proteins is at least 10 ppm. It is likely that most of this range is due to variations in σ_{22}. With use of eq 4, rough approximation of the σ_{22}'s for peptide carbonyls could be combined with assignment techniques such as $^{13}C/^{15}N$ double labeling[39] or site-specific mutations[40] to create a list of approximate shift tensors. We have used the data of Kainosho et al.[39] to estimate σ_{22} for several peptide carbonyl carbons in *Streptomyces subtilisin* inhibitor. These values range from −78.4 to −50.8 ppm (relative to liquid benzene), with several values falling significantly downfield of the values observed in peptides **1-4**. Although no direct correlation between isotropic carbonyl shifts (and therefore predicted σ_{22}'s) and protein secondary structure is apparent,[39] there may be other structure-dependent effects, such as hydrogen bond strengths, which determine peptide carbonyl σ_{22}'s.

Conclusion

The data presented here demonstrate that there is significant variation in both the magnitude and orientation of carbonyl ^{13}C chemical shift tensors of several synthetic peptides. Most of this variation is due to interactions in the crystal lattice and not to purely intramolecular interactions. As previously observed with other carbonyl ^{13}C chemical shift tensors, σ_{22} is the most variable of the three principal values and σ_{33} is perpendicular to the peptide plane, to within a few degrees.

Acknowledgment. We thank Paul Engelking, Russell Desiderio, Ernest Curto, and Jim VanZee for their contributions to the development of the powder pattern fitting procedures. We also thank Alan Deese, Edward Dratz, Dennis Hare, and Tom Pratum for their assistance in the collection and analysis of the data. This work was supported in part by PHS GM35787 and PHS GM32681-02. T.G.O. was supported by National Institutes of Health Training Grant PHS GM07759.

Registry No. **1**, 109929-74-0; **1** (ethyl ester), 109929-78-4; **2**, 109929-75-1; **2** (ethyl ester), 109929-80-8; **3**, 88815-61-6; **3** (ethyl ester, free base), 109929-81-9; **4**, 109929-29-5; **4** (ethyl ester), 109929-82-0; **5**, 109929-76-2; **5** (ethyl ester), 109929-83-1; **6**, 543-24-8; ethyl [^{15}N]-DL-alanate hydrochloride, 109929-77-3; ethyl [^{15}N]-DL-tyrosinate, 109929-79-5; benzyloxycarbonyl-[1-^{13}C]-glycine, 67739-37-1; [^{15}N]-glycine ethyl ester hydrochloride, 58420-99-8; ethyl [^{15}N]-L-phenylalanate, 94601-37-3.

The Amide ^{15}N Chemical Shift Tensors of Four Peptides Determined from ^{13}C Dipole-Coupled Chemical Shift Powder Patterns

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Abstract: The ^{15}N chemical shift tensors of a homologous series of peptides of the form *N*-acetyl[1-^{13}C]glycyl[^{15}N]-X-amide (X = glycine, alanine, and tyrosine) and the unprotected dipeptide [1-^{13}C]glycyl[^{15}N]glycine hydrochloride have been determined from ^{13}C dipole-coupled ^{15}N powder patterns. It was found that the shift tensor principal values differ greatly while their molecular orientation does not. The common shift tensor orientation places σ_{22} perpendicular to the peptide plane, and σ_{33} at a 99° angle with respect to the C-N bond. The orientations of σ_{11} and σ_{22} were previously unknown for ^{15}N chemical shift tensors of amides. Comparison of magic angle spinning (MAS) spectra with solution spectra shows significantly different solid and solution isotropic chemical shifts for several of the peptides studied, demonstrating that at least part of the variation in principal values is due to lattice effects. This conclusion is borne out by the MAS spectrum of *N*-acetyl[1-^{13}C]glycl-[^{15}N]phenylalaninamide, which shows at least three peaks corresponding to different lattice environments.

Solid-state ^{15}N nuclear magnetic resonance (NMR) has recently proven very useful in the structural, chemical, and dynamic characterization of proteins.[2-8] In many of these studies the chemical shift anisotropy of the ^{15}N nucleus played a crucial role, and in several cases a knowledge of the chemical shift tensor of the ^{15}N was critical to the interpretation of the spectra.[3,6-8] Unfortunately, relatively few ^{15}N chemical shift tensors have been determined,[9,10] thus limiting the accuracy of the structural in-

Table I. Peptide Molecules Used in This Study

	A-NHCH$_2$^{13}C(O)^{15}NC(R)HC(O)-B			
	A	R	B	abbreviation
1	CH_3CO	DL-CH_3(DL-Ala)	NH_2	AcGlyAlaNH_2
2	CH_3CO	DL-$CH_2C_6H_4OH$(DL-Tyr)	NH_2	AcGlyTyrNH_2
3	H	H(Gly)	OH	GlyGly·HCl
4	CH_3CO	H(Gly)	NH_2	AcGlyGlyNH_2
5	CH_3CO	L-$CH_2C_6H_5$(L-Phe)	NH_2	AcGlyPheNH_2

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(2) (a) MacKenzie, N. E.; Fagerness, P. E.; Scott, A. I. *J. Chem. Soc., Chem. Commun.* **1985**, 635-637. (b) Limbach, H. H.; Hennig, J.; Kendrick, R.; Yannoni, C. S. *J. Am. Chem. Soc.* **1984**, *106*, 4059-4060.

