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Double-Nucleus Enhanced Recoupling for Efficient ^{13}C MAS NMR Correlation Spectroscopy of Perdeuterated Proteins

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Recent progress in solid-state magic-angle-spinning (MAS) NMR methodology has significantly contributed to the prospects for obtaining structural insight into "difficult", biologically relevant materials such as membrane proteins and protein fibrils.[1] In the quest for higher resolution, the use of perdeuterated proteins has been advocated, yielding spectra with liquid-state-like resolution in both the ^{1}H and $^{15}N/^{13}C$ dimensions.[2] This approach, combined with an optimization of the proton/deuterium ratio to maximize sensitivity,[3] enables the use of scalar-coupling-based NMR experiments. As a beneficial side-effect of perdeuteration, low-power decoupling of ^{1}H and ^{2}H is sufficient to obtain ^{13}C line widths less than 0.5 ppm.

Experiments that transfer magnetization in a spin diffusion (SD)-type process are important to provide constraints for the assignment and structure calculation of proteins.[1] SD is relatively insensitive to dipolar truncation effects and allows detection of weak couplings sufficient for full structure determination of proteins.[4] Two commonly used SD-based experiments for obtaining $^{13}C-^{13}C$ correlations are proton-driven spin diffusion (PDSD)[5] and radio-frequency-assisted diffusion (RAD,[6] also known as DARR[7]). These mixing sequences are "proton-aided" and are strongly affected by the reduced proton density in the perdeuterated proteins.[8] In particular, the reduced number of $^{1}H-^{1}H$ dipolar couplings makes efficient long-range magnetization exchange between heteronuclei difficult.

In this communication, we introduce a new strategy for improving the efficiency of ^{13}C SD in perdeuterated proteins. This is achieved by exploiting both the proton and deuterium dipolar coupling networks. The method is called *do*uble-*n*ucleus *e*nhanced *r*ecoupling (DONER), and it provides significantly improved transfer efficiency for carbon SD at extremely low proton density. We have used a uniformly $^{2}H/^{13}C/^{15}N$-labeled α-spectrin SH3 domain sample to demonstrate the efficiency of the DONER sequence.

The efficiency of PDSD transfer is significantly reduced at high levels of deuteration.[8] The effect of deuteration in this respect is similar to fast MAS, since both cases slow SD by reducing dipolar couplings. The RAD experiment is a modified version of the PDSD experiment in which proton irradiation is applied under the rotary resonance (RR) condition, $\omega_{1H} = n\omega_R$ ($|n| = 1, 2$), during ^{13}C mixing period. Here ω_{1H} is the field strength of the proton irradiation and ω_R is the spinning frequency. For protonated proteins, application of RAD significantly broadens the ^{13}C line width because of the recoupling of $^{1}H-^{13}C$ dipolar interactions, which enhances the efficiency of the SD process.[6−8] The new pulse scheme presented in this work is an improved version of the RAD experiment for application to deuterated proteins. The schematic representation of the DONER pulse scheme is shown in Figure 1. Simultaneous proton and deuterium irradiation is applied under the RR conditions ($\omega_{1H} = \omega_{2H} = n\omega_R$) during the ^{13}C mixing period. The effect of irradiation at the deuterium Larmor frequency under the RR condition is very similar to that of ^{1}H irradiation in that it broadens

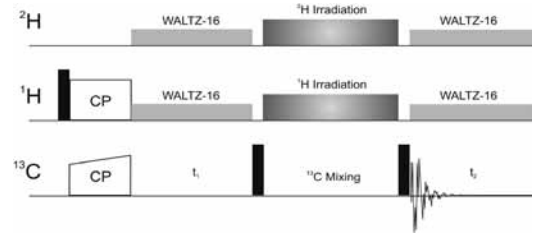

Figure 1. Schematic representation of the *do*uble-*n*ucleus *e*nhanced *r*ecoupling (DONER) pulse sequence. Solid bars indicate 90° pulses. ^{13}C magnetization is created by cross-polarization (CP) from protons. Low-power ^{1}H and ^{2}H WALTZ decoupling (2.5 kHz) was applied during the acquisition and evolution periods. During the ^{13}C mixing period, ^{1}H and ^{2}H irradiations are simultaneously applied under the rotary resonance condition ($\omega_{1H} = \omega_{2H} = n\omega_R$).

the ^{13}C lines. Despite the fact that dipolar couplings of deuterons are much smaller than those of protons ($D_{1H-13C}/D_{2H-13C} \approx 6.5$), the extensive amount of deuterium in the sample compensates for this effect, and sufficient recoupling is achieved. Interestingly, the line-broadening effect of irradiation at either the ^{1}H or ^{2}H frequency alone or at both frequencies simultaneously qualitatively provides a very similar picture, and only minor differences were observed in one-dimensional (1D) ^{13}C spectra recorded under different irradiation conditions (Supporting Figure 1 in the Supporting Information).

