Local Structure and Relaxation in Solid-State NMR: Accurate Measurement of Amide N−H Bond Lengths and H−N−H Bond Angles

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Solid-state nuclear magnetic resonance (SSNMR) provides important approaches for elucidating molecular structure in situations that cannot be studied by solution NMR or X-ray diffraction. Hence, membrane proteins,[1,2] large enzyme inhibitor complexes,[3] and reaction intermediates[4] represent classes of systems that can be uniquely examined with SSNMR. In these investigations structural data has been gathered either from chemical shifts, or more recently from a variety of dipolar recoupling techniques that restore ^{13}C−^{13}C and/or ^{13}C−^{15}N dipolar interactions during magic angle spinning (MAS).[1] Here we describe an experimental approach based on an MREV pulse sequence[5] that actively recouples the dipolar interaction between a low-γ nucleus (^{13}C, ^{15}N, etc.) and ^{1}H while decoupling the stronger ^{1}H−^{1}H interactions. With this type of recoupling we have performed high-resolution measurements of ^{15}N−^{1}H bond lengths (to ± 0.003 Å) and H−N−H bond angles (to ±3°) at relatively high MAS frequencies ($\omega_r/2\pi = 6-12$ kHz). Appropriate inclusion of relaxation effects proved to be crucial in the data analysis.

Previous SSNMR approaches for measuring ^{13}C− and ^{15}N−^{1}H bond lengths,[6] although very accurate, are limited by constraints on the MAS frequency since high-frequency spinning averages the dipolar coupling and attenuates the dipolar sidebands, that are the source of information in the spectra. The experiment described here produces a *powder line shape* in the indirect dimension that is, in principle, independent of the MAS frequency. Furthermore, because the technique is γ-encoded[7] the line shape exhibits two sharp singularities (cf. Figure 3), spaced at the scaled dipolar coupling. Unresolved couplings, experimental artifacts, and effects from relaxation predominantly appear around zero frequency and are easy to distinguish from the dipolar coupling in this line shape. This kind of recoupling can be realized by a C5 type pulse sequence[8,9] shown in Figure 1a, consisting of *n C* elements,[8] spanning one rotor period and successively phase shifted by $2\pi/n$. The *C* element here is based upon MREV-8, which eliminates the ^{1}H−^{1}H dipolar coupling,[5] but with an important modification: the effective field due to the ^{15}N−^{1}H dipolar coupling is purely transverse (see Figure 1b); hence we refer to the sequence as T-MREV (*t*ransverse *MREV*-8).

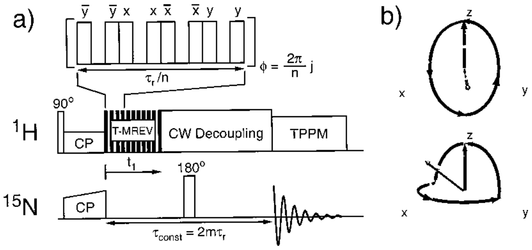

Figure 1. (a) Transverse MREV-8 (T-MREV) pulse sequence. The sequence is used as a *C* element[8] in a rotor-synchronized phase-shifting scheme, where each *C* element has the overall phase $j(2\pi/n)$, $j = 0, 1, ..., n - 1$, and one rotor period accommodates *n C* elements. In our experiments we used $n = 5$. (b) Illustration of the transverse (top) and tilted (bottom) effective precession axes produced by T-MREV and MREV-8 sequences.

The first-order average Hamiltonian[10] for the T-MREV experiment is

$$\tilde{H}_{IS}^{(1)} = (\kappa\omega^{(-1)}I_+ + \kappa^*\omega^{(1)}I_-)S_z \tag{1}$$

where κ is a complex scaling factor determined by the symmetry number *n* and the details of the *C* elements in the sequence (see Supporting Information) and $\omega^{(1)} = (\omega^{(-1)})^* = (b_{IS}/2\sqrt{2})\sin(2\beta)e^{i\gamma}$ is a singly rotating Fourier component in the MAS Hamiltonian for the dipolar coupling. *I* and *S* operators refer to the ^{1}H and low-γ spin, respectively. The dipolar coupling constant is given by $b_{IS} = -\gamma_I\gamma_S\hbar/r_{IS}^3\ (\mu_0/4\pi)$, where r_{IS} is the $I-S$ internuclear distance.

