Determination of the Peptide Torsion Angle $\phi$ by $^{15}$N Chemical Shift and $^{13}$C$^{\alpha}$-$^{1}$H$^{\alpha}$ Dipolar Tensor Correlation in Solid-State MAS NMR

M. Hong,* J. D. Gross,* W. Hu,† and R. G. Griffin*+2

*Francis Bitter Magnet Laboratory and Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139; †Department of Polymer Science and Engineering, University of Massachusetts, Amherst, Massachusetts 01003

Received February 11, 1998; revised July 31, 1998

We demonstrate a dipolar-chemical shift correlation technique for sign-sensitive determination of the torsion angle $\phi$ in solid peptides and proteins under magic-angle spinning. The indirect dimension of the experiment is obtained by separate but synchronous evolution of the magnetization under the $^{15}$N chemical shift and the C-H dipolar coupling. The resulting sum and difference spectrum of the two frequencies, with more than ten independent sidebands, depends strongly on the relative orientation of the $^{15}$N chemical shift tensor and the C-H bond. The technique can distinguish $\phi$ angles over the full range of 360° when the amide $^{15}$N chemical shift tensor does not possess reflection symmetry with respect to the peptide plane. Thus it complements our previous HNCH experiment, in which two mirror-symmetric conformers of the H$^{\alpha}$-N bond relative to the C$^{\alpha}$-H$^{\alpha}$ bond around the N-C$^{\alpha}$ axis cannot be distinguished. © 1998 Academic Press

INTRODUCTION

The elucidation of the conformational structure of biological molecules such as polypeptides by nuclear magnetic resonance (NMR) spectroscopy has traditionally relied on the measurement of internuclear distances. Recently, however, several techniques were introduced which determine torsion angles by correlating two orientation-dependent interactions across the torsion axis of interest (1–7). By exploiting relatively strong spin interactions such as the chemical shift anisotropy or the uniaxial spin interaction, the torsion angle techniques yield precise conformational information such as the secondary structure of polypeptides. Such information would be obtained with less precision by distance techniques, since they require measuring very weak dipolar couplings with high accuracy. For determining the peptide torsion angle $\phi$ in the C(O)$_{a-1}$-N-C$^{\alpha}$-C(O)$_{a}$ segment, a 2D magic-angle spinning HNCH technique that we proposed recently seems particularly promising (8, 9). The $\phi$ torsion angle is extracted from the relative orientation of the N-H$^{\alpha}$ and C$^{\alpha}$-H$^{\alpha}$ bonds, which is probed by evolving double- and zero-quantum (DQ/ZQ) N-C$^{\alpha}$ spin states under the N-H and C-H dipolar interactions. The torsion-angle dependent dipolar sideband spectra are manifested in the $\omega_1$ dimension and are separated according to the C$^{\alpha}$ isotropic chemical shift in the $\omega_2$ dimension. Thus, the technique is capable of simultaneously measuring torsion angles for spectrally resolved residues. In addition, it can be shown the technique has higher angular resolution in biologically relevant conformational regions than distance measuring techniques (10).

Using dipolar couplings as structural probes has the advantage that the unique axis of the dipolar tensor in the molecular segment is known, a priori, to be oriented along the internuclear vector. This makes it straightforward to convert the NMR spectral parameters into molecular parameters. However, the axial symmetry of the dipolar interactions and the symmetry of the dipolar spectra with respect to $\omega_1 = 0$ also have drawbacks. The latter reduces the number of independent sidebands by a factor of two, which represents a significant reduction of the information content of the spectra. The axial symmetry of the interactions makes it impossible to distinguish H$^{\alpha}$-N-C$^{\alpha}$-H$^{\alpha}$ conformations that are equidistant from the trans (H$^{\alpha}$-N-C$^{\alpha}$-H$^{\alpha}$ = 180°) or cis (H$^{\alpha}$-N-C$^{\alpha}$-H$^{\alpha}$ = 0°) configurations in opposite directions. Thus, H$^{\alpha}$-N-C$^{\alpha}$-H$^{\alpha}$ torsion angles with the same magnitude but opposite signs yield identical dipolar spectra. In terms of the $\phi$ torsion angle, this corresponds to a degeneracy for pairs of angles ($\phi$, $\phi'$) that are symmetric with respect to $-120^\circ$ or $60^\circ$, i.e., $\phi + \phi' = -240^\circ$. Since this inability to distinguish mirror symmetric conformations stems from the uniaxiality of the dipolar interaction, it is also found in the dipolar-coupling-based distance measuring techniques.

