Simultaneous Characterization of the Amide ^{1}H Chemical Shift, ^{1}H−^{15}N Dipolar, and ^{15}N Chemical Shift Interaction Tensors in a Peptide Bond by Three-Dimensional Solid-State NMR Spectroscopy

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Three-dimensional correlation spectra[1] of polycrystalline samples can be used to characterize the ^{1}H, ^{13}C, and ^{15}N chemical shift and dipolar interaction tensors essential for interpretation of experimental NMR studies, including structure determination by solid-state NMR spectroscopy,[2] applications of chemical shifts as restraints[3] for structure determination by multidimensional solution NMR spectroscopy, and descriptions of dynamics by relaxation analysis.[4] The spin interactions associated with the amide N−H groups of peptide bonds are particularly important in studies of proteins. The magnitudes of the principal elements of the amide ^{1}H chemical shift, ^{1}H−^{15}N dipolar, and ^{15}N chemical shift interaction tensors in a peptide bond can be measured directly from the discontinuities observed along the three independent frequency axes of the three-dimensional powder pattern spectrum of Ala-^{15}N-Leu[5] in Figure 1; the relative orientations of the tensors can be determined by comparisons of experimental and simulated two-dimensional spectra along selected planes, and their molecular orientations by reference to the heteronuclear dipolar interaction associated with the N−H bond. The three-dimensional powder pattern spectrum in Figure 1 represents the distribution of signal intensity from all orientations of the three operative spin interactions. The full spans of the ^{1}H and ^{15}N chemical shift interactions are present in two of the dimensions; however, since the ^{1}H−^{15}N dipolar coupling is symmetric about zero, only positive frequencies are shown in the third dimension. The distinctive appearance of this spectrum is due to the low-intensity "hole", which is a consequence of none of the principal elements of a chemical shift tensor being collinear with the dipolar coupling vector. These data are most readily analyzed by comparing selected experimental and calculated two-dimensional planes.[6] Notably, the two-dimensional ^{1}H separated local field spectrum can be extracted from the experimental three-dimensional spectrum. The experimental (Figure 2E) and calculated (Figure 2F) two-dimensional ^{1}H separated local field spectra have a much simpler appearance than the corresponding ^{15}N spectra in panels A and B of Figure 2 because σ_{11H} of the ^{1}H chemical shift tensor is collinear with the N−H bond axis. The experimental two-dimensional PISEMA[7] spectrum in Figure 2A, which is equivalent to the ^{15}N chemical shift/^{1}H−^{15}N dipolar coupling plane of the three-dimensional spectrum, is well simulated by the calculated spectrum in Figure 2B where σ_{33N} is tilted 17° from the N−H bond axis. The principal values of the chemical shift tensors can be measured with a precision of about 1 ppm from the spectra in Figures 1 and 2; $\sigma_{11} = 3$, $\sigma_{22} = 8$, and $\sigma_{33} = 17$ ppm (relative to TMS) for ^{1}H, and $\sigma_{11} = 64$, $\sigma_{22} = 77$, and $\sigma_{33} = 217$ ppm (relative to NH_3) for ^{15}N. Both of the experimental two-dimensional chemical shift/dipolar coupling spectra in Figure 2 are best simulated with a ^{1}H−^{15}N dipolar coupling of 19.5 ± 0.4 kHz, which corresponds to an N−H bond length of 1.07 ± 0.02 Å.

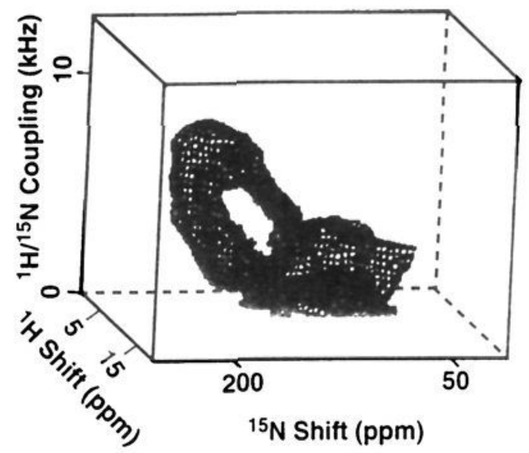

Figure 1. Three-dimensional ^{1}H chemical shift/^{15}N−^{1}H dipolar coupling/^{15}N chemical shift correlation spectrum of a 50 mg polycrystalline sample of Ala-^{15}N-Leu obtained on a home-built four-channel spectrometer with ^{1}H and ^{15}N resonance frequencies of 550 and 55.7 MHz. Twenty-four transients were coadded for each of 24 t_1 experiments with a dwell of 21.6 μs and 32 t_2 experiments with a dwell of 27 μs. The recycle delay of 5 s gave a total experimental time of 25.6 h.