(3) Harbison, G. S.; Herzfeld, J.; Griffin, R. G. *Biochemistry* **1985**, *22*, 1-5.

(4) Huang, T. H.; Bachovchin, W. W.; Griffin, R. G.; Dobson, C. M. *Biochemistry* **1984**, *23*, 5933-5937.

(5) Munowitz, M.; Bachovchin, W. W.; Herzfeld, J.; Dobson, C. M.; Griffin, R. G. *J. Am. Chem. Soc.* **1982**, *104*, 1192-1196.

(6) Cross, T. A.; Opella, S. J. *J. Mol. Biol.* **1982**, *159*, 543-549.

(7) Cross, T. A.; Tsang, P.; Opella S. J. *Biochemistry* **1983**, *22*, 721-726.

(8) Cross, T. A.; Opella, S. J. *J. Mol. Biol.* **1985**, *182*, 367-381.

(9) Mehring, M. *High Resolution NMR in Solids*; Springer-Verlag: New York, 1983; p 258.

(10) Harbison, G. S.; Jelenski, L. W.; Stark, R. E.; Torchia, D. A.; Herzfeld, J.; Griffin, R. G. *J. Magn. Reson.* **1984**, *60*, 79-82.

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formation obtained in these studies. In addition, the complete orientation of an amide ^{15}N shift tensor has not previously been reported.

The peptide amide ^{15}N chemical shift anisotropy, combined with a second tensorial interaction such as ^{13}C or ^{1}H dipolar coupling to the ^{15}N, has the potential to provide very precise information about the relative orientation of peptide planes in oriented proteins, as demonstrated by Opella and co-workers for bacteriophage fd coat protein.[6-8] However, an accurate knowledge of the principal values and orientation of the ^{15}N chemical shift tensor is necessary. To date, the only known amide ^{15}N shift tensor is that of glycylglycine hydrochloride monohydrate. Unfortunately, it was not possible for the authors to accurately determine the orientations of the σ_{11} and σ_{22} principal axes of this tensor.[10]

The question arises as to whether the tensor of this compound adequately describes amide ^{15}N chemical shift tensors in general. How sensitive are the principal values and tensor orientation to the lattice environment? Do the principal values and/or tensor orientation vary as a function of residue type or crystal packing?

To answer these questions we have synthesized a homologous series of end-protected dipeptides of the form *N*-acetyl[1-^{13}C]-glycyl[^{15}N]-X-amide (see Table I) for the purpose of determining their ^{15}N chemical shift tensors, including the orientations of σ_{11} and σ_{22}.

One reason for the scarcity of known ^{15}N chemical shift tensors is that the standard method of determining them requires large (tens of milligrams) single crystals of the molecules of interest and a knowledge of the crystal structure. Since there are many molecules that are difficult to crystallize in this quantity, ^{15}N shift tensors have only rarely been determined by this method.[9] An alternative to the single-crystal method is the use of a microcrystalline powder of a molecule in which there is a single nucleus dipole-coupled to the ^{15}N. This dipolar coupling can then be exploited in a variety of one- and two-dimensional static and magic angle spinning (MAS) experiments to determine the orientation of the dipolar vector in the chemical shift tensor's principal axis system.[11] Often, this information is sufficient to define a limited number of tensor molecular orientations. Because we have been unable to grow large single crystals of our end-protected dipeptides, we have chosen this approach in the present study. We have obtained ^{13}C dipole coupled ^{15}N chemical shift powder patterns of four of the peptides listed in Table I and have used an iterative least-squares fitting procedure to determine the principal values and orientations of the ^{15}N chemical shift tensors of these molecules.

We have found large variations in the principal values of the chemical shift tensors of the four peptides. On the basis of comparison of the isotropic chemical shifts of these molecules in the solid form (as determined by MAS) with their solution chemical shifts, we conclude that much of this variation is due to the lattice environment of the molecule.

Experimental Section

Peptide Synthesis. The doubly labeled peptides shown in Table I were synthesized by methods described previously.[13] The purity of all of the peptides was monitored by high-resolution ^{1}H, ^{13}C, and ^{15}N NMR spectroscopy and was found to be greater than 95%. In addition, the presence of a single component in our sample of AcGlyPheNH_2 was confirmed by HPLC with use of a reverse-phase C-18 column and acetonitrile/water elution gradient.