To determine torsion angles unambiguously, at least one non-uniaxial spin interaction is required in the correlation scheme. Further, the orientation of this non-uniaxial interaction tensor must be such that no principal axis is perpendicular to the torsional axis (2). In the case of the torsion angle $\phi$, the anisotropic chemical shift of the backbone amide nitrogen is a suitable choice. The molecular orientation of the amide $^{15}$N chemical shift tensor has been studied in several model peptides (11–19) and was found...
to exhibit relatively small variations. The $\sigma_{22}$ axis lies close to, but not necessarily along, the normal to the peptide plane. Among the experimental studies found, deviations from normality range from 0° to 20° in different peptides. The $\sigma_{33}$ axis lies in the peptide plane and forms an angle of 135° to 140° with respect to the N-C$^\alpha$ bond. Once the $^{15}$N tensor orientation is known, correlation of the $^{15}$N chemical shift with an interaction at the C$^\alpha$ site should yield sign-specific information on the intervening torsion angle $\phi$.

In this paper, we demonstrate the correlation of the $^{15}$N chemical shift and the C$^\alpha$-$H^\alpha$ dipolar coupling tensor orientations under magic-angle spinning (MAS) to determine the $\phi$ torsion angle. The technique resolves different residues by detecting the $^{13}$C$^\alpha$ isotropic chemical shift in the second dimension of the 2D experiment. To obtain MAS sideband patterns of the sum and difference of the $^{15}$N chemical shift anisotropy (CSA) and the C$^\alpha$-$H^\alpha$ dipolar coupling, separate but synchronous evolution under these two interactions (9, 20) is employed. Using the well-characterized model peptide N-acetyl-D, L-valine (NAV), we show that the technique exhibits the highest sensitivity to $\phi$ torsion angles in the $\beta$-sheet structure region. The requirements for a full determination of the $\phi$ torsion angle, including its sign, are discussed.

**THEORY**

In this section, we first describe the pulse sequence for correlating the backbone $^{15}$N chemical shift interaction with the C$^\alpha$-$H^\alpha$ dipolar coupling. We then show mathematically how the $\phi$ torsion angle affects the time and frequency signals of the indirect dimension of the 2D spectrum. Last, we present the simulated $^{15}$N CSA/C$^\alpha$H$^\alpha$ spectra as a function of the $\phi$ angle.

**Pulse sequence with synchronous $^{15}$N CSA/$^{13}$C-$^1$H evolution.** The radio frequency (RF) pulse sequence for the experiment is shown in Fig. 1. The main feature of the experiment is that the magnetization evolves rotor synchronously under the C-H dipolar coupling and the $^{15}$N chemical shift interaction during two separately but synchronously incremented $t_1$ periods.

