J|A|C|S COMMUNICATIONS

Published on Web 12/10/2008

High-Resolution Double-Quantum Deuterium Magic Angle Spinning Solid-State NMR Spectroscopy of Perdeuterated Proteins

Vipin Agarwal,[†] Katja Faelber,[†] Peter Schmieder,[†] and Bernd Reif*[,‡]

Leibniz-Institut für Molekulare Pharmakologie (FMP), Robert-Rössle-Strasse 10, 13125 Berlin, Germany, and Charité Universitätsmedizin, 10115 Berlin, Germany

Received May 15, 2008; E-mail: reif@fmp-berlin.de

Magic angle spinning (MAS) solid-state NMR has developed rapidly over the past decade. Advances in its application to biological samples have led to the determination of the structure of uniformly isotopically enriched crystalline model systems[1,2] as well as of amyloidogenic peptides and proteins.[3−6] Recently, we have focused on ^{1}H NMR spectroscopy of back-exchanged amide protons in perdeuterated peptides and proteins as a means of increasing resolution and sensitivity in MAS solid-state NMR experiments.[7−10] In addition, we have shown that ^{1}H NMR spectra of selectively protonated methyl groups yield even higher sensitivity, while maintaining excellent resolution (with ^{1}H line widths below 30 Hz).[11] Furthermore, residual protonation in a perdeuterated protein at nonexchangeable sites can be exploited spectroscopically.[12] Deuterons contain similar information on the chemical environment compared to protons. In solution-state, direct observation of deuterons is, however, hampered by their large quadrupolar coupling in combination with overall tumbling. There, methyl deuteron line widths are found to be on the order of >150 Hz.[13,14] In the solid-state, narrow ^{2}H resonance lines can in principle be achieved given the inhomogeneous nature of the deuterium quadrupolar interaction under MAS, and the fact that molecular tumbling is absent.

In this manuscript, we present solid-state ^{2}H,^{13}C correlation spectra for a protein with unprecedented resolution in the ^{2}H dimension. Figure 1 represents ^{2}H,^{13}C correlation spectra recorded for the ^{2}H,^{13}C,^{15}N isotopically enriched peptide Nac-Val-Leu-OH which was used as a model compound. A simple ^{2}H single-quantum (SQ) correlation is shown in Figure 1A, whereas a deuterium double-quantum (DQ) excitation and reconversion scheme has been employed to record the spectrum represented in Figure 1B. Experimentally, the simplest pulse scheme consists of two 90° hard pulses separated by a delay τ for generation of ^{2}H DQ coherence (DQC).[15,16] DQCs are allowed to evolve during t_1. Reconversion to ^{2}H single-quantum is achieved in a symmetric way. A ^{2}H z-filter is applied before magnetization is transferred to carbons to eliminate unwanted coherences. In particular, care must be taken to rotor synchronize the full duration of the DQ-excitation-evolution-reconversion period (see Supporting Information). To avoid MQ spinning side bands, t_1 is incremented in multiples of a rotor period. Signal-to-noise improves significantly if ^{2}H RF fields >70 kHz are employed for hard pulses and CP. The time required for optimum ^{2}H-DQ excitation is inversely proportional to the size of the quadrupolar coupling. Short DQ excitation and reconversion times (1 μs) yield exclusively correlations for C−D$^\alpha$ deuterons associated with large quadrupolar couplings (e^2qQ/h = 170 kHz,[17,18] black), whereas longer delays (9 μs) are necessary to allow observation of correlations involving methyl deuterons which are associated with a scaled quadrupolar coupling (57 kHz, blue/red). In the experiment which employs τ = 9 μs, C−D resonances appear with negative intensity as the spin operator evolves and changes sign for deuterons having a large quadrupolar coupling. Clearly, the resolution in the ^{2}H−DQ experiment is significantly increased compared to the SQ correlation experiment. Three arguments can be invoked to explain the gain in resolution for the ^{2}H DQ compared to ^{2}H SQ spectra: First, the resolution is doubled due to evolution of DQCs while at the same time the absolute line width is maintained.[19] Second, ^{2}H DQCs are insensitive to magic angle offset.[20,21] Third, the ^{2}H DQC line width is unaffected by dynamics which can exert a significant broadening on deuterium SQCs.[22−24] ^{2}H DQ experiments have been carried out previously for small molecules and polymers.[15,22] However, so far no application to perdeuterated peptides and proteins was presented.