Powder Spectra. The ^{15}N powder pattern spectra were obtained at 20.3 MHz with use of a Bruker CXP-200 Console and 4.7-T magnet with a probe equipped with a triply tuned solenoid coil[14] (6 × 20 mm). The spectra were collected with use of cross polarization with a mixing time of 1 ms and high power CW ^{1}H decoupling with $(\gamma H_1)/2\pi \simeq 90$ kHz. A spin-echo pulse sequence was used to minimize distortions caused by pulse "breakthrough". The time between the cross-polarization period and the ^{13}C π pulse was 50 μs. The pulses and receiver were phase cycled to remove artifacts caused by phase inaccuracies in the ^{13}C π pulse and residual, nonechoing FID. Recycle delays ranged from 8 to 30 s and the number of acquisitions from 1500 to 3000, depending on the dipeptide, to give spectra with comparable signal-to-noise ratios. The total sweep width was 100 kHz, and 512 data points in each of the two channels in quadrature were collected. The sample sizes were approximately 150 mg. External $^{15}NH_4Cl$ was used as a chemical shift standard, and chemical shifts were referenced to liquid $^{15}NH_3$ at −50 °C[19] assuming that the chemical shift of solid $^{15}NH_4Cl$ on this scale is 38.5 ppm (downfield).

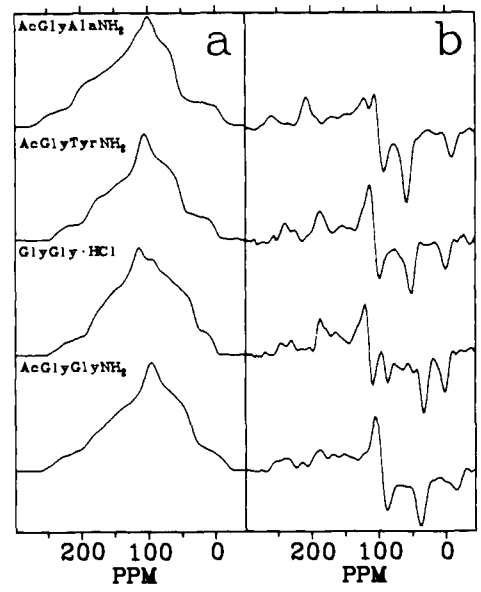

Figure 1. The ^{13}C dipole-coupled powder spectra of the ^{15}N-labeled central peptide amide nitrogen in peptides **1**–**4**. The absorption spectra are shown in part a and the first derivative with respect to frequency in part b. Chemical shifts are relative to liquid NH_3 assuming solid NH_4Cl (external standard) has a shift of 38.5 ppm (low ppm is upfield).

Solution Spectra. The high-resolution ^{15}N spectra were collected at 36.5 MHz on a standard Nicolet NT-360 instrument. The spectra were collected with NOE enhancement, with ^{1}H broadband decoupling. The dipeptides were dissolved in D_2O at a concentration of ca. 50 mM with 0.1% $^{15}NH_4Cl$ as an internal chemical shift reference. ^{13}C was not decoupled.

MAS Spectra. The ^{15}N magic angle sample spinning (MAS) spectra were collected at 20.3 MHz on a modified NT200 instrument equipped with a magic angle spinning probe with an Andrew-Beams type spinner.[15] Data were collected with use of cross polarization with a mixing time of 5 ms and high-power ^{1}H decoupling with $(\gamma H_1/2\pi) = 45$ kHz. Spinning speeds were ~2.8 kHz.

Data Analysis. Analysis of the solid-state NMR spectra was done on a VAX 11/750, using a command driven NMR data analysis software package (FTNMR) as well as original FORTRAN programs whose algorithms are described below. In order to produce derivative spectra (see below) with sufficient signal-to-noise ratios for use in least-squares fitting it was necessary to apodize the time-domain spectra with a Kaiser window digital filter ($\alpha = 4$, $N = 2.5$ ms).[16] This apodization function effectively removed the noise at long times in the time domain spectrum

(11) (a) Stoll, M. E.; Vega, A. J.; Vaughan, R. W. *J. Chem. Phys.* **1976**, *65*, 4093-4098. (b) Hester, R. K.; Ackerman, J. L.; Neff, B. L.; Waugh, J. S. *Phys. Rev.* **1976**, *36*, 1081-1083. (c) Linder, M.; Hohener, A.; Ernst, R. R. *J. Chem. Phys.* **1980**, *73*, 4959-4970. (d) Munowitz, A.; Aue, W. P.; Griffin, R. G. *J. Chem. Phys.* **1982**, *77*, 1686-1689. (e) Zilm, K. W.; Grant, D. M. *J. Am. Chem. Soc.* **1981**, *103*, 2913-2922.