After cross polarization from $^1$H to $^{13}$C, antiphase magnetization $C\phi N_\phi$ is created by recoupling the N-C dipolar interaction with a REDOR sequence, which consists of alternating $180^\circ$ pulses on $^{13}$C and $^{15}$N channels every half rotor cycle for two rotor periods (21). The magnetization evolves under the C-H dipolar interaction for a period defined by an MREV-8 multiple-pulse train, which attenuates the homonuclear $^1$H-$^1$H coupling (22). The maximum MREV-8 duration or evolution time is one rotor period. The $^{13}$C evolution period is part of a two-rotor-cycle constant time period that serves to refocus the $^{13}$C chemical shift interaction and to maintain rotor phase coherence between the $^{13}$C and the $^{15}$N evolution periods. At the end of the constant time, the $^{13}$C magnetization is transferred to $^{15}$N spins by two simultaneous $90^\circ$ pulses on the $^{13}$C and $^{15}$N channels. The resulting $N_\phi C\phi_\phi$ magnetization evolves under the $^{15}$N chemical shift interaction with proton decoupling. A moving $^{15}$N $180^\circ$ pulse not only matches the $^{15}$N evolution time to the C-H coupling period but also doubles the apparent magnitude of the $^{15}$N CSA. The doubling makes the effective strengths of the $^{15}$N CSA and C-H coupling comparable, thus leading to enhanced spectral variation with respect to the torsion angle (9, 23). Again, to maintain synchronization with sample rotation, the $^{15}$N evolution period is placed in a constant time of one rotor cycle. At the end of the $^{15}$N evolution, another pair of $90^\circ$ pulses and a REDOR mixing period convert the coherence $C\phi N_\phi$ into observable $^{13}$C magnetization, which is detected during $t_2$. Absorptive, sign-sensitive spectra in $\omega_1$ are obtained by recording the cosine and sine components of the $^{15}$N chemical shifts in separate data sets according to the method of Ruben and co-workers (25).

Similar to the double- and zero-quantum HNCH technique introduced earlier (8), the current experiment gives rise to the sum of two interaction frequencies in the $\omega_1$ dimension. However, this is achieved by separate evolutions of the $^{13}$C and $^{15}$N antiphase magnetizations rather than the simultaneous evolution of the double- and zero-quantum N-C coherence. The separation of the two evolution periods is necessary since the protons need to be coupled to $^{13}$C$^\alpha$ in order to measure the C-H
couplings but be decoupled from $^{15}\text{N}$ for measuring the $^{15}\text{N}$ chemical shift. The advantage of the synchronous evolution can be understood by comparing it with an alternative 3D experiment with two independently incremented evolution periods. Synchronous evolution reduces signal-averaging time considerably, thus making the experiment more applicable to large biomolecules. It also avoids undesirable dispersive sideband lineshapes that could be present in the anisotropic planes of the 3D experiment (26).

Alternatively, in-phase magnetizations ($C_x, N_x$) could be employed in the evolution periods. This would require the use of cross polarization (CP) between the two low-$\gamma$ nuclei (27). Several variable-amplitude spin lock methods should be considered in implementing such a double CP experiment in order to obtain maximum transfer efficiency (28–30).

**Torsion-angle dependence of the time signal.** The pulse sequence modulates the spin density operator during $t_1$ according to $\cos(\Psi_{\text{CH}}(t_1))\exp(i\Psi_{\text{N}}(t_1))$, where $\Psi_{\text{CH}}(t_1) = \int_0^{t_1} dt \omega_{\text{CH}}(t)$ is the dynamic phase accumulated under the interaction $\lambda$. This modulation can be rewritten in terms of the sum and difference of the two phases,

$$\frac{1}{2}[\cos(\Psi_{\text{CH}}(t_1)) + \cos(\Psi_{\text{N}}(t_1))]. \quad [1]$$

where the sum and difference phases

$$\Psi_{\text{N}}(t_1) \pm \Psi_{\text{CH}}(t_1) = \int_0^{t_1} dt (\omega_{\text{N}}(t) \pm \omega_{\text{CH}}(t)) \quad [2]$$

contain integrals over the sum and difference frequencies of the chemical shift and dipolar coupling. Such sum and difference frequencies depend on the relative orientation of the $^{15}\text{N}$ chemical shift and C-H interaction tensors. Since the two principal axes systems (PAS) are fixed in the two segments across the torsion axis N-C$^{\alpha}$, and neither tensor is uniaxial along this axis, the relative orientation of the two tensors reflects the torsion angle $\phi$ around this bond. If the orientation of each interaction PAS is expressed by Euler angles $\Omega_{\lambda} = (\alpha_{\lambda}, \beta_{\lambda}, \gamma_{\lambda})$, and if the N-C$^{\alpha}$ bond is chosen as the $z$-axis of the molecular frame, then $\gamma_{\text{N}} - \gamma_{\text{CH}}$ corresponds to the torsion angle $\phi$, apart from a constant that corresponds to the $\gamma$-difference in the $\phi = 0^\circ$ conformation.

