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Communication

Solid-state nitrogen-14 nuclear magnetic resonance enhanced by dynamic nuclear polarization using a gyrotron

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ABSTRACT

By combining indirect detection of ^{14}N with dynamic nuclear polarization (DNP) using a gyrotron, the signal-to-noise ratio can be dramatically improved and the recovery delay between subsequent experiments can be shortened. Spectra of glassy samples of the amino acid proline doped with the stable bi-radical TOTAPOL rotating at 15.625 kHz at 110 K were obtained in a 400 MHz solid-state NMR spectrometer equipped with a gyrotron for microwave irradiation at 263 GHz. DNP enhancement factors on the order of $\varepsilon \sim 40$ were achieved. The recovery delays can be reduced from 60 s without radicals at 300 K to 6 s with radicals at 110 K. In the absence of radicals at room temperature, the proton relaxation in proline is inefficient due to the absence of rotating methyl groups and other heat sinks, thus making long recovery delays mandatory. DNP allows one to reduce the acquisition times of ^{13}C-detected ^{14}N spectra from several days to a few hours.

1. Introduction

The high natural abundance of ^{14}N makes it an attractive target to characterize nitrogen-containing compounds [1,2]. However, the detection of ^{14}N is a difficult task because of its quadrupolar coupling and its spin $I = 1$. Although quadrupolar couplings can be a rich source of structural and dynamic information, their magnitude can lead to wide ^{14}N spectra spanning several MHz, which are difficult to detect directly. Significant advances have recently been made in ^{14}N NMR in solids using two-dimensional (2D) methods based on indirect detection of ^{14}N via spy nuclei (^{1}H or ^{13}C) with spin $S = 1/2$ [1,2]. These methods tend to be rather time-consuming because of low signal-to-noise ratios and long recovery delays, particularly if the proton relaxation times are long. The coherence of the spy nucleus can be transferred to the quadrupolar nucleus using residual dipolar splittings [2,3] or 'recoupled' heteronuclear dipolar interactions [4–12], and finally transferred back to the spy nucleus for detection. These indirect detection methods require several parameters to be optimized empirically in order to achieve the most efficient coherence transfer. These experiments often suffer from poor signal-to-noise ratios. In solid samples with long proton relaxation times, such as proline ($T_1(^1\mathrm{H}) \sim 60$ s at room temperature), the duration of 2D experiments for indirect ^{14}N detection can be on the order of days. As we shall show in the Communication, the combination of dynamic nuclear polarization (DNP) with the indirect detection of nitrogen-14 can improve both the signal-to-noise for each scan and reduce the recovery delays, because the proton magnetization (which is transferred to carbon-13 by cross-polarization) builds up with DNP on a short time-scale $T_{\mathrm{DNP}} \sim 6$ s at 110 K.

2. Results and discussion

The gain in signal-to-noise achieved through DNP [13,14] is accomplished through the transfer of polarization from a highly polarized spin, usually the unpaired electron spin of a stable radical such as TEMPO or TOTAPOL [15–17], to nuclear spins such as protons. Dynamic nuclear polarization can be combined with magic angle spinning (MAS) at low temperatures (*ca.* 100 K), using a high power microwave source (usually a gyrotron), and suitable glass-forming solvents [18–21]. Our experiments are performed using a solid-state DNP-NMR spectrometer designed by Bruker Biospin [22]. In addition to a 400 MHz wide-bore spectrometer, the system includes a low-temperature MAS probe capable of spinning 3.2 mm rotors at frequencies up to 17 kHz at temperatures around 100 K, and a 263 GHz gyrotron providing ~5 W microwave irradiation to the sample. This microwave power is high enough to saturate the DNP enhancement in typical samples used and has a sufficient stability for long term 2D spectroscopy (several days). Acquisition times are not limited by the duty cycle or stability of the gyrotron, which can operate continuously for an indefinite period. The spinning frequency of 15.625 kHz was stable to ±2 Hz for the duration of the experiments and is comparable to a standard

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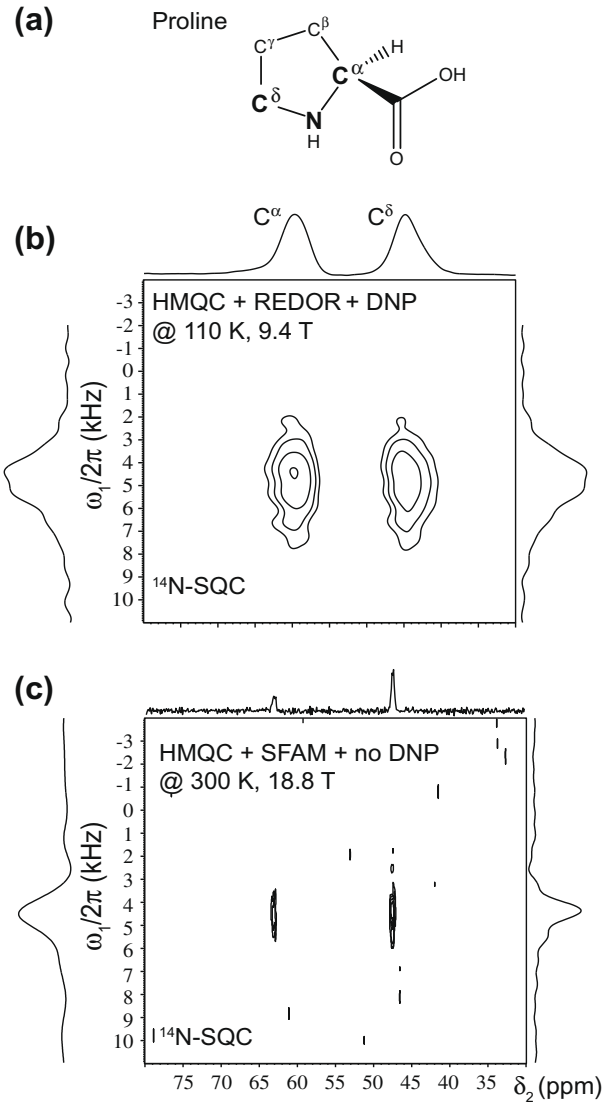