At the onset of the T-MREV irradiation (see Figure 1a) the system is in a transverse ^{15}N spin state ($|\rho(0)\rangle = |S_x\rangle$). In combination with the ^{15}N−^{1}H dipole coupling, T-MREV generates an effective Hamiltonian under which the spin system evolves from the ^{15}N state into a combined ^{15}N and ^{1}H state. In the absence of relaxation, this nutation is described by a simple rotation, $|\rho(t)\rangle = \cos(\omega t)|S_x\rangle + \sin(\omega t)|2S_yI_\psi\rangle$, where $\omega = |\kappa\omega^{(-1)}|$ is the scaled dipolar nutation frequency, and $I_\psi = \cos(\psi)I_x + \sin(\psi)I_y$, $\psi = \gamma - \lambda - \pi/2$, where γ is the powder angle and λ is the phase of the complex scaling factor κ. In solids the line width of the ^{1}H spectrum under homonuclear decoupling is typically an order of magnitude larger than for low-γ nuclei, such as ^{15}N. Therefore, during T-MREV the spin system evolves between two states characterized by different relaxation rates. This asymmetry will perturb the ideal unitary evolution of the density operator in a nontrivial way. Therefore, phenomenological relaxation must be included in the data analysis in order to extract quantitative molecular parameters from the experiment. Using an approach described elsewhere,[11] the average Liouvillian, *W*, in the equation $d/dt|\rho(t)\rangle = W|\rho(t)\rangle$, is constructed as the sum of the commutator superoperator, associated with the average Hamiltonian, and an average relaxation superoperator, calculated from a phenomenological transverse relaxation operator. The two-spin Liouville

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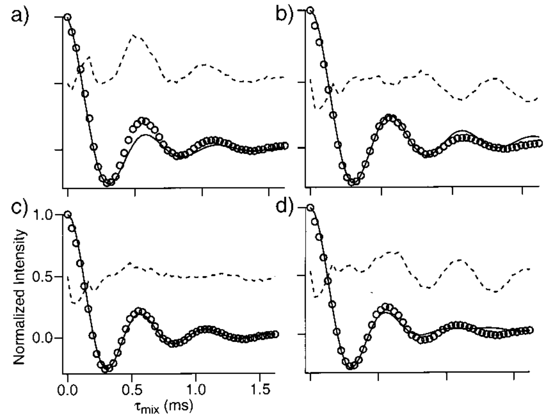

Figure 2. Indirect dimension (time domain) of the T-MREV experiment (○) on ^{15}N-acetyl-D,L-valine (NAV), simulations (—), and residuals (- - -) magnified by a factor 3 for clarity. (a) The best fit to the model with relaxation treated as a simple exponential damping of the time-domain data. (b) Best-fit to the analytical model including relaxation, using only one ^{15}N−^{1}H coupling. (c) Best-fit with inclusion of couplings to the two protons nearest to the ^{15}N. (d) As in (c) but with a fixed incorrect geometry ($\theta = 180°$). The data were acquired at a ^{1}H frequency of 360.3 MHz and at 6.173 kHz MAS, corresponding to a ^{1}H rf field of 92.59 kHz and 5-fold symmetry of the T-MREV supercycle. The best fits in (a)−(d) were obtained for direct N−H couplings of 10.53, 11.14, 10.98, and 11.22 kHz which correspond to N−H distances of 1.050, 1.030, 1.035, and 1.028 Å. The couplings given in this work were calibrated by using experimentally determined multiple pulse scaling factors obtained from NH HETCOR on $^{15}NH_4Cl$ with T-MREV as the homonuclear decoupling sequence (see Supporting Information). The inclusion of relaxation effects is imperative for extracting accurate molecular parameters, whereas the inclusion of the weaker coupling has a modest but detectable effect on the measured distance. The NAV data shown represents a favorable case since the H^{N}−N−H$^{\alpha}$ spin system is well isolated from other protons.

equation in the subspace spanned by the relevant states, $|S_x\rangle$ and $|2S_yI_\psi\rangle$, can be written

$$\frac{\mathrm{d}}{\mathrm{d}t}\begin{pmatrix}\langle S_x\rangle \\ \langle 2S_yI_\psi\rangle\end{pmatrix} = \begin{pmatrix}-1/T_2^S & -\omega \\ \omega & -1/T_2^{IS}\end{pmatrix}\begin{pmatrix}\langle S_x\rangle \\ \langle 2S_yI_\psi\rangle\end{pmatrix} \qquad (2)$$

where the effective relaxation constant $1/T_2^S$ can be set to zero, since the experiment is constant time and relaxation of the ^{15}N spin state is slow. Explicit calculation yields the relation $T_2^{IS} = (^3/_2)T_2^{IS,0}$, for the T-MREV sequence, where $T_2^{IS,0}$ is directly related to the line width of the ^{1}H spectrum. Equation 2 can be solved analytically (a detailed treatment will be given elsewhere) or numerically for a multiple spin system.