In exact analogy to standard MAS theory, it can be shown that the sidebands obtained from the sum and difference frequencies for an isotropic sample are absorptive and positive (31, 32), as long as the two evolution periods $t_1$ are equal in length and start at identical rotor orientations. This is ensured in the pulse sequence by constant-time evolution of the $^{13}\text{C}$ and $^{15}\text{N}$ coherences.

**Simulated $^{15}\text{N}$ CSA/C$^{\alpha}$-H$^\alpha$ correlation spectra.** To demonstrate the sensitivity of the $^{15}\text{N}$ CSA/C$^{\alpha}$-H$^\alpha$ spectra to $\phi$, we calculated the rotational sideband spectra as a function of $\phi$. The spectra are calculated in the time domain by analytical evaluation of Eq. [1] over the course of the evolution period. The N-C dipolar coupling, which is not averaged out during the $^{15}\text{N}$ chemical shift evolution, is also included. Each FID is calculated for one rotor period of 360 $\mu$s using a $t_1$ dwell time of 18 $\mu$s. It is replicated many times to produce an apparent acquisition time of 18.4 ms, then exponentially broadened by 150 Hz, and finally Fourier transformed to give the frequency spectrum.

As shown in Fig. 2, the common molecular frame in the simulation is chosen with the $z$-axis along the N-C$^{\alpha}$ bond and with the $x$-axis in the H$^\alpha$C$^{\alpha}$N plane. In this common frame, the Euler angles describing the C-H bond orientation are $\Omega_{\text{CH}} = (0^\circ, 109.5^\circ, 0^\circ)$, while those for the $^{15}\text{N}$ chemical shift tensor orientation are $\Omega_{\text{N}} = (\alpha_{\text{N}}, \beta_{\text{N}}, \gamma_{\text{N}}) = (-20^\circ, 137^\circ, 240^\circ - \phi)$. The choice of $\alpha_{\text{N}} = -20^\circ$ is based on a recent report of the $^{15}\text{N}$ shift tensor orientation in Ala-$^{15}\text{N}$-Leu (13), where correlation of the N-H coupling, $^1\text{H}$ shift, and $^{15}\text{N}$ shift tensors in a 3D spectrum yielded $\alpha_{\text{N}}$ of 10$^\circ$ to 30$^\circ$. It differs from a previous result on NAV ($\alpha_{\text{N}} \approx 0$), which was obtained by correlating the N-H dipolar coupling with the $^{15}\text{N}$ tensor (11). However, as stated in that paper, the experiment mainly determined the angle between the N-H bond and the $\sigma_{33}$ axis and could not constrain the orientations of the $\sigma_{11}$ and $\sigma_{22}$ axes sufficiently due to the small asymmetry of the tensor. A detailed RMSD analysis shown below in Fig. 8 indicates that $\sigma_{54} = 0$ is inconsistent with the experimental spectrum of NAV (Fig. 6).

Other simulation parameters include the effective C-H coupling (10.4 kHz), the $^{15}\text{N}$ CSA ($-8.51$ kHz), the N-C coupling (1.0 kHz), the REDOR mixing time (720 $\mu$s), and the spinning
speed (2.778 kHz). These parameters are taken from the experimental spectrum of NAV shown in Fig. 6. The number of crystallites in the powder average is 5000.

Figure 3 exhibits the calculated $^{15}$N CSA/C$\alpha$-H$^a$ rotational sideband spectra as a function of $\phi$. The spectra show significantly asymmetric sideband envelopes with respect to the zero frequency, in marked contrast to the symmetric spectra of the dipolar HNCH experiment. Correspondingly, nearly twice as many independent sidebands are available from these CSA-based spectra, which aids the determination of the best-fit $\phi$ angle. Pairs of spectra with complementary $\phi$ angles $\phi_1 + \phi_2 = 120^\circ, -240^\circ$ that cannot be distinguished by pure dipolar correlation (8) are displayed side by side. Though similarities still remain, the spectral differences are well visible, especially when $\phi$ corresponds to the $\beta$-sheet conformation ($-160^\circ$ and $-80^\circ, -140^\circ$ and $-100^\circ$). The polarity of the spectra reverses between $-20^\circ$ and $-60^\circ$, and between $140^\circ$ and $180^\circ$.