(12) (a) Reference 9, pp 233-257. (b) Maric, M.; Waugh, J. S. *J. Chem. Phys.* **1979**, *70*, 330. (c) Herzfeld, J.; Berger, A. E. *J. Chem. Phys.* **1980**, *73*, 6021-6030.

(13) Oas, T. G.; Hartzell, C. J.; McMahon, T. J.; Drobny, G. P.; Dahlquist, F. W. *J. Am. Chem. Soc.*, previous paper in this issue.

(14) Kan, S.; Fan, M.; Courtieu, J. *Rev. Sci. Instrum.* **1980**, *51*, 887-890.

(15) Bartuska, V. J.; Maciel, G. E. *J. Magn. Reson.* **1981**, *42*, 312-317.

(16) Hamming, R. W. *Digital Filters*; Prentice-Hall: Englewood Cliffs, NJ, 1983; pp 171-176.

(17) Oas, T. G.; Drobny, G. P.; Dahlquist, F. W. *J. Magn. Reson.*, submitted for publication.

(18) The dipolar coupling tensor is Hermitian (ref 10) and can be transformed via a unitary transformation to the principal axis systems of the chemical shift tensor. Under these conditions, the dipolar couplings of orientations along these axes are invariant and because the dipolar tensor is traceless must sum to zero. This means that the dipolar splittings at the regions of the powder pattern corresponding to σ_{11}, σ_{22}, and σ_{33} are not linearly independent. This precludes the independent determination of D, α, and β.

(19) (a) Srinivasan, P. R.; Lichter, R. L. *J. Magn. Reson.* **1977**, *28*, 227-234. (b) Briggs, J. M.; Randall, E. W. *Mol. Phys.* **1973**, *26*, 699.

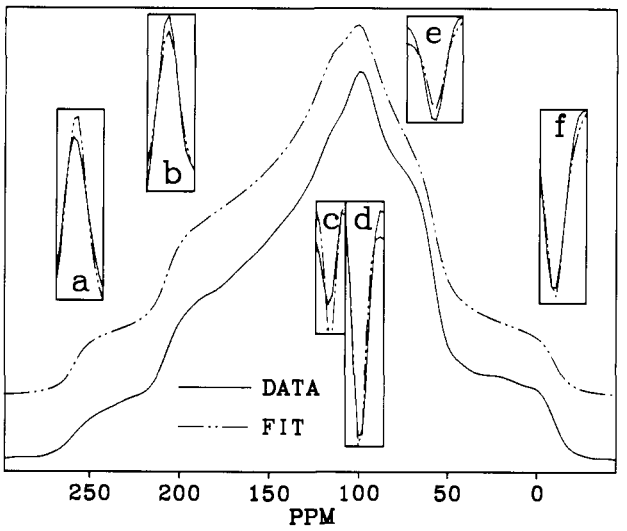

Figure 2. A comparison of the actual and simulated powder spectra for $AcGlyAlaNH_2$. Insets a–c show regions used to fit σ_{33} and the polar angle of the C–N bond, β, in the first derivative form. Insets c and e contain second and first derivative peaks, respectively, corresponding to the dipole-split σ_{22} edge. Insets d and f contain second and first derivative peaks, respectively, corresponding to the σ_{11} doublet. Regions c–f were used to fit σ_{11}, σ_{22}, and the azimuthal angle of the C–N bond, α. The integral of the simulation was normalized to that of the data for each region and is displayed as such in the figure. Chemical shifts are relative to liquid NH_3 assuming solid NH_4Cl (external standard) has a chemical shift of 38.5 ppm (low ppm is upfield).

(to which derivative spectra are very sensitive) without significantly broadening the frequency domain spectrum. No other line broadening was used.

Results

The ^{15}N NMR powder spectra of peptides **1–4** (see Table I) are shown in Figure 1. The absorption spectra are shown in Figure 1a, and the first derivatives of these spectra with respect to frequency are shown in Figure 1b. The derivative forms of the powder patterns are shown because they better emphasize the frequencies of the important features, both visually and for the purpose of least-squares fitting analysis.[17] The shapes of the powder spectra in Figure 1 reflect both ^{15}N chemical shift and ^{13}C–^{15}N dipolar interactions present in these peptides. Because of this, it is possible to analyze these spectra in terms of the principal values of the chemical shfit tensor and the orientation of the N–C vector in the shift tensor frame.[11]

This approach has been used in a study of the ^{13}C chemical shift tensors of the same molecules and is described more completely therein.[13] One important difference between the ^{13}C and ^{15}N spectra is that the maximum dipolar coupling is a much larger fraction of the chemical shift anisotropy for ^{15}N (~75%) than it is for ^{13}C (~30%). This results in ^{15}N powder patterns that are much more sensitive to the orientation of the dipolar vector and the size of the dipolar coupling constant.

