the peptide plane, respectively. This prediction is in good agreement with the results obtained from a three-dimensional experiment correlating ^1H chemical shift, $^1H-^{15}N$ dipolar coupling, and ^{15}N chemical shift interactions on a polycrystalline sample of Ala-^{15}N-Leu dipeptide.[35]

The ^{15}N chemical shift and $^1H-^{15}N$ dipolar interaction tensors at the peptide bond of the glycine residue in type I collagen were also characterized by using the approach described above. It has been reported that there are inherently large amplitude dynamic motions present in the backbone of the collagen fibrils at room temperature.[45,46] Therefore, to freeze all the backbone motions, all of the NMR experiments on unoriented collagen

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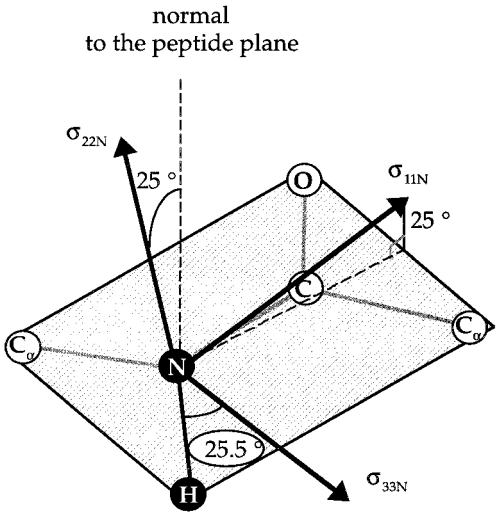

Figure 6. Orientations of the principal axes of the ^{15}N chemical shift and ^{1}H−^{15}N dipolar tensors, associated with the peptide bond, in the molecular frame of ^{15}*N*-acetyl glycine. σ_{33N} is in the peptide plane, σ_{11N} and σ_{22N} are 25°, 155°, 205°, or 335° away from the peptide plane and the normal to the peptide plane, respectively.

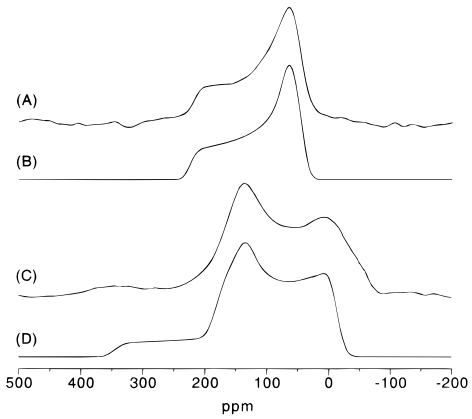

Figure 7. One-dimensional NMR spectra of a randomly oriented [^{15}N-Gly]collagen sample. Experimental (A) and simulated (B) ^{15}N chemical shift spectra. Experimental (C) and simulated (D) ^{1}H−^{15}N dipolar ^{15}N chemical shift spectra. Spectra (A) and (C) are the results of 1 200 and 10 600 scans, respectively.

were performed at −40 °C.[45,46] The experimental and the best-fitting simulated ^{15}N chemical shift spectra are given in parts A and B of Figure 7, respectively. The principal values of the ^{15}N chemical shift tensor are $\sigma_{11N} = 42.3 \pm 2$ ppm, $\sigma_{22N} = 67 \pm 2$ ppm, and $\sigma_{33N} = 223.4 \pm 2$ ppm. These values are in good agreement with the results reported in the literature.[54] The experimental and the best-fitting simulated dipolar chemical shift spectra are shown in parts C and D of Figure 7, respectively. A value of 10.5 ± 0.5 kHz was used for D_{NH} which corresponds to the N−H bond length of 1.05 ± 0.02 Å. Calculated powder patterns for various values of the angles α_N and β_N are shown in Figures 8 and 9, respectively. The simulated dipolar chemical shift powder patterns for the 35°, 145°, 215°, and 325° values of the α_N angle closely matched the experimental spectrum (Figure 7C); simulated spectra for α_N angles 35° and 145° are shown in Figure 9. The value of β_N was determined as (24.5° or 155.5°) ± 1°. The orientations of the tensors are shown in Figure 10. σ_{11N} and σ_{22N} make an angle of 35° (or 145°, 215°, or 325°) with the peptide plane and the normal to the peptide plane, respectively. The properties of the tensor elements are summarized in Table 1.