RESULTS AND DISCUSSIONS

C-H dipolar coupling. The correlation of the $^{15}$N CSA and C$\alpha$-H$^a$ dipolar coupling requires knowledge of the size and the orientation of these interactions. The size of the C$\alpha$-H$^a$ dipolar coupling is scaled from the one-bond coupling constant by the MREV-8 sequence. Since only the product of the rigid-limit coupling strength and the MREV-8 scaling factor is relevant for the current experiment, it is unnecessary to have precise knowledge of each parameter independently. In fact, the one-bond C-H coupling constant is known to be smaller than the theoretical value due to vibrational averaging effects (33), and the scaling factor of MREV-8 is cumbersome to measure. Thus, we determined the scaled C-H dipolar couplings using a DIPSHIFT experiment in which the C-H couplings are separated according to the $^{13}$C chemical shift (34). The MREV-8 parameters and the $t_i$ dwell time are set to be the same as in the torsion angle experiment. Figure 4a shows the $\omega_2$ projection of the 2D spectrum, which exhibits the expected five aliphatic resonances and the carbonyl sideband manifold. Taking the $\omega_2$ spectrum of the C$\alpha$ resonance at 55 ppm (Fig. 4b), we observe a symmetric rotational sideband pattern, whose intensities are fit to yield a dipolar coupling strength of 10.4 ± 0.5 kHz (Fig. 4c).
The principal values of the $^{15}$N chemical shift tensor exhibit non-negligible variations among different peptides. Fortunately, they can be measured conveniently by one-dimensional (1D) CP experiments in the case of a single $^{15}$N site or by two-dimensional (2D) anisotropic-isotropic chemical shift separation techniques (35–38) in the case of multiple $^{15}$N sites. The asymmetry parameter ($\eta$) of the amide $^{15}$N chemical shift tensor in peptides varies from as low as $<0.1$ (mainly glycine) to as high as $>0.35$ (14, 16, 17, 19).

The $^{15}$N chemical shift anisotropy of NAV was measured by a static $^{15}$N CP experiment. The powder pattern (Fig. 5a) is characterized by an anisotropy of $\delta = \sigma_3 - \sigma_{iso} = 106$ ppm and an asymmetry parameter of $\eta = 0.27$. In the 9.4-T magnetic field used, this CSA (4.3 kHz) amounts to less than half of the one-bond C-H dipolar coupling constant (ca. 11 kHz). This justifies the doubling procedure used in the pulse sequence (9), since the maximal spectral variation with the torsion angle occurs when the two interaction strengths are comparable.

To ensure that the CSA-doubled $^{15}$N spectrum obtained under MAS is consistent with the static spectrum, we performed a control experiment using the pulse sequence of Fig. 1 but without CH dipolar evolution under MREV-8 decoupling. This yields a pure $^{15}$N CSA spectrum without the C-H dipolar coupling. As shown in Fig. 5c, the $^{15}$N sideband spectrum indicates that the width of the shift anisotropy is indeed doubled with respect to the powder pattern. Further, the sense of asymmetry of the sideband spectrum is the same as the static spectrum under the phase cycles for the $^{15}$N 180° pulse and for the receiver. Knowledge of the spectral asymmetry is important for simulating the torsion angle spectra with the correct sign. A best-fit simulation of the pure $^{15}$N CSA sideband spectrum is shown in Fig. 5d. In this simulation, the unrefocused N-C dipolar coupling with a double magnitude (2 kHz) is taken into account.