There are eight adjustable parameters that are required to simulate the dipole-coupled ^{15}N spectra of peptides **1–4**. These are the Lorentzian and Gaussian line widths of the line assumed to come from a given crystal orientation, the three principal values of the chemical shift tensor, the dipolar coupling constant ($\gamma_C\gamma_N\hbar/r_{CN}^3$), and the polar angles β and α describing the orientation of the C–N bond in the axis system of the chemical shift tensor. As defined in this frame, β is the angle between the C–N bond and the σ_{33} axis and α is the angle between the projection of the C–N bond onto the σ_{11}–σ_{22} plane and the σ_{11} axis.

In prinicple, these parameters can be extracted from the data in Figure 1 by iterative least-squares fitting of simulated spectra to the data. However, least-squares fitting of complete powder patterns that contain even minor deviations from ideal line shapes can often lead to erroneous "best-fit" parameter values. To avoid this problem, we have chosen to fit selected regions of the spectra in their first- or second-derivative forms. We have found that this method, described in more detail elsewhere,[17] leads to simulated spectra which, in terms of the frequencies of the important features, are excellent fits to the data. Figure 2 illustrates this approach for $AcGlyAlaNH_2$. Comparison of the actual and best-fit absorption spectra reveals differences in intensity across the powder patterns. However, as shown in insets a–f in Figure 2, the frequencies of the six derivative peaks are identical for the actual and simulated spectra. These derivative peaks correspond to the dipole-split edges due to σ_{11}, σ_{22}, and σ_{33}. In Figure 2 regions a and b contain the first derivatives of the σ_{33} doublet, regions c and e contain the σ_{22} doublet, and regions d and f contain the σ_{11} doublet. These were the regions used in least-squares analysis. The parameters were fit as follows: (1) σ_{33}, the line shape (Lorentzian and Gaussian linewidth), and β were fit to the first derivative forms of the spectra in regions a and b in Figure 2 (each independently normalized) with the other parameters held fixed; (2) σ_{11}, σ_{22}, α, and line shape were fit to the second derivative forms of the spectra in regions c and d in Figure 2 and the first derivative forms of the spectra in regions e and f in Figure 2. These fits were performed with the dipolar coupling constant (D) fixed to several values from 1150 to 1250 Hz (corresponding to C–N bond lengths of 1.39–1.35 Å) producing essentially identical fits. This is due to the fact that D, α, and β are inherently covariant in the regions fit[18] and cannot be determined independently.

Table II. ^{15}N Amide Chemical Shift Tensors of Five Peptides

molecule	principal values (ppm)[a] σ_{11}	σ_{22}	σ_{33}	angle between σ_{33} and the ^{13}C–^{15}N bond[b] β(deg)
$AcGlyAlaNH_2$	44.6	85.1	229.4	100
$AcGlyTyrNH_2$	52.1	77.1	209.3	98
GlyGly·HCl	57.3	59.8	210.0	99
$AcGlyGlyNH_2$	40.7	64.2	210.6	100

[a] Relative to liquid $^{15}NH_3$ assuming solid $^{15}NH_4Cl$ (external standard) has a chemical shift of 38.5 ppm (low ppm upfield). Uncertainties are ±3.0 ppm. [b] σ_{11} and σ_{33} are assumed to lie in the peptide plane (see text). Uncertainties are ±2°.

The powder spectra of peptides **1–4** were subjected to this sort of analysis, and the resulting best fit values for σ_{11}, σ_{22}, σ_{33}, and β are summarized in Table II. In all cases, the sensitivity of the fits to values of α was low, particularly when σ_{11} and σ_{22} were close in value. This resulted in uncertainties in α of ±5–10°. However, a value of α = 0° always produced acceptable fits and it is therefore assumed to have a value of 0 ± 10° in all of the peptides studied. This is consistent with the notion that, due to the planar symmetry of the peptide bond, one of the three principal axes of the chemical shift tensor (in this case σ_{22}) is perpendicular to the peptide plane. The values of β listed in Table II are identical, within experimental error, and equal to 99 ± 2°. This value agrees with the results of a previous single-crystal study of glycyglycine hydrochloride monohydrate that found β to be 98.6°.[10] Unlike β, the best-fit values of σ_{11}, σ_{22}, and σ_{33} differ significantly among peptides **1–4**. The values of σ_{33} for peptides **2–4** are identical within experimental error, but they differ significantly from that of $AcGlyAlaNH_2$. The values of σ_{11} and σ_{22} are significantly different among all the peptides. The value of σ_{11} ranges from 40.7 ppm for $AcGlyGlyNH_2$ to 57.3 ppm for GlyGly·HCl, a range of 16.6 ppm, or ~10% of the total chemical shift anisotropy (σ_{33}–σ_{11}). The value of σ_{22} ranges from 59.8 ppm for GlyGly·HCl to 85.1 ppm for $AcGlyAlaNH_2$, a range of 25.3 ppm (16% of the anisotropy). In addition to these variations, there is also a large range in the asymmetry of the shift tensors, ranging form GlyGly·HCl, which is almost axially symmetric, to $AcGlyAlaNH_2$, which is much more asymmetric.