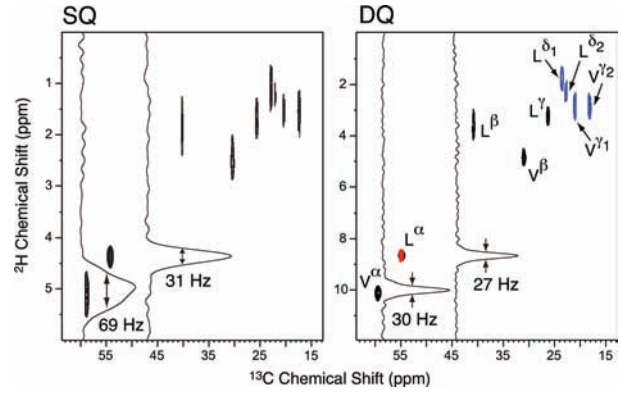

Figure 1. ^{2}H,^{13}C correlation recorded for ^{2}H-,^{13}C-,^{15}N-labeled NAc-Val-Leu-OH in the solid-state. The ^{2}H single-quantum (SQ) spectrum is represented on the left; ^{2}H double-quantum correlation (DQ) is on the right. DQ spectra were recorded using excitation times of 1 (black) and 9 μs (blue/red). These spectra are superimposed in the figure. The average ^{2}H line widths for CD$^\alpha$/CD$_2$/CD$_3$ groups in the SQ and DQ spectra are 47/150/90 Hz and 28/120/80 Hz, respectively. All experiments are recorded on a Bruker 600 MHz Avance spectrometer, setting the MAS rotation frequency to 20 kHz.

Figures 2 and 3 represent ^{2}H-DQ,^{13}C correlation spectra recorded for a triply labeled sample of the SH3 domain of chicken α-spectrin. Figure 2 shows the methyl region of the spectrum. For comparison, a ^{1}H,^{13}C correlation recorded on the same sample employing protons at natural abundance (HANAH) is represented.[12] The methyl ^{2}H isotopic shifts are almost identical to the ^{1}H chemical shifts. In contrast to proton correlation experiments, the obtainable sensitivity is decreased by a factor of $\gamma(^1\text{H})/\gamma(^2\text{H})$ $[I_{1\text{H}}(I_{1\text{H}}+1)]^{1/2}/[I_{2\text{H}}(I_{2\text{H}}+1)]^{1/2}$ = 4.0 as magnetization originates from deuterium in the experiment. The significantly reduced T_1 relaxation rate of deuterons, however, enables faster recycle delays which result in an >2-fold increase in sensitivity per unit time.[25] A further increase in signal-to-noise is achieved, as the deuterium density is ap-

[†] Leibniz-Institut für Molekulare Pharmakologie (FMP).
[‡] Charité Universitätsmedizin.

10.1021/ja803620r CCC: $40.75

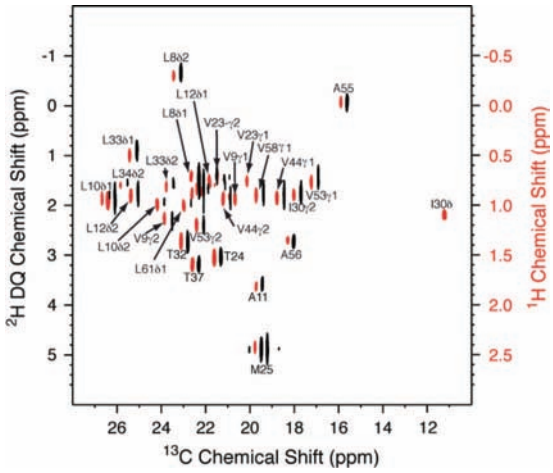

Figure 2. Methyl region of the ^{13}C-detected ^{1}H,^{13}C (red) and the ^{2}H-DQ,^{13}C (black) correlation spectra recorded for a ^{2}H-,^{13}C-,^{15}N-labeled sample of the SH3 domain of chicken α-spectrin. The ^{1}H,^{13}C correlation is recorded at ^{1}H natural abundance[12] using the same sample. The spectra are offset in the ^{13}C dimension due to the ^{1}H/^{2}H isotopic effect. The base contour level is set to 20% of maximum intensity in all spectra. Subsequent contour levels display 1.1-fold increased intensity with respect to the previous contour. *J*-Deconvolution was employed for processing to remove one bond ^{13}C,^{13}C scalar couplings in the acquisition dimension.[26]