$^{15}$N CSA/C$_a$-H$_a$ correlation spectra. The $^{15}$N CSA/C$_a$-H$_a$ correlation spectrum of NAV is shown in Fig. 6. In the $\omega_2$ dimension (Fig. 6a), only the C$_a$ and the acetyl carbonyl resonances survive due to the relatively short N-C transfer time used. The $\omega_1$ cross section of the C$_a$ peak (Fig. 6b) displays an asymmetric spectral envelope with much higher intensities in the first three orders of sidebands than those in Fig. 5c due to the contribution of the C-H coupling. The best-fit simulation of the spectrum (Fig. 6c), obtained with $\alpha_N = -25^\circ$, yields $\phi = -140^\circ$, which agrees well with the x-ray crystal structure of $\phi = -136.5^\circ$ (40). The choice of $\alpha_N$ in the best-fit simulation is discussed in more detail below.

Angular resolution. In order to quantify the angular resolution of the $^{15}$N CSA/C$_a$-H$_a$ correlation technique, i.e., the

![FIG. 5](image)

![FIG. 6](image)
degree of difference between spectra of different torsion angles, we calculated the root-mean-square deviations (RMSD) between pairs of simulated spectra. As described previously (9), the RMSD is defined as

$$\text{RMSD}(\phi, \phi') = \left( \frac{1}{N} \sum_{i=1}^{N} \left( S_i(\phi) - S_i(\phi') \right)^2 \right)^{1/2}, \tag{3}$$

where $S_i(\phi)$ is the normalized intensity of the $i$th sideband, out of $N = 14$ sidebands in a spectrum of torsion angle $\phi$. The result for $72^2$ pairs of $\phi$-angle spectra, calculated in increments of $5^\circ$ for $-180^\circ \leq \phi \leq 180^\circ$ and with $\alpha_N = -25^\circ$, is shown in a 2D RMSD contour plot (Fig. 7) as a function of $\phi$ and $\phi'$. Each spectrum is averaged over 648,000 crystallite orientations. The intensities of the contour lines reflect the spectral variation due to the $\phi$ angle. The higher the contour intensity, the larger the spectral difference. Since RMSD($\phi, \phi$) = 0, the contour intensity along the main diagonal of the 2D is zero. Near the diagonal between about $-180^\circ$ and $-15^\circ$, the contour lines are more densely spaced, indicating significant spectral changes, or high angular resolution. Several cross sections through the 2D contour plot are also shown. In these cross sections, the relative widths of the global minima at a fixed RMSD value indicate the relative angular resolution. For example, the angular resolution at $\phi = -140^\circ$, which corresponds to the $\beta$-sheet structure, is better than that around $\phi = -60^\circ$, which corresponds to the $\alpha$-helix conformation. It can also be seen that the traces for $-100^\circ$ and $-140^\circ$, which are identical in the pure dipolar HNCH correlation experiment, are clearly different here. This shows that even a relatively small deviation of the $^{15}$N $\sigma_{11}$ axis from the peptide plane ($\alpha_N = -25^\circ$) can effectively remove the spectral degeneracy around $\phi = -120^\circ$. The similarity between the $-60^\circ$ and the $-180^\circ$ traces results from one of the three discrete degeneracies in the full 2D contour plot. Finally, in both the $-60^\circ$ and the $-140^\circ$ cross sections, the RMSD values differ greatly between $\phi = -140^\circ$ and $\phi = -60^\circ$, indicating that the $\beta$-sheet conformation and the $\alpha$-helix structure can be clearly distinguished.

**Requirements for full-range $\phi$ determination.** In our previous HNCH correlation experiments, the spectra for pairs of $\phi$ angles centered around $-120^\circ$ and $+60^\circ$ are identical. These degeneracies occur because the relative orientation of the unique axes of two dipolar tensors is characterized by only one parameter, the angle between the axes. The correlation involving the non-uniaxial $^{15}$N chemical shift interaction is less prone to such degeneracies, since the relative tensor orientation depends on two angles, which are the polar coordinates of the C$^\alpha$-H$^\alpha$ dipolar axis in the $^{15}$N chemical shift principal axes system. As discussed previously (1), systematic degeneracies occur only if a principal axis of either tensor is perpendicular.