Figure 3 shows the magic angle spinning (MAS) spectrum of a sample of $AcGlyPheNH_2$, which, based on previous ^{13}C experiments, was known to contain at least three magnetically inequivalent lattice environments.[13] Figure 3 demonstrates that these environments are also detectable in the ^{15}N spectrum, which contains at least three peaks with approximately the same relative intensities as found in the ^{13}C spectrum, although the order is

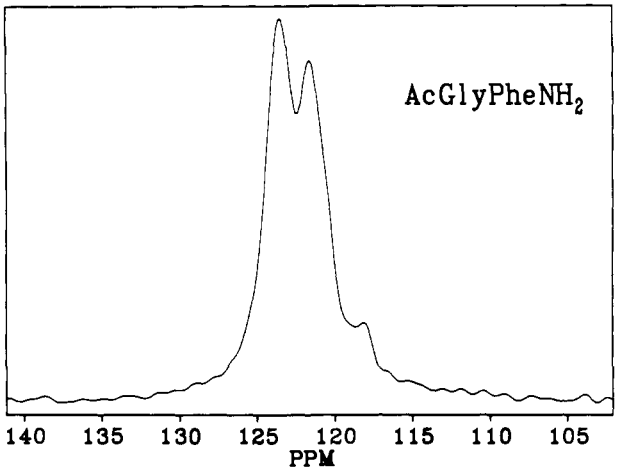

Figure 3. The MAS spectrum of $AcGlyPheNH_2$ showing at least three distinguishable isotropic chemical shifts present in the sample. This figure demonstrates the existence of at least three different chemical shift tensors for the same molecule (see text). Chemical shifts are relative to liquid NH_3 assuming solid NH_4Cl (external standard) has a chemical shift of 38.5 ppm (low ppm is upfield).

Table III. Comparison of Isotropic Chemical Shifts (ppm) of Five Peptides As Determined from MAS and Solution NMR Spectra

peptide	MAS[a]	solution[b]
$AcGlyAlaNH_2$	122.8	124.7
$AcGlyTyrNH_2$	116.9	120.4
GlyGly·HCl	111.5	109.5
$AcGlyGlyNH_2$	109.4	109.4
$AcGlyPheNH_2$-1	123.3	121.0
$AcGlyPheNH_2$-2	121.4	121.0

[a] Relative to liquid NH_3 assuming solid NH_4Cl (external standard) has a chemical shift of 38.5 ppm (low ppm upfield). Uncertainties are ±0.5 ppm. [b] Determined in D_2O with dilute $^{15}NH_4^+$ as an internal standard. Chemical shift of $^{15}NH_4^+$ is assumed to be 21 ppm downfield of liquid NH_3.[19] Uncertainties are ±0.5 ppm.

reversed, with the most intense peak being the most downfield in the ^{15}N spectrum instead of the most upfield, as it was in the ^{13}C spectrum.[13] These results indicate that the ^{15}N chemical shift tensor principal values are affected by the lattice environment of the nucleus.

This conclusion is supported by the data in Table III, which lists the isotropic ^{15}N chemical shifts of peptides **1–5** as determined in the solid form from the MAS spectra and in solution by high-resolution NMR. These data reveal significant differences in the isotropic chemical shifts of the solid and solution forms of $AcGlyAlaNH_2$, $AcGlyTyrNH_2$, GlyGly·HCl, and one of the two forms of $AcGlyPheNH_2$. In the case of $AcGlyAlaNH_2$ and $AcGlyTyrNH_2$, the solution shift is downfield of the solid shift, whereas for the other two it is upfield. These data indicate that the principal values of the ^{15}N shift tensors of these molecules are different in their solution and solid forms. Because of the existence of both downfield and upfield discrepancies in isotropic shifts, these differences are not likely to be caused by a simple solvent–induced shift.

Discussion

The planar symmetry of the peptide bond suggests that one principal axis of the chemical shift tensor is constrained to be perpendicular to the peptide plane. This constraint is consistent with the results of previous studies of sp^2 ^{15}N shift tensors.[20] The orientation of the C–N bond in the σ_{11}/σ_{22} plane can be most accurately determined when the principal values of σ_{11} and σ_{22} are much different. Least-squares fits of peptides **1**, **2**, and **4** indicate that σ_{22} is perpendicular to the C–N bond, leading to the conclusion that this is the principal axis, which is perpendicular to the peptide plane. Although orientations corresponding to rotation of the tensor around the C–N bond would produce identical dipole-coupled powder patterns, molecular symmetry suggests that σ_{11} and σ_{33} lie in the peptide plane. The orientation of σ_{33} with respect to the C–N bond is given by the angle β which is 99 ± 2° for all the peptides studied. This value is the same, within experimental error, as that given by Harbison et al. in a single-crystal study of GlyGly·HCl. The shift tensor orientation suggested by these results is shown in Figure 4. This orientation places σ_{11} in the peptide plane at an angle of 9° from the C–N bond and σ_{33} in the peptide plane forming an angle of approximately 20° from the N–H bond.