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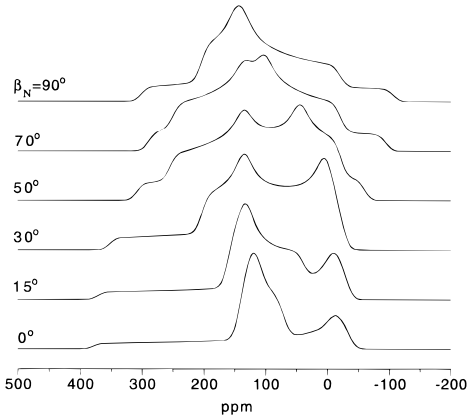

Figure 8. Simulated one-dimensional ^{1}H−^{15}N dipolar ^{15}N chemical shift spectra of a randomly oriented [^{15}N-Gly]collagen sample for various values of β_N with $\alpha_N = 35°$, 145°, 215°, or 325°. Powder pattern spectra for β_N values *x*° and *180*° − x° are identical.

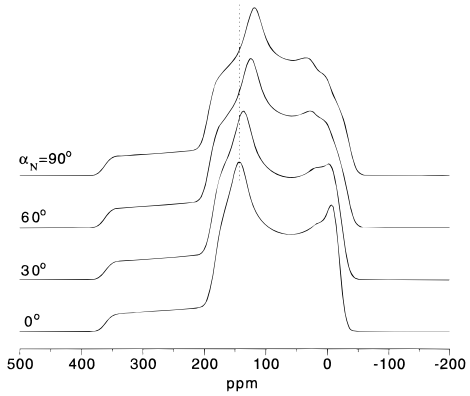

Figure 9. Simulated one-dimensional ^{1}H−^{15}N dipolar ^{15}N chemical shift spectra of a randomly oriented [^{15}N-Gly]collagen sample for various values of α_N with $\beta_N = 24.5°$. Powder patterns spectra for α_N values 0 ± *x*° and 180° ± *x*° are identical.

To demonstrate the application of the one-dimensional dipolar chemical shift solid-state NMR spectroscopy to study the conformation of biological solids, we performed experiments on the oriented fibers of type I [^{15}N-Gly]collagen. The ^{15}N chemical shift spectrum of the [^{15}N-Gly]collagen fibers oriented parallel to the direction of the external magnetic field is shown in Figure 11(A). A single sharp line at 60 ppm in the spectrum indicates that the sample is uniaxially oriented along the fiber axis. The line width may be attributed to the degree of randomness of the collagen fibers in the sample and also to the differences in the conformations of glycine residues in collagen. Since the observed NMR frequency depends only on the projection of the principal tensor components on the applied field axis, we can immediately point out that σ_{33N} is nearly orthogonal to the fiber axis. The best-fitting calculated spectrum in Figure 11B was simulated with the values $\sigma_{11N} = 42.3 \pm 2$ ppm, $\sigma_{22N} = 67 \pm 2$ ppm, $\sigma_{33N} = 223.4 \pm 2$ ppm, $\theta = 82°$ and $\phi = 53°$. The angles θ and ϕ were given a variation of 18° in the Gaussian function of the simulation program to account for the inherent range of the orientation of the N−H bonds of different glycine residues relative to the helical axis of the

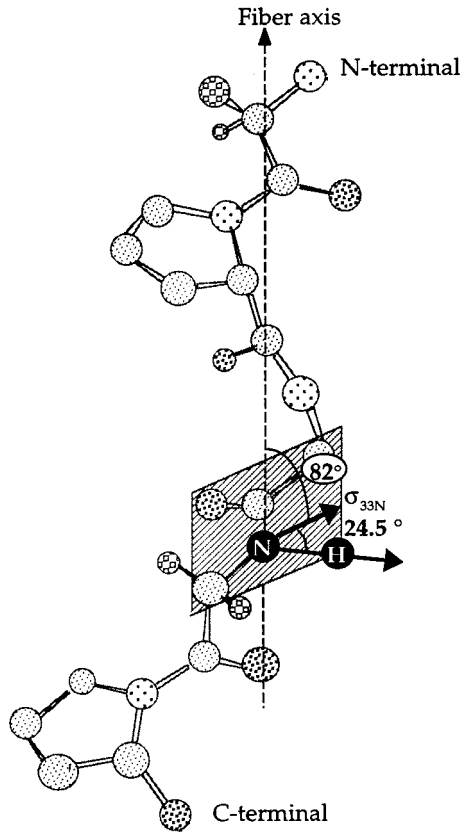

Figure 10. Orientations of the principal axes of the ^{15}N chemical shift and $^1H-^{15}N$ dipolar tensors, associated with the peptide bond of glycine, in the molecular frame of collagen.