**FIG. 7.** The 2D RMSD contour plot for quantifying the angular resolution of the $^{15}$N CSA/C$^\alpha$-H$^\alpha$ technique. The $^{15}$N CSA/C$^\alpha$-H$^\alpha$ spectra were calculated with $\alpha_N = -25^\circ$ and as a function of $\phi$ at $5^\circ$ increments, giving $72^2$ points in the 2D plot. The contour heights correspond to the difference between spectra for the pair of $\phi$ angles defined by the $x$- and $y$-position. Three points of exactly vanishing RMSD in the lower right half of the plot are indicated by dots. Regions of $\beta$-sheet and $\alpha$-helix conformations are emphasized by bars. Several cross sections through the 2D plot at the indicated $\phi$ values are shown on the right.
to the torsion bond. For a uniaxial interaction, one of the degenerate principal axes always fulfills this condition. For the peptide $^{15}$N chemical shift, one principal axis would be perpendicular to the N-C$^\alpha$ bond if the azimuthal angle $\alpha_N$ of that bond in the chemical shift PAS is 0°, 90°, 180°, or 270°. The additional case of the polar angle $\beta_N = 90°$ can be safely excluded based on the literature data for the $^{15}$N chemical shift tensor orientation, where $\beta_N \approx 140°$ (13, 14). For $\alpha_N = 0°$, $\pm90°$, or $180°$, the degeneracy is the same as in the pure dipolar HNCH experiments. It is centered around $\phi = −120°$ and $+60°$, so that, for instance, the spectra for $\phi = −140°$ and $−100°$ are identical. For $\alpha_N \neq 0°$ and the given orientation of the $^{15}$N CSA tensor, only three discrete degeneracies occur. They can be identified as isolated minima at the zero level in Fig. 7.

While the angle $\beta_N$ between the N-C$^\alpha$ bond and the $\alpha_{33}$ axis of the $^{15}$N chemical shift tensor does not vary significantly among different amino acid residues, $\alpha_N$ values between 0° and 20° have been estimated from correlation of the $^{15}$N chemical shift anisotropy with a segment-fixed dipolar coupling (13, 16). If the peptide group were strictly planar and the chemical shift only reflected the local symmetry, then $\alpha_N = 0°$, $180°$ or $\pm90°$ would be required. However, the peptide group is not completely planar in NAV according to the x-ray crystal structure (39). In addition, long-range $\gamma$-gauche effects in $\beta$-branched amino acid sidechains can shift the $^{15}$N tensor orientation further away from $\alpha_N = 0°$ (40).

We investigated the effect of the angle $\alpha_N$ on the determination of the best-fit $\phi$ torsion angle by calculating the RMSD between the experimental and the simulated torsion angle spectra as a function of $\phi$ for several different values of $\alpha_N$ (Fig. 8). As expected, the traces for $\alpha_N = 0°$ and 90° exhibit the undesired symmetry around $\phi = −120°$ and $\phi = 60°$, which is the same as that observed in the HNCH experiment. The orientation of $\alpha_N = 0°$ can be safely excluded because the fits indicate relatively large RMSD values, while $\alpha_N = −90°$ is completely inconsistent with the literature. For $\alpha_N$ between $−20°$ and $−90°$, a pronounced minimum is observed near $\phi = −140°$, which is close to the value found in the x-ray crystal structure ($\phi = −136.5°$). The exact position of the RMSD minima for $\alpha_N$ between $−25°$ and $−90°$ are not very different. This can be understood because changing $\alpha_N$ with fixed $\beta_N$ leaves the $\alpha_{33}$ axis unaffected but changes the orientations of the $\alpha_{22}$ and $\alpha_{11}$ principal axes. Since these two principal values are relatively similar, the resulting spectral variations are small. However, we point out that for $\alpha_N$ values of the opposite sign the minimum appears at $\phi = −100°$. Thus, a full-range determination of $\phi$ requires knowledge of the sign of $\alpha_N$. For NAV, given the x-ray crystal structure and the small absolute values of $\alpha_N$ in the literature, the sign of $\alpha_N$ must be negative and its most probable value is $−25°$. Note that other NMR techniques that correlate the $^{15}$N CSA tensor with the N-C$^\alpha$, N-C$^\beta$, or N-H dipolar couplings cannot yield the sign of $\alpha_N$. The observed insensitivity of the best-fit $\phi$ torsion to $\alpha_N$ within the relevant angular range means that it is possible to determine $\phi$ even without precise knowledge of $\alpha_N$. Due to the significant difference in the N-H dipolar and $^{15}$N chemical shift tensor orientations, their correlation with the C-H dipolar coupling responds differently to $\phi$-angle changes. This fact, combined with the asymmetry of the CSA/dipolar sideband spectrum, makes the $^{15}$N CSA/C$\alpha$-H$^\alpha$ experiment useful for corroborating the result of the HNCH experiment and increasing its precision in regions where the $\phi$ resolution of the latter is low.

