Measuring Amide Nitrogen Quadrupolar Coupling by High-Resolution $^{14}$N/$^{13}$C NMR Correlation under Magic-Angle Spinning

Zhehong Gan

National High Magnetic Field Laboratory, 1800 East Paul Dirac Drive, Tallahassee, Florida 32310

Received November 30, 2005; E-mail: gan@magnet.fsu.edu

Along with hydrogen, carbon, and oxygen, the nitrogen atom constitutes one of the most important elements in organic, inorganic, and biological molecules. Among the two nitrogen isotopes, $^{15}$N NMR spectroscopy (0.37% natural abundance) has been used extensively with favorable NMR properties of a spin-1/2, while the more abundant $^{14}$N isotope (99.6%) has rarely been studied. The useful information on nitrogen electric field gradient (EFG) is accessible only through $^{14}$N quadrupolar coupling which usually dominates over other spin interactions.\(^1\) Direct NMR detection of $^{14}$N suffers from poor sensitivity and broad lines due to low-$\gamma$ properties and large quadrupolar interaction. The integer $^{14}$N spin precludes the presence of a single-quantum central-transition as observed for half-integer quadrupolar nuclei without the broadening from the first-order quadrupolar interaction. Observation of the overtone (1 ↔ −1) transition, both directly\(^8\)−\(^11\) and indirectly\(^12,13\) can avoid the first-order quadrupolar broadening, but the overtone signal intensity and excitation are second-order effects that dwindle at high magnetic fields. This communication shows that indirect $^{14}$N detection through nearby $^{13}$C under magic-angle spinning (MAS) can overcome the resolution and sensitivity limitations for acquiring $^{14}$N NMR spectra of solids. $^{14}$N peaks under MAS display large isotropic second-order quadrupolar shifts with relatively small anisotropic broadening, such that $^{14}$N quadrupolar coupling can be measured precisely from the $^{14}$N peak positions relative to their chemical shifts. The high-resolution $^{14}$N/$^{13}$C correlation experiment under MAS and the measurement of amide nitrogen quadrupolar coupling are demonstrated with a natural abundant tripeptide Ala-Gly-Gly (AGG).

Figure 1 shows the slightly modified HMQC-type pulse sequence used for two-dimensional (2D) $^{14}$N/$^{13}$C correlation under magic-angle spinning. Both $J$ and dipolar couplings contribute to the heteronuclear correlation. The scalar coupling is invariant under MAS, and typical coupling constants in proteins and polypeptides are $J_{	ext{SCD}} \sim 11$ Hz, $J_{	ext{SCA}} \sim 5$−8 Hz, and $J_{	ext{NCA}} \sim 3$−6 Hz (note the scaling by $\gamma$ relative to $^{15}$N).\(^14\) The slow buildup of two-spin coherence $C_i \sim \sin(\pi\delta t)C_iS_i$ competes with the decay from $^{13}$C $T_2$ relaxation. The much larger dipolar coupling is modulated by the sample rotation. The secular dipolar coupling term is averaged to zero under MAS. For $^{14}$N/$^{13}$C spin pairs, the nonsecular dipolar coupling term is modulated by the sample rotation. The secular dipolar coupling term is averaged to zero under MAS. For $^{14}$N/$^{13}$C spin pairs, the nonsecular dipolar coupling term is averaged to zero under MAS. For $^{14}$N/$^{13}$C spin pairs, the nonsecular dipolar coupling term is averaged to zero under MAS. For $^{14}$N/$^{13}$C spin pairs, the nonsecular dipolar coupling term is averaged to zero under MAS. The first-order quadrupolar interaction is averaged to zero by rotor synchronized $t_1$ and a precisely set magic-angle, similar to the satellite-transition magic-angle spinning (STMAS) experiment of half-integer quadrupolar spins.\(^24\) As the second-order quadrupolar shift has been well studied for half-integer quadrupolar nuclei, the $l = 0, 2, 4$ expansion coefficients for $S = 1$ spin are expressed here by their ratios to that of the central transition of half-integer spins\(^24\)

$$R_0 = -4/5, R_2 = 1/10, R_4 = -2/15 \quad (2)$$

It is important to note that the ratios for the anisotropic terms $R_2$ and $R_4$ are much smaller than the isotropic term $R_0$. This difference makes the isotropic second-order quadrupolar shift\(^24\)

$$\delta \nu_{Q}^{\text{iso}} = \frac{1}{20} \left( \frac{Z_q}{v_0} \right)^2, Z_q = \frac{eQ}{h} V_{xx}^{\nu} V_{yy}^{\nu} + V_{zz}^{\nu} = \sqrt{\frac{(3 + \eta^2)}{2}} C_q \quad (3)$$

much larger than the anisotropic broadening. For nitrogen, the downfield quadrupolar shift can be easily separated from the chemical shift obtained by a $^{15}$N NMR MAS experiment. For amide nitrogen, the quadrupolar shift can be over 600 ppm at 14.1 T (43.35 MHz $v_0$) with quadrupolar coupling constant in the range of 3−4 MHz. Such large shifts can cause alias along the indirect $^{14}$N dimension as the spectral window is restricted to the spinning frequency with rotor-synchronized evolution time $t_1$. Spectral de-aliasing may be necessary when measuring the quadrupolar shift. With the large shifts and small broadening, the...
modification to the HMQC pulse sequence is that the $^{13}$C acquisition starts immediately after the last $^{14}$N pulse. Full-echo acquisition with a long $\tau$ increases the signal/noise ratio by $\sim\sqrt{2}$.28

In conclusion, the $^{14}$N/$^{13}$C correlation experiment via $J$ and residual dipolar couplings under MAS overcomes the resolution and sensitivity limitations in measuring $^{14}$N NMR spectra. The result of a model polyepptide demonstrates that large quadrupolar couplings up to 4 MHz, such as those of amide nitrogen, can be measured precisely from the isotropic second-order quadrupolar shift. In the past, little has been learned on nitrogen quadrupolar effects for molecules with multiple nitrogen sites and large quadrupolar coupling constants. The indirect detection method has the advantages of high spectral resolution and sensitivity, making the measurement and the use of $^{14}$N quadrupolar coupling possible for large molecules. It should be mentioned that the measurement with AGG has been carried out with only $\sim1\%$ ($^{14}$C natural abundance) of the $^{14}$N spins in the sample. Isotope enrichment can increase the sensitivity by an order of magnitude for the use of $^{14}$N quadrupolar coupling in large and complex systems, such as proteins and nucleic acids.

Acknowledgment. This work has been supported by the National High Magnetic Field Laboratory through Cooperative Agreement (DMR-0084173) with the National Science Foundation and the State of Florida, and is dedicated to Professor David M. Grant, University of Utah, for his 75th birthday.

References


JT058597