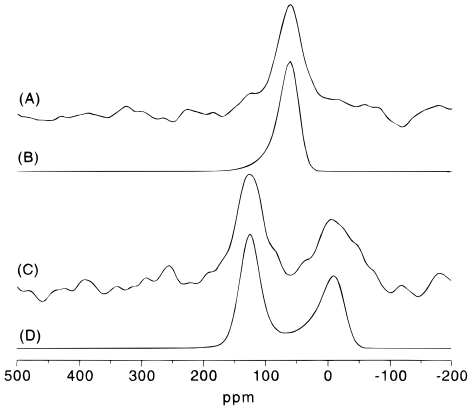

Figure 11. One-dimensional NMR spectra of [^{15}N-Gly]collagen fibers oriented parallel to the direction of the external magnetic field. Experimental (A) and simulated (B) ^{15}N chemical shift spectra. Experimental (C) and simulated (D) $^1H-^{15}N$ dipolar ^{15}N chemical shift spectra. Spectra A and C are the results of 6 000 and 20 000 scans, respectively.

collagen.[44] The experimental ^{15}N chemical shift $^1H-^{15}N$ dipolar spectrum of the sample oriented parallel to the magnetic field is given in Figure 11C. The $^1H-^{15}N$ dipolar splitting (6.3 ± 0.5 kHz) scaled due to the Lee-Goldburg sequence by a factor of 0.58 was measured directly from the spectrum in Figure 11C. Thus, the actual $^1H-^{15}N$ dipole coupling frequency for the collagen fiber oriented parallel to the applied field was easily calculated as 10.8 kHz. The N−H bond length of 1.05 Å was used in the simulations. This indicates that the N−H bond is nearly perpendicular to the triple helical axis or to the direction

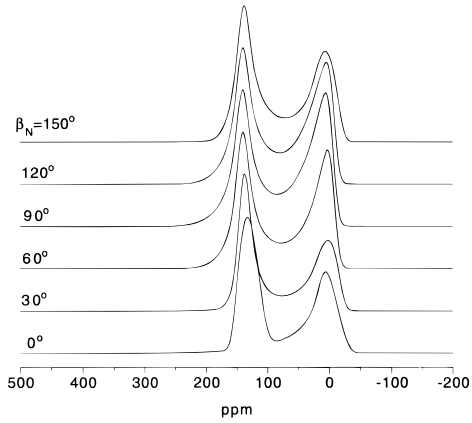

Figure 12. Simulated one-dimensional $^1H-^{15}N$ dipolar ^{15}N chemical shift spectra of [^{15}N-Gly]collagen fibers oriented parallel to the direction of the external magnetic field for various values of β_N with $\alpha_N = 145°$.

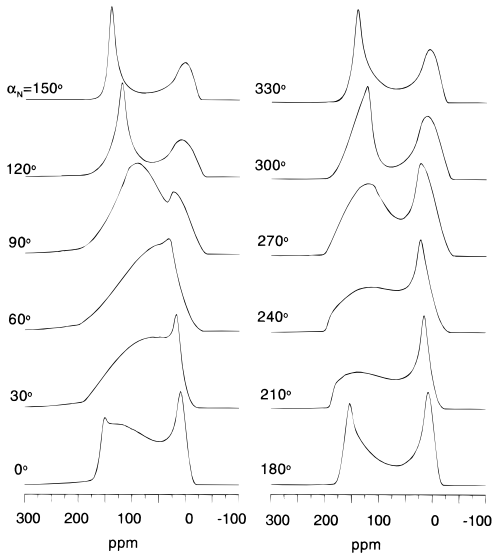

Figure 13. Simulated one-dimensional $^1H-^{15}N$ dipolar ^{15}N chemical shift spectra of [^{15}N-Gly]collagen fibers oriented parallel to the direction of the external magnetic field for various values of α_N with $\beta_N = 24.5°$.