Fig. 1. (a) Chemical structure of proline with the $^{13}C^{\alpha}$, $^{13}C^{\delta}$, and ^{14}N shown in bold. (b) ^{13}C–^{14}N correlation spectrum of U–^{13}C-enriched proline with 20 mM TOTAPOL in a glass-forming mixture, obtained in a few hours with the HMQC method [6] using DNP, leading to an enhancement $\varepsilon \sim 40$ after cross-polarization from protons to ^{13}C in a 400 MHz solid-state DNP-NMR spectrometer with a 3.2 mm sapphire rotor spinning at 15.625 kHz at 110 K. For recoupling the REDOR sequence [25] was used to transfer the coherences from ^{13}C to ^{14}N and back. (c) ^{13}C–^{14}N correlation spectrum of a polycrystalline powder of pure ^{13}C-enriched proline without any radicals, also recorded with the HMQC method [6], but without DNP in 7 days at 800 MHz with a 2.5 mm zirconium rotor spinning at 31.25 kHz at 300 K. For recoupling the SFAM sequence [26,27] was used to transfer coherences from ^{13}C to ^{14}N and back, because it is more efficient than REDOR [25] at high spinning frequencies.

state-of-the-art MAS probe. Fully ^{13}C-enriched L-proline was dissolved in D8-glycerol:D_2O:H_2O (60:30:10 v/v) [23] which gives a glassy matrix at temperatures near 100 K. Three samples were prepared with the bi-radical TOTAPOL [17] at concentrations of 5, 10, and 20 mM. These concentrations resulted in DNP enhancement factors $\varepsilon = 20$, 26 and 40. These enhancement factors are defined as the ratios of ^{13}C signal amplitudes observed after cross-polarization from protons with and without microwaves. At 100 K, the three samples with increasing biradical concentrations had much reduced proton longitudinal relaxation times that were very close to the time constants of the DNP process, i.e., $T_1(^1H) \sim T_{DNP} \sim 14$, 8, and 6 s for 5, 10, and 20 mM TOTAPOL, respectively. Two-dimensional experiments with indirect detection of ^{14}N via ^{13}C in combination with DNP begin with cross-polarization from the DNP enhanced protons to carbons and then proceed as previously described [6].

A 2D spectrum of proline using DNP at 110 K with 15.625 kHz MAS at 9.4 T (400 MHz for protons) is shown in Fig. 1a. With a recycle delay of 8 s and 128 scans for each of $N = 16$ t_1 increments (the signal has decayed at $t_1^{max} = 1.024$ ms), we were able to acquire a 2D spectrum within a few hours. When including the optimization of the coherence transfer steps, the experiment can be completed in less than a day. At 9.4 T (400 MHz for protons) and 15.625 kHz, we could not obtain any meaningful spectrum of proline without DNP. The DNP-enhanced spectrum (Fig. 1a) may be compared with the spectrum of a poly-(but not micro-) crystalline sample of proline acquired without DNP in 7 days (Fig. 1b). The latter non-enhanced spectrum was recorded at 18.8 T (800 MHz for protons) and 31.25 kHz, i.e., at twice the magnetic field and twice the spinning frequency as the DNP-enhanced spectrum. The linewidths in both ^{13}C and ^{14}N dimensions are broadened in the DNP spectrum. This broadening is believed not to be due to paramagnetic effects, since the linewidths do not change with increasing radical concentration. As expected, the linewidth in the ^{14}N dimension is roughly twice as broad at 9.4 T than at 18.8 T because of the second-order quadrupole interaction, which is inversely proportional to the static field. This problem can only be overcome by using DNP at 526 GHz in a field of 18.8 T. In the ^{13}C dimension, the broadening may be due to static disorder in the frozen glass in which the small molecules are embedded or to the lack of dynamics on a suitable time scale. It is possible that the ^{13}C linewidths can be improved by optimizing the preparation of the sample, which was not microcrystalline. It is known that proteins in glassy matrices can exhibit lines that are significantly narrower. Indeed, Bajaj et al. [24] have shown narrow linewidths in the center of a large protein where the structure is less perturbed by the glass forming solvent. In conclusion, we have shown that DNP can dramatically reduce the experimental time for indirect detection of nitrogen-14 signals in solid-state NMR. With further improvements in DNP efficiency and faster magic angle spinning at low temperatures it may be possible to extract important structural parameters from unlabeled biomolecules through ^1H–^{14}N correlations. Currently, the resolution in the ^1H spectra is limited by sample preparation conditions and insufficient magic angle spinning frequencies, thus we are limited to ^{13}C–^{14}N correlations where the ^{13}C resolution is better. Alternatively, one could use selectively labelled ^{13}C in large biomolecules to determine targeted quadrupolar coupling parameters.

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