The ^{15}N T-MREV dephasing curve and simulations obtained from ^{15}N-acetyl-D,L-valine (NAV) in Figure 2 demonstrate that the relaxation model accurately reproduces the experimental data. However, it is also clear that the T-MREV spectrum is sensitive to the coupling to the nondirectly bonded H$^{\alpha}$ and the H^{N}−N−H$^{\alpha}$ angle θ, which is directly related to the torsion angle ϕ. The protons of the acetyl group in NAV have a motionally averaged distance to the amide ^{15}N of over 3 Å, and the N−H$^{\beta}$ distance is ~3.33 Å, whereas the N−H$^{\alpha}$ distance is ~1.98 Å. The H$^{\alpha}$ proton was therefore included in the simulation as the only distant proton, and the N−H^{N} and N−H$^{\alpha}$ distances were determined to be 1.035 and 1.97 Å, respectively. The angle θ was determined to be 130° ± 15°. As expected the N−H^{N} distance measured is longer than that obtained from diffraction studies[12] due to vibrational averaging.[13] However, the experimental N−H$^{\alpha}$ distance is almost identical to that obtained from the X-ray structure, and θ agrees well with the 141.4° found by X-ray diffraction. The dependence of the experiment on ϕ is subtle since this parameter affects θ in a nonlinear fashion. A full rotation through ϕ varies θ within a fairly narrow range, but even in this case there is a significant dependence as shown by the RMSD plot (see Supporting Information).

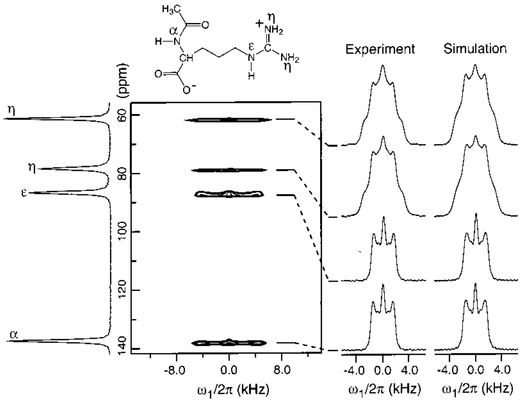

Figure 3. 2D T-MREV spectrum of [U-^{15}N]*N*-acetyl-L-arginine acquired at 500.0 MHz ^{1}H frequency. The line shapes of the NH$_2$ groups are very sensitive to the H−N−H angle in the range 100°−120°, ensuring an accurate determination of the bond angle. Best-fits of 118° and 119° agree well with the neutron diffraction structure of the closely related L-arginine[15] (117.7° and 118.3°). For the NH groups, the same dipolar coupling was found as in NAV, but with different relaxation parameters. A more accurate interpretation using multiple couplings was not attempted in this case. We note that the zero-frequency feature in the spectra, also observed in other studies,[7,9] is due to relaxation.

To investigate the sensitivity of the technique to the relative orientation of two N−H vectors, corresponding to couplings of equal magnitude, we applied T-MREV to [U-^{15}N]*N*-acetyl-L-arginine. The analysis of the resulting 2D T-MREV spectrum, shown in Figure 3, revealed a very strong dependence on the H−N−H angles in the guanidinium group (see Supporting Information). The large dynamic range of the experiment ensured an accuracy of ~±3° in the measurement of the H−N−H angles in the two NH$_2$ groups, despite the presence of weaker couplings not incorporated in the calculation.

In conclusion, T-MREV recoupling is useful for accurately constraining the local structure of NH and NH$_2$ groups at high MAS frequencies that are compatible with high magnetic fields. We expect the pulse sequence to be widely utilized as a general building block for NH or CH dephasing in composite experiments such as torsion angle experiments involving the NH or CH vector as a structural director.[14] Immediate applications of this methodology to characterization of NH hydrogen bonding in systems of biological relevance can be envisioned. The high angular resolution exemplified in Figure 3 translates into enhanced resolution of the structural parameters determined by these experiments.

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Supporting Information Available: Figures and Table (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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