**CONCLUSIONS**

We demonstrated a dipolar-chemical shift correlation technique for determining the peptide torsion angle $\phi$ potentially over the full range of 360°. The technique breaks the spectral degeneracy in previous HNCH correlation experiments by employing the asymmetric chemical shift interaction of the peptide backbone $^{15}$N. Correlation of the $^{15}$N CSA and C$\alpha$-H$^\alpha$ coupling interaction yields the relative orientation of the two tensors and hence the H$^\alpha$-N-C$\alpha$-H$^\alpha$ torsion angle. Doubling of the magnitude of the $^{15}$N CSA relative to the C$\alpha$-H$^\alpha$ dipolar coupling achieves an angular resolution of better than $±10°$ in the biologically interesting $\beta$-sheet region, as estimated by an RMSD analysis. Because of the asymmetry of the $^{15}$N-CSA/C$\alpha$-H$^\alpha$ spectrum, the number of independent conformation-sensitive sidebands is doubled compared to the pure dipolar...
HNCH experiment. If the orientation of the $\sigma_{11}$ axis of the $^{15}\text{N}$ chemical shift principal-axes system is exactly known, then the twofold degeneracy of the $\phi$ angle is removed and the torsion angle can be determined unambiguously in the full 360° range. Even if the orientation of the $\sigma_{11}$ axis is not known, the best-fit procedure still provides a good estimate of $\phi$, but with a twofold degeneracy. This $^{15}\text{N}$ CSA/C$^\text{a}$-H$^\text{m}$ experiment should be used in conjunction with the HNCH experiment to increase the angular precision of the $\phi$ determination. The experiment, demonstrated here on $^{15}\text{N}$-enriched but $^{13}\text{C}$-natural abundance NAV, has sufficient sensitivity to be applied to larger $^{13}\text{C}$- and $^{15}\text{N}$-labeled peptides and proteins for the simultaneous determination of many $\phi$ torsion angles.

**EXPERIMENTAL**

Powder NAV was obtained from Cambridge Isotope Laboratories, Inc. (Andover, MA) and was recrystallized in aqueous solution. The spectra were measured on a homebuilt spectrometer operating at 397.8 MHz for $^1\text{H}$, 100.0 MHz for $^{13}\text{C}$, and 40.3 MHz for $^{15}\text{N}$ nuclei. A homebuilt triple resonance probe with a transmission line design and equipped with a 4-mm Chemagnetics (Fort Collins, CO) MAS spinning module was used. Proton RF fields of about 100 kHz were used for excitation and CW decoupling. The $^1\text{H}$ 90° pulse length for MREV-8 multiple-pulse decoupling was 3 $\mu$s. Semi-window-less MREV-8 was used and incremented in half cycles of 18 $\mu$s during the C-H evolution period. The spinning speed was 2778 Hz and was controlled by a Doty (Columbia, SC) spinning speed controller to within 2 Hz.

**ACKNOWLEDGMENTS**

This research at MIT was supported by the National Institute of Health (GM-23403, GM-23289, and RR-00995). M. Hong was supported by a NIH postdoctoral fellowship (GM-18870). The authors thank Prof. K. Schmidt-Rohr for helpful discussions.

**REFERENCES**

23. T. Gullion and J. Schaefer, Detection of weak heteronuclear dipolar coupling by rotational-echo double-resonance nuclear magnetic