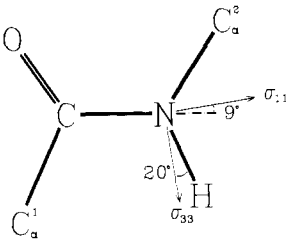

Figure 4. The orientation of σ_{11} and σ_{33} in the peptide plane, assuming σ_{22} is perpendicular to the peptide plane and positive above the page. The uncertainty in the angles is ±2°.

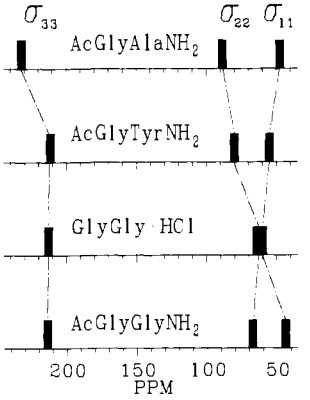

Figure 5. Summary of the principal values of the ^{13}C chemical shift tensors of peptides **1–4** as determined by iterative fitting of the powder patterns. The uncertainty in the fitting results is indicated by the width of the bars (±3.0 ppm). Chemical shifts are relative to liquid NH_3 assuming solid NH_4Cl (external standard) has a chemical shift of 38.5 ppm (low ppm is upfield).

Within the limits of accuracy to which β could be determined (±2°) there is no significant difference between the orientations of the shift tensors of peptides **1–4**. This is in contrast to the orientation of the ^{13}C tensors for the same molecules which vary by 12° and suggests that other peptide amide ^{15}N chemical shift tensors may have the same orientation. In contrast to the shift tensor orientations of peptides **1–4**, the principal values vary widely. Figure 5 summarizes the principal values obtained for peptides **1–4**. $AcGlyAlaNH_2$ has significantly greater anisotropy ($\sigma_{33}-\sigma_{11}$) than the other peptides. It is also the only peptide whose σ_{33} value is not within the experimental error of 210 ppm. There are significant differences in σ_{22} and σ_{11} for all four peptides **1–4**. These data demonstrate that a single chemical shift tensor cannot be used to accurately represent the chemical shift tensor of all peptide amide ^{15}N.

The multiple peaks in the MAS spectrum of $AcGlyPheNH_2$ and the difference between solids (MAS) and solution isotropic shifts for peptides **1–4** indicate that at least part of the variation in the chemical shift tensors is due to variations in the lattice environment. Variation in hydrogen bonding by the amide proton is a likely source of these lattice-dependent effects. It has been shown that various hydrogen bond strengths in a Schiff's base ^{15}N lead to significant variations in the principal values of its chemical shift tensor.[3] In addition, hydrogen bonds to either the amide proton or the peptide carbonyl oxygen would change the electronic structure of the peptide bond, thus giving rise to changes in the

(20) Harbison, G.; Herzfeld, J.; Griffin, R. G. *J. Am. Chem. Soc.* **1981**, *103*, 4752. (b) Schweitzer, D.; Speiss, H. W. *J. Magn. Reson.* **1974**, *15*, 529. (c) Reference 3.

chemical shift tensor. Other possible lattice-dependent effects would include intermolecular ring current shifts in the case of the aromatic peptides and deviations in the planarity of the peptide group caused by crystal-induced packing forces.

Whatever the nature of these lattice-dependent effects, they clearly preclude the use of model compound chemical shift tensors to determine the shift tensors of peptide ^{15}N in proteins, even if the side chain composition of the model compound matches that of the protein peptide of interest. In studies that utilize peptide ^{15}N chemical shift measurements of oriented proteins to deduce protein backbone structure,[7,8] variations in the ^{15}N shift tensor such as those reported here can be extremely important. Assume, for instance, that a peptide group in such a protein is oriented with the magnetic field perpendicular to the peptide plane. Assume also that the ^{15}N shift tensor of this peptide is the same as that of AcGlyAlaNH$_2$. Under these conditions, the observed ^{15}N chemical shift would be 85.1 ppm. If this chemical shift were interpreted assuming that the tensor for GlyGly·HCl applied, the predicted orientation would be in error by 24.6°.[21] There are many circumstances when assuming the incorrect tensor would make it impossible to deduce any orientation at all.[22]

The alternative to assuming a canonical shift tensor is to determine the principal values of the ^{15}N tensor for the actual peptide group in the protein being studied. This could be accomplished by obtaining the spectrum of a motionless powder of the protein, either directly or by slow-speed MAS and fitting the powder spectrum or by analyzing the sideband intensities.[12] By labeling the adjacent amino acid with ^{13}C in the carbonyl position, the ^{15}N can be selectively detected in static[23] or MAS[11d] spectra.