Figure 2A depicts a conventional two-dimensional (2D) PDSD $^{13}C-^{13}C$ correlation spectrum recorded with 50 ms mixing. Only very few cross-peaks are present, observed for the residues S19, S36, T32, and T37. The higher number of residual protons in these residues forms a larger homonuclear dipolar coupling network as a result of their exchangeable sites (NH and OH) close to their C_α and C_β carbons. The spectrum in Figure 2B was recorded with 50 ms RAD mixing and proton irradiation at 10 kHz. Clearly, more cross-peaks are observed in the spectrum, arising mostly from asparagines, aspartic acids, lysines, and arginines as well as from short-range $C_\alpha-CO$ and $C_\alpha-C_\beta$ transfer. Because of the larger proton content in various side-chain NH and OH sites, cross-peaks involving nearby spins gain intensity with the help of ^{1}H irradiation in the RAD experiment. However, most of the ^{13}C signals from residues that are further away from the exchangeable sites are missing in the spectrum. Remarkably, the application of simultaneous ^{1}H and ^{2}H irradiation during the ^{13}C mixing period results in more intense cross-peaks from many additional ^{13}C sites involving the intensively or fully deuterated ones (Figure 2C). In this spectrum, the $C_\alpha-C_\beta$, $C_\alpha-C_\gamma$, and $C_\beta-C_\gamma$ cross-peaks are more intense than in the previous two spectra. The integral of the signal that appears at ∼55 ppm in the extracted slice, arising mainly from leucine and aspartic acid $C_\alpha-C_\beta$ cross-peaks, has been used to globally quantify the increase in efficiency. The cross-peak intensity in the DONER experiment is ∼3 times stronger than in the RAD experiment and ∼5 times stronger than in the PDSD experiment.

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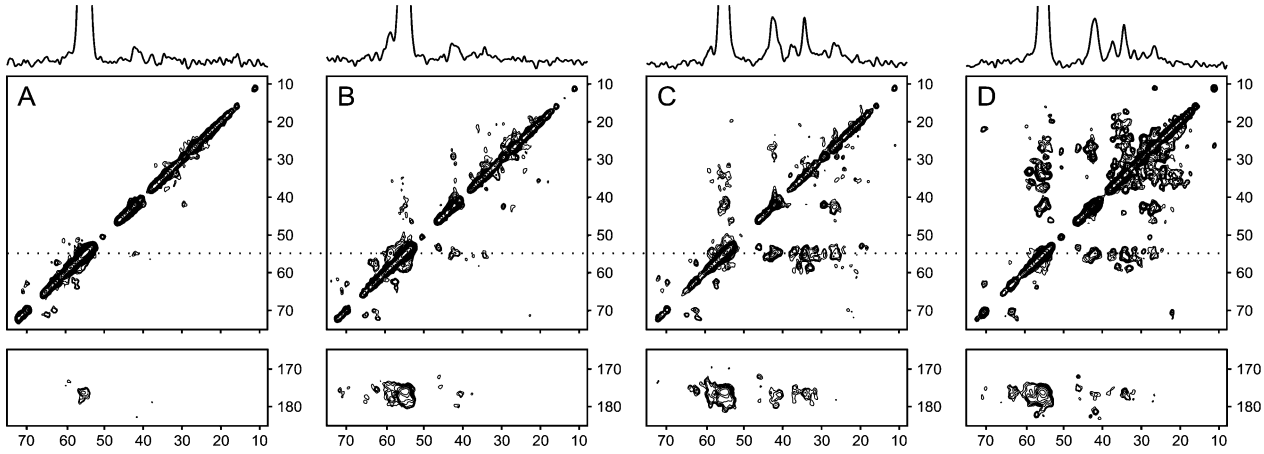

Figure 2. 2D $^{13}C-^{13}C$ MAS NMR spectra (in ppm) recorded on uniformly $^{2}H/^{13}C/^{15}N$-labeled α-spectrin SH3. Various ^{13}C homonuclear mixing schemes are compared: (A) PDSD, (B) RAD with ^{1}H irradiation during mixing, (C) DONER with both ^{1}H and ^{2}H irradiation during mixing, and (D) DONER with direct excitation. The spectra in (A−C) were recorded with initial $^{1}H-^{13}C$ CP. The spectrum in (D) had a longer recycle delay of 5 s. A 50 ms mixing time was used for all four experiments. The experimental conditions were comparable for the four spectra: For ^{1}H and ^{13}C, 4.0 and 5.0 μs 90° pulses, respectively, were used. Low-power proton and deuterium decoupling (WALTZ16) at 2.5 kHz were applied during both evolution and acquisition. CP was achieved with a contact time of 3 ms, $\omega_{1H}/2\pi = 53$ kHz, and $\omega_{13C}/2\pi = 33$ kHz (A−C). In the indirect dimension, 512 increments were recorded, with 16 scans per t_1 increment. The total acquisition times were 10 and 20 ms for the indirect and direct dimensions, respectively. All of the experiments were performed on a Bruker Avance 2 spectrometer operating at a ^{1}H Larmor frequency of 400 MHz. The MAS frequency was set to 10 kHz, and the effective temperature was 275 °C for the four spectra. The positions of the 1D slices (top) are indicated with dashed lines in the 2D plots. The scaling of the 1D spectra and the contour levels in the 2D spectra are absolutely comparable.