of the external magnetic field. This is consistent with the results obtained from the oriented chemical shift spectrum. The experimental spectrum in Figure 11C was further simulated to characterize the chemical shielding tensor orientation relative to the dipolar axis of N−H bond. In the calculation, there are four variable angles to be adjusted to simulate the experimental spectrum. However, θ and ϕ values were uniquely determined from the ^{15}N chemical shift spectrum of the collagen sample oriented parallel and perpendicular relative to the applied field.[44] Therefore, θ and ϕ values are fixed, and only α_N and β_N values are need to be determined. The best-fitting simulated ^{15}N chemical shift $^1H-^{15}N$ dipolar spectrum of the sample oriented parallel to the magnetic field given in Figure 11D was obtained with the angles $\alpha_N = 145° \pm 10°$ and $\beta_N = 24.5° \pm 2°$. Spectral patterns calculated with different sets of α_N and β_N values are shown in Figures 12 and 13. Interestingly, the splitting patterns are dependent on both α_N and β_N angles. The angle, β_N, is consistent with the result obtained from the powder dipolar chemical shift spectrum (Figure 7C), while the value of α_N is confirmed to be 145° ± 10° rather than 35°, or 215°, or 325°. The simulated (Figure 11D) and experimental dipolar chemical shift spectra (Figure 11C) of the oriented collagen sample match only when $\alpha_N = 145 \pm 10°$.

Discussion

The properties of the one-dimensional dipolar chemical shift spectrum provide dramatic illustrations of how heteronuclear dipole−dipole couplings are manifested in NMR spectra. It enables the ^{15}N chemical shift and $^{1}H-^{15}N$ dipolar coupling tensors to be characterized for systems that are specifically labeled with ^{15}N. The magnitudes of the principal elements of the ^{15}N chemical shift tensor for the glycine residue are significantly different for various systems as shown in Table 1. The range of the variation of σ_{11N}, σ_{22N}, and σ_{33N} values are 25, 27, and 26 ppm, respectively. The angle β_N varies from 18 to 158° for glycine residues. On the other hand, the variation of α_N is large. This is because most of the studies based on experiments on powder samples assumed that σ_{11N} is in the peptide plane, and thus, α_N was predicted to be 0°. As illustrated in our studies, powder patterns are not very sensitive to the variation of α_N values. The effect of the variation of the α_N angle depends on the frequency difference between σ_{11N} and σ_{22N}. For example, α_N was determined with a higher accuracy for NAG (±5°) than in the case of [^{15}N-Gly]collagen (±10°) because the difference between σ_{11N} and σ_{22N} is large for NAG (45 ppm) and small for [^{15}N-Gly]collagen (25 ppm). Further, the powder patterns generated with the α_N values $0° \pm x°$ and $180 \pm x°$ are identical.

It is worth mentioning that the dipolar chemical shift spectrum of the oriented collagen is more sensitive (Figure 13) to the angle α_N than the unoriented powder spectrum of the same sample (Figure 8). Thus, the oriented sample enables the measurement of α_N accurately (145° ± 10°). This accuracy is of great importance in the structural studies of uniaxially oriented biological solids using solid-state NMR spectroscopy.

Nuclear spin interaction tensors associated with the peptide bond are useful in the study of cross-correlation between chemical shift and heteronuclear dipolar interactions in proteins using solution NMR methods.[3,10] The parameters R, defined as $(\sigma_{33N} - \sigma_{iso})^2/(1 + \eta^2/3)$, and $\Delta\sigma$, defined as $\sigma_{33N} - 0.5(\sigma_{11N} + \sigma_{22N})$, are important in the interpretation of relaxation studies of proteins in solution. Therefore, it is important to consider any variations in the values of R and $\Delta\sigma$. The data in Table 1 indicate a variation of 3400 in R values and a variation of 24 ppm in $\Delta\sigma$ values for glycine residues in various systems that have been studied using solid-state NMR methods.

The one-dimensional dipolar chemical shift spectrum of an oriented sample of type I collagen fiber demonstrates, by itself, several important aspects of the feasibility of applying solid-state NMR spectroscopy to uniaxially oriented biological systems. First of all, it shows that the one-dimensional experiment utilizing the Lee-Goldburg pulse sequence[48] to suppress the $^{1}H-^{1}H$ dipolar couplings can be performed on macromolecules in solid-state. Second, it demonstrates that the two spectral parameters required for the determination of peptide plane orientations can be measured in a single spectrum. Third, it shows that the one-dimensional dipolar chemical shift spectrum is sufficient to characterize the tensors associated with a peptide bond completely in a polycrystalline sample. And, fourth, it shows the potential for studying the local as well as the backbone dynamics from a specifically labeled site of proteins. Further, compared to the traditional two-dimensional separated local field approaches, this one-dimensional method significantly reduces the experimental time and is easy to implement in a spectrometer, and the results are simple to interpret.[18,19,35,39] Thus, this approach should be of use in the study of structure and dynamics of fibrous macromolecules which are immobile about each of three orthogonal axes on NMR time scales[45,55−62] and of membrane-associated proteins labeled with ^{15}N isotope at a single site embedded in phospholipid bilayers which are immobile about one or two axes on NMR time scales.[63−66]

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