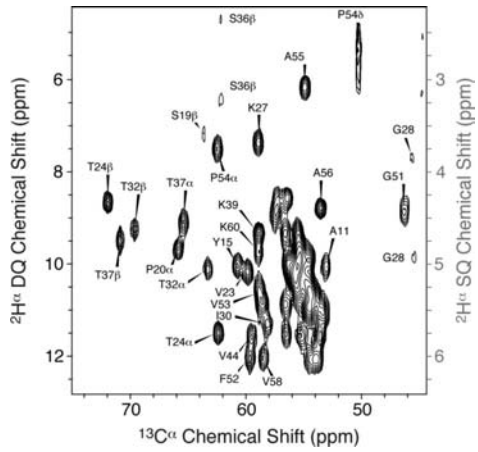

Figure 3. C^α spectral region of the ^{13}C-detected ^{2}H-DQ,^{13}C correlation spectrum of the SH3 domain. D^α resonances are as narrow as 16 Hz (A56) in the ^{2}H DQ dimension.

proximately 30-fold higher compared to the proton density in a methyl group of a deuterated protein, given a 10% likelihood for a CD_2H moiety.[12] In total, an improvement by a factor of 8.6 of the ^{2}H,^{13}C correlation experiment compared to the ^{1}H,^{13}C correlation is expected. We obtain an experimental gain in signal-to-noise ratio by a factor of 2.46 ± 0.82. The lower than expected improvement in sensitivity can be explained by the limited efficiency of ^{2}H-DQ excitation and reconversion, and variations in the ^{2}H line width. Typical ^{2}H methyl line widths are on the order of (38 ± 14) Hz, with T37 displaying the smallest line width of 19 Hz.

Figure 3 displays the C^α−D^α spectral region of the ^{2}H−DQ,^{13}C correlation experiment for the SH3 domain. In general, C−D groups pose a greater challenge to spectroscopy as they are associated with an effectively 3-fold larger quadrupolar coupling. Typically, we find ^{2}H line widths in the α-spectral region to be on the order of (30 ± 9) Hz, with A56 having the most narrow line width of 16 Hz in the DQ dimension. As the sample was prepared by growing *E.coli* in D_2O (isotopic purity >99.9%), the H^α proton density is negligible. No signal is therefore observed in the respective ^{1}H,^{13}C correlation (see Supporting Information).

To the best of our knowledge, this is the first example of high-resolution deuterium NMR spectroscopy performed on a perdeuterated protein. Taken together, we find the smallest ^{2}H line widths for C−D moieties. In the peptide sample, no individual CD_2 resonances are resolved. In SH3, however, distinct D^β chemical shifts are observed for deuterons in the methylene group of, for example, G28/S36, yielding similar resolution compared to D^α. In general, methyl groups tend to exhibit larger line widths (compared to CD and resolved CD_2). The increased line width for nonresolved CD_2 and CD_3 groups most likely originates from a $n = 0$ rotational resonance condition.[27] In cases of degenerate methylene and methyl deuteron chemical shifts, the dipolar interaction among these deuterons is reintroduced, which in turn induces line broadening. We believe that the suggested scheme will have a broad impact for correlation spectroscopy as the deuteron can be used as an additional nucleus for chemical shift dispersion and as a probe of dynamics.

Acknowledgment. This work was supported by the Leibniz-Gemeinschaft (WGL) and the DFG (Re1435, SFB 449, SFB 740).