In Figure 3, σ_{33N} is shown to be tilted by 17° away from the N−H bond based on the data in Figure 2A,B. Since the internal molecular reference is the single vector defined by the N−H bond, the placement of σ_{33N} in the peptide plane must utilize additional information, in this case the results from single-crystal studies on another dipeptide.[8] Any deviation of the orientation of σ_{33N} from the peptide plane would result in an equivalent rotation of the other two principal elements about the N−H bond axis. σ_{11N} is tilted by about 20° from the peptide plane based on the data in Figure 2,[9] and σ_{22N} is perpendicular to the σ_{11N}/σ_{33N} plane by symmetry. By contrast, the ^{1}H chemical shift/^{1}H−^{15}N dipolar coupling spectra in Figure 2E,F show, within the limits of the experimental signal to noise ratio, that σ_{11H} of the ^{1}H chemical shift tensor is collinear with the N−H bond vector. Further, the orientations of the ^{1}H and ^{15}N tensors are related by the Euler angles 45°, 90°, and 75°, which were determined from the analysis of the two-dimensional ^{1}H chemical shift/^{15}N chemical shift correlation spectrum in Figure 2C,D. This confirms the orientations of the principal elements determined from the spectra involving the dipolar couplings while showing that σ_{22N} and σ_{22H} project from the peptide plane in opposite directions and that σ_{33H} is in the peptide plane.

Both the magnitudes and orientations of the amide ^{1}H chemical shift tensor of Ala-^{15}N-Leu are in good agreement with

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(1) (a) Ramamoorthy, A.; Wu, C. H.; Opella, S. J. *J. Magn. Reson., Ser. B* **1995**, *107*, 88. (b) This pulse sequence separates and correlates spectral parameters associated with chemical shift and dipolar interactions of a pair of heteronuclei, and can be used with single-crystal, uniaxially oriented, and spinning samples, in addition to stationary powder samples.

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(5) The dipeptide was synthesized using solution-phase methods. (9-Fluorenylmethoxycarbonyl)-L-alanine (Peptides International, Louisville, KY) was coupled to *p*-tolylsulfonyl-^{15}N-leucine benzyl ester prepared from ^{15}N-leucine (Cambridge Isotope Laboratories, Woburn, MA) by a mixed anhydride coupling reaction. The resulting dipeptide was deprotected by catalytic hydrogenation using ammonium formate and 10% palladium on carbon in methanol and acetic acid. The final sample was crystallized from aqueous ethanol.

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(9) Although the simulations indicate that the spectra are not very sensitive to variations in the α angle when the β angle and the frequency difference between σ_{11} and σ_{22} are small, it is possible to estimate α_N to be 10°−30°.

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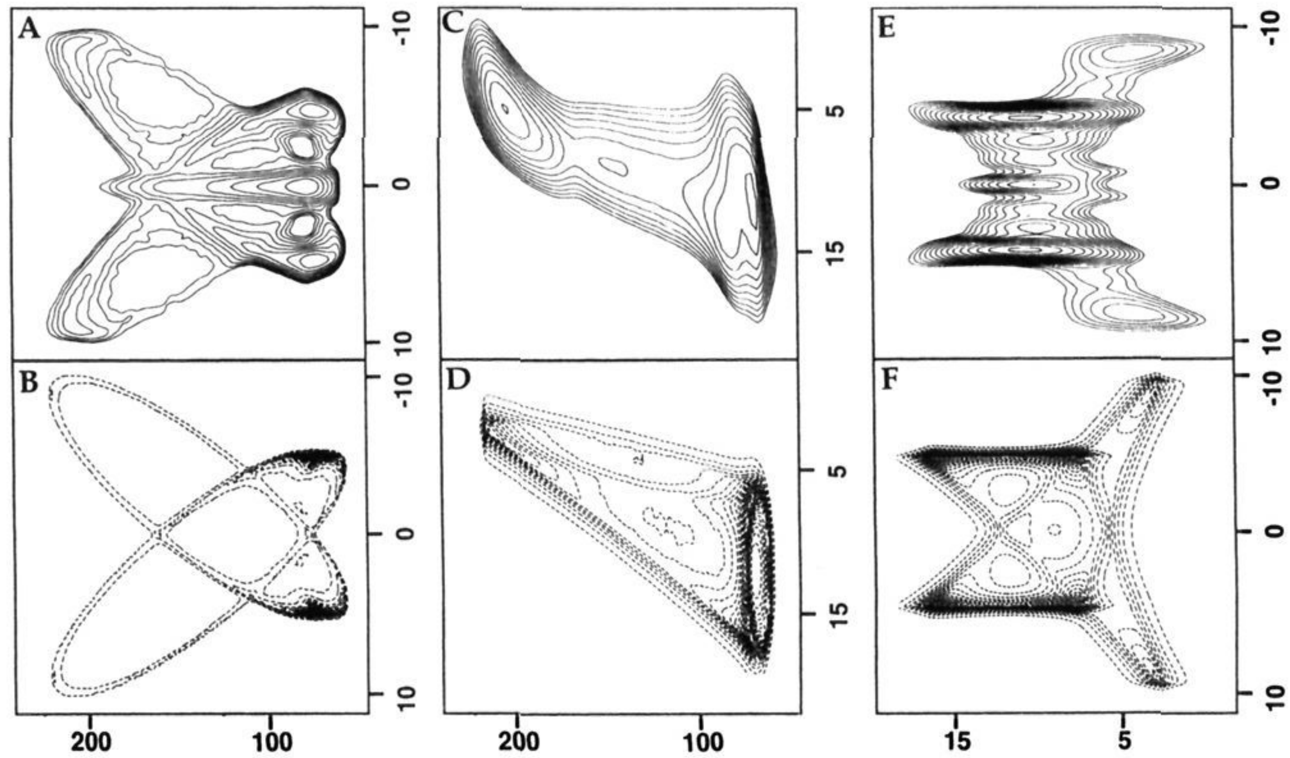