Conclusion

We have shown that there are large, lattice-dependent variations in the ^{15}N chemical shift tensor principal values of several model dipeptides. However, there are no significant differences in the molecular orientations of these tensors. These conclusions have led us to suggest a new approach for the use of ^{15}N peptide chemical shift measurements to determine protein structure based on the direct measurement of the principal values of the shift tensor in the molecule of interest using $^{15}N/^{13}C$ double-labeling and selective detection.[11d,23] These measurements would greatly improve the reliability of peptide ^{15}N chemical shift anisotropy in the determination of protein structure.

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(21) There is an infinite set of orientations that have a chemical shift of 85.1 ppm, assuming the chemical shift tensor of GlyGly·HCl. We have chosen a specific orientation by assuming that a second tensorial interaction can be observed. The $^{15}N-^{13}C$ dipolar coupling of a doubly labeled peptide oriented with its N–C bond parallel to the magnetic field would be 1300 Hz, assuming standard peptide geometries. The orientation calculated in this example corresponds to the intersection of the 85.1 ppm chemical shift and 1300 Hz dipolar coupling isochromats in the principal axis systems of the GlyGly·HCl chemical shift tensor.

(22) These cases correspond to occasions in which no intersection between the chemical shfit and dipolar isochromats (see ref 21) exists due to the fact that the incorrect shift tensor does not yield a physically reasonable chemical shift for a given orientation.

(23) Oas, T. G.; Hartzell, C. J.; Drobny, G. P.; Dahlquist, F. W. *J. Magn. Reson.*, submitted for publication.

Determination of the ^{15}N and ^{13}C Chemical Shift Tensors of L-[^{13}C]Alanyl-L-[^{15}N]alanine from the Dipole-Coupled Powder Patterns

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Abstract: The ^{13}C and ^{15}N chemical shift tensors of L-[1-^{13}C]alanyl-L-[^{15}N]alanine have been determined from the dipole-coupled powder patterns and verified with the decoupled spectra. The principal values of the ^{13}C tensor are $\sigma_{11} = -115.5$, $\sigma_{22} = -42.3$, and $\sigma_{33} = 33.5$ ppm, and the polar angles relating the $^{13}C-^{15}N$ bond to the chemical shift tensor are $\beta = 90°$ and $\alpha = -39.5°$. Although the ^{13}C powder pattern widths of AlaAla and AcGlyAlaNH$_2$ differ by only 2%, the anisotropy and asymmetry parameters differ by 10 and 25%, respectively. The principal values for the ^{15}N chemical shift tensor are $\sigma_{11} = 65.3$, $\sigma_{22} = 78.1$, and $\sigma_{33} = 215.5$ ppm, and the orientation of the $^{13}C-^{15}N$ bond to the σ_{33} axis is 106°. AlaAla and AcGlyAlaNH$_2$ show a striking difference in asymmetry parameters (0.06 vs. 0.16) and anisotropy (144 vs. 165).

We report here the ^{13}C and ^{15}N chemical shift tensors of polycrystalline L-[1-^{13}C]alanyl-L-[^{15}N]alanine (AlaAla). This is the first determination in a nonglycine dipeptide of the polar angles relating the $^{13}C-^{15}N$ bond to the ^{13}C and ^{15}N chemical shift tensors. Absolute orientation to the molecular frame is dependent on analogy to single-crystal studies which place σ_{33} of the ^{13}C chemical shift tensor perpendicular to the peptide plane[2] and σ_{33} of the ^{15}N chemical shift tensor in the peptide plane.[3] Although ^{13}C chemical shift tensors have been studied extensively,[4] the only complete acyl ^{13}C tensor determined in an amide group is reported for the dipeptide GlyGly·HCl.[2] Studies of ^{15}N chemical shift

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(2) Stark, R. E.; Jelinski, L. W.; Ruben, D. J.; Torchia, D. A.; Griffin, R. G. *J. Magn. Reson.* **1983**, *55*, 266.

(3) Harbison, G. S.; Jelinski, L. W.; Stark, R. E.; Torchia, D. A.; Herzfeld, J.; Griffin, R. G. *J. Magn. Reson.* **1984**, *60*, 79.

(4) Mehring, M. *Principles of High Resolution NMR in Solids*; Springer-Verlag: New York, **1983**; p 231.

0002-7863/87/1509-5966$01.50/0