Supporting Information Available: NMR pulse schemes; off MAS simulations; ^{1}H,^{13}C, and ^{2}H,^{13}C correlation spectra; S/N and ^{2}H line width analysis for individual amino acids of SH3. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Rienstra, C. M.; Tucker-Kellogg, L.; Jaroniec, C. P.; Hohwy, M.; Reif, B.; McMahon, M. T.; Tidor, B.; Lozano-Pérez, T.; Griffin, R. G. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 10260–10265.
(2) Castellani, F.; van Rossum, B.-J.; Diehl, A.; Schubert, M.; Rehbein, K.; Oschkinat, H. *Nature* **2002**, *420*, 98–102.
(3) Petkova, A. T.; Ishii, Y.; Balbach, J. J.; Antzutkin, O. N.; Leapman, R. D.; Delaglio, F.; Tycko, R. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 16742–16747.
(4) 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*, 711–716.
(5) Petkova, A. T.; Leapman, R. D.; Guo, Z. H.; Yau, W. M.; Mattson, M. P.; Tycko, R. *Science* **2005**, *307*, 262–265.
(6) Wasmer, C.; Lange, A.; Van Melckebeke, H.; Siemer, A. B.; Riek, R.; Meier, B. H. *Science* **2008**, *319*, 1523–1526.
(7) Reif, B.; Jaroniec, C. P.; Rienstra, C. M.; Hohwy, M.; Griffin, R. G. *J. Magn. Reson.* **2001**, *151*, 320–327.
(8) Chevelkov, V.; van Rossum, B. J.; Castellani, F.; Rehbein, K.; Diehl, A.; Hohwy, M.; Steuernagel, S.; Engelke, F.; Oschkinat, H.; Reif, B. *J. Am. Chem. Soc.* **2003**, *125*, 7788–7789.
(9) Chevelkov, V.; Rehbein, K.; Diehl, A.; Reif, B. *Angew. Chem., Int. Ed.* **2006**, *45*, 3878–3881.
(10) Hologne, M.; Chevelkov, V.; Reif, B. *Prog. NMR Spect.* **2006**, *48*, 211–232.
(11) Agarwal, V.; Diehl, A.; Skrynnikov, N.; Reif, B. *J. Am. Chem. Soc.* **2006**, *128*, 12620–12621.
(12) Agarwal, V.; Reif, B. *J. Magn. Reson.* **2008**, *194*, 16–24.
(13) Ishima, R.; Louis, J. M.; Torchia, D. A. *J. Biomol. NMR* **2001**, *21*, 167–171.
(14) Millet, O.; Muhandiram, D. R.; Skrynnikov, N. R.; Kay, L. E. *J. Am. Chem. Soc.* **2002**, *124*, 6439–6448.
(15) Chandrakumar, N.; von Fircks, G.; Gunther, H. *Magn. Reson. Chem.* **1994**, *32*, 433–435.
(16) Kristensen, J. H.; Bildsøe, H.; Jakobsen, H. J.; Nielsen, N. C. *J. Magn. Reson.* **1999**, *139*, 314–333.
(17) Emsley, J. W. *Solid-State NMR Spectroscopy—Principles and Applications*; Duer, M. J., Ed.; Blackwell Science: Oxford, U.K., 2002.
(18) Hologne, M.; Chen, Z.; Reif, B. *J. Magn. Reson.* **2006**, *179*, 20–28.
(19) Vega, S.; Shattuck, T. W.; Pines, A. *Phys. Rev. Lett.* **1976**, *37*, 43–46.
(20) Eckman, R.; Muller, L.; Pines, A. *Chem. Phys. Lett.* **1980**, *74*, 376–378.
(21) Hoffmann, A.; Schnell, I. *ChemPhysChem* **2004**, *5*, 966–974.
(22) Cutajar, M.; Ashbrook, S. E.; Wimperis, S. *Chem. Phys. Lett.* **2006**, *423*, 276–281.
(23) Thrippleton, M. J.; Cutajar, M.; Wimperis, S. *Chem. Phys. Lett.* **2008**, *452*, 233–238.
(24) Cavadini, S.; Abraham, A.; Ulzega, S.; Bodenhausen, G. *J. Am. Chem. Soc.* **2008**, *130*, 10850–10851.
(25) Hologne, M.; Faelber, K.; Diehl, A.; Reif, B. *J. Am. Chem. Soc.* **2005**, *127*, 11208–11209.
(26) Hoch, J. C.; Stern, A. S., Maximum Entropy Reconstruction in NMR: An Alternative to DFT. In *NMR Data Processing*; Wiley-Liss: New York, 1996; pp 102−135.
(27) Levitt, M. H.; Raleigh, D. P.; Creuzet, F.; Griffin, R. G. *J. Chem. Phys.* **1990**, *92*, 6347–6364.

JA803620R