Moreover, the intensities of the $C_\alpha-C_\beta$, $C_\alpha-C_\gamma$, and $C_\beta-C_\gamma$ cross-peaks are comparable to those observed in fully protonated SH3 recorded under similar conditions (Supporting Figure 2). Since the spectrum in Figure 2C was recorded with cross-polarization (CP), carbon sites further away from the exchangeable protons lacked initial magnetization, and hence, the ^{13}C population was not uniform at the beginning of t_1. Symmetric spectra can be obtained by either inserting a DONER period directly after CP or by using direct ^{13}C excitation, as shown by the spectrum in Figure 2D. This spectrum is qualitatively highly similar to PDSD spectra recorded on fully protonated SH3 (Supporting Figure 2). In contrast, combining direct excitation with RAD mixing yields only very few cross-peaks (Supporting Figure 3). A more detailed study comparing site-specific buildup rates for the different types of cross-peaks is currently in preparation.

It has been demonstrated previously that ^{13}C line widths less than 0.5 ppm can be achieved in an extensively deuterated protein by the simultaneous application of only low-power proton and deuterium scalar decoupling.[9] We observed that at high levels of deuteration at the exchangeable sites, ^{2}H scalar decoupling is more crucial than ^{1}H scalar decoupling (Supporting Figure 4). High-power dipolar decoupling is not needed in such deuterated systems, since the dipolar-coupled network formed by the proton spins collapses to a great extent as a result of deuteration, which strongly contributes to the low efficiency of ^{13}C SD experiments on highly deuterated proteins. A very similar ^{13}C line-broadening effect is observed irrespective of whether ^{1}H or ^{2}H RR irradiation or both simultaneously are used (cf. Supporting Figure 1). Currently, the process underlying the improved transfer is not fully understood. With MAS, dipolar $^{2}H-^{2}H$ couplings are partly reintroduced.[10] It might even be conceivable that a complex dipolar-coupled network among ^{13}C, ^{1}H, and ^{2}H spins plays a role in the improved ^{13}C transfer. However, extensive calculations are needed to fully understand the observed effects.

The use of simultaneous deuterium and proton irradiation under the RR condition to assist ^{13}C homonuclear SD increases spectral sensitivity and allows use of SD-like experiments on highly deuterated samples. This may provide a useful building block for ^{13}C transfer in multidimensional experiments for assignment and structure-calculation purposes by using the same deuterated sample. The high resolution obtained for perdeuterated preparations in combination with the DONER approach to increase the sensitivity for correlations between heteronuclei will open up new experimental schemes in the research of perdeuterated proteins.

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Supporting Information Available: Line-broadening effect of ^{1}H and ^{2}H under RR irradiation, 2D $^{13}C-^{13}C$ spectra of protonated and deuterated SH3, comparison of direct-excitation RAD and DONER, and comparison of ^{1}H and ^{2}H WALTZ decoupling. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Castellani, F.; van Rossum, B.-J.; Diehl, A.; Schubert, M.; Rehbein, K.; Oschkinat, H. *Nature* **2002**, *420*, 98. Jaroniec, C. P.; MacPhee, C. E.; Bajaj, V. S.; McMahon, M. T.; Dobson, C. M.; Griffin, R. G. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 711. Wasmer, C.; Lange, A.; Van Melckebeke, H.; Siemer, A. B.; Riek, R.; Meier, B. H. *Science* **2008**, *319*, 1523.
(2) Chevelkov, V.; Rehbein, K.; Diehl, A.; Reif, B. *Angew. Chem., Int. Ed.* **2006**, *45*, 3878.
(3) Akbey, U.; Lange, S.; Franks, W. T.; Linser, R.; Rehbein, K.; Diehl, A.; van Rossum, B.-J.; Reif, B.; Oschkinat, H. *J. Biomol. NMR* [Online early access]. DOI: 10.1007/s10858-009-9369-0. Published Online: Aug 22, 2009.
(4) Grommek, A.; Meier, B. H.; Ernst, M. *Chem. Phys. Lett.* **2006**, *427*, 404. Manolikas, T.; Herrmann, T.; Meier, B. H. *J. Am. Chem. Soc.* **2008**, *130*, 3959.
(5) Gan, Z. H.; Ernst, R. R. *Chem. Phys. Lett.* **1996**, *253*, 13.
(6) Zilm, K. W. Presented at the 40th Experimental NMR Conference, Orlando, FL, 1999.
(7) Takegoshi, K.; Nakamura, S.; Terao, T. *Chem. Phys. Lett.* **2001**, *344*, 631.
(8) Morcombe, C. R.; Gaponenko, V.; Byrd, R. A.; Zilm, K. W. *J. Am. Chem. Soc.* **2004**, *126*, 7196–7197.
(9) Agarwal, V.; Diehl, A.; Skrynnikov, N.; Reif, B. *J. Am. Chem. Soc.* **2006**, *128*, 12620.
(10) Gan, Z.; Robyr, P. *Mol. Phys.* **1998**, *95*, 1143.

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