Figure 2. Comparison of experimental (top) and calculated (bottom) two-dimensional powder pattern spectra corresponding to orthogonal planes of the three-dimensional spectrum in Figure 1: (A, B) correlations of ^{15}N chemical shift and ^{1}H$-^{15}$N dipolar couplings; (C, D) correlations of ^{1}H chemical shift and ^{15}N chemical shift; (E, F) correlations of ^{1}H chemical shift and ^{1}H$-^{15}$N dipolar couplings. Spectra C and E were extracted from the experimental three-dimensional data in Figure 1 by adding together all of the planes along the third dimension. Spectrum A was obtained from a separate two-dimensional PISEMA[7] experiment since that yielded somewhat better resolution than the equivalent data out of the three-dimensional spectrum. $\alpha_i(i{=}\mathrm{N,H})$ is the angle between σ_{11i} and the projection of the N$-$H bond on the $\sigma_{11i}-\sigma_{22i}$ plane, while $\beta_i(i{=}\mathrm{N,H})$ is the angle between the σ_{33i} and the N$-$H bond. The principal axis systems of the ^{1}H and ^{15}N chemical shift tensors are related by the Euler angles α_{NH}, β_{NH}, and γ_{NH}. The external magnetic field orientations were generated randomly using 10^6 iterations in the Monte Carlo calculations for each two-dimensional spectrum. Each simulation took several minutes on a Silicon Graphics work station using a program written in C++.

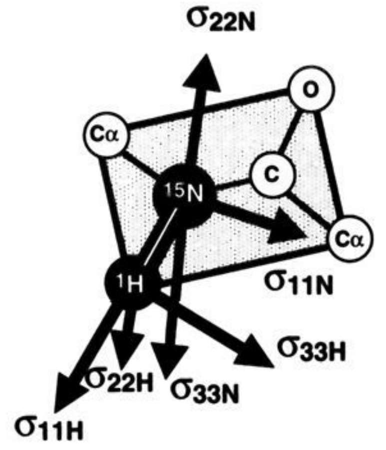

Figure 3. Orientations of the principal axes of the amide ^{1}H chemical shift, ^{1}H$-^{15}$N dipolar, and ^{15}N chemical shift interaction tensors in a peptide bond.

our previous determination on *N*-acetyl-valine.[10] The magnitudes of the principal values of the amide ^{15}N chemical shift tensors have been measured in a wide variety of peptide environments,[8,11,12] and the values for Ala-^{15}N-Leu are typical. Although we previously suggested on the basis of symmetry and chemical properties of amides and some of the initial data on peptides and proteins that $\sigma_{33\mathrm{N}}$ would be, on average, collinear with the N$-$H bond,[13] especially for residues other than Gly, as it is for the indole N$-$H of Trp and the π N$-$H of His[14,15] side chain sites, there now appears to be agreement among the data presented here and other examples in the literature[8,11] indicating that the ^{15}N tensor in peptide bonds has $\sigma_{33\mathrm{N}}$ oriented 15$-$20° away from the N$-$H bond axis. The N$-$H bond length, as reflected in the magnitude of the heteronuclear dipolar coupling, has been the subject of some controversy because most solid-state NMR measurements appear to give values somewhat longer than those arrived at by neutron diffraction.[16] The value determined from the data in Figures 1 and 2 is in good agreement with previous solid-state NMR measurements showing relatively long N$-$H bond lengths in model peptides,[17-19] but differs from the shorter bond lengths derived from larger dipolar couplings.[20] Since the magnitudes and orientations of the principal values of dipolar and chemical shift tensors can be determined in readily available powder samples with these experiments, it is now possible to characterize their variations among peptide sequences.

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