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Probing Proton−Proton Proximities in the Solid State: High-Resolution Two-Dimensional ^{1}H−^{1}H Double-Quantum CRAMPS NMR Spectroscopy

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Solid-state NMR has identified itself as a valuable probe of local molecular structure, in particular because of its ability to accurately determine distances or identify close proximities between specific nuclei. Most solid-state NMR techniques that exploit dipolar couplings in this way probe rare spins such as ^{13}C and ^{15}N. However, the potential of techniques that directly probe ^{1}H−^{1}H dipolar couplings is being increasingly recognized.[1−3] Indeed, two-dimensional (2D) ^{1}H−^{1}H double-quantum (DQ) magic-angle spinning (MAS) experiments have become a method of choice for probing proton−proton proximities and, as such, have provided much insight into the solid-state structures adopted by various materials.[1]

For organic solids containing networks of many dipolar-coupled protons, a key consideration in multiple-quantum ^{1}H solid-state NMR is resolution, since the strong proton−proton dipolar couplings usually obscure detailed chemical shift information. Employing fast MAS alone (typically 30 kHz) has been shown to yield, in many cases, resolution sufficient to resolve specific DQ peaks. The proton−proton proximities identified in this way have allowed, for example, the identification of intramolecular hydrogen bonds or $\pi-\pi$ packing arrangements in a variety of organic materials.[4] Pure MAS is, however, often insufficient to achieve resolution in the crowded regions of ^{1}H DQ MAS spectra. Enhanced resolution of ^{1}H spectra can be obtained by combining the rotation of the sample with the application of carefully synchronized multiple-pulse sequences in the so-called CRAMPS approach.[3,5,6] Along these lines, Schnell et al.[7] and more recently Vega and co-workers[8] have reported, by employing windowless and windowed homonuclear decoupling in the indirect (t_1) and direct (t_2) dimensions, respectively, an improvement in resolution of proton DQ spectra. However, a major problem in such spectra was so far the presence of artifacts as compared to the fast MAS approach.

In this communication, we show that by a careful design of the NMR experiment and by the application of optimized homonuclear decoupling sequences, high-resolution and high-sensitivity clean DQ spectra can be recorded. These spectra provide sufficient resolution to observe *all expected inter- and intramolecular short-range correlations*, in particular in the alkyl region, of a model dipeptide.

Figure 1 presents the pulse sequence for the ^{1}H DQ CRAMPS experiment employed in this work. Two phase-modulated homonuclear decoupling schemes, carefully designed to provide optimal proton resolution in strongly coupled systems, the windowless eDUMBO-1_{22}[9] and windowed-DUMBO-1,[6] were applied in the t_1 and t_2 dimensions, respectively. The length, together with the phases (relative to that of the eDUMBO scheme) of the prepulses θ_1, was carefully calibrated to minimize axial peaks and quadrature images in F_1. In the same way, the orientation of the effective field of the

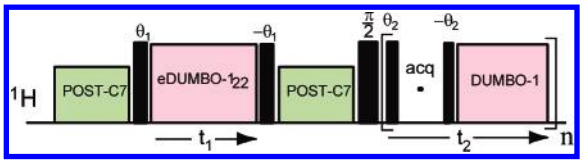

Figure 1. Pulse sequence for the ^{1}H DQ CRAMPS experiment. The pulse sequence is available from our website.[12]

DUMBO-1 sequence was adjusted to suppress zero frequency peaks in F_2. In addition, prepulses θ_2 were inserted before and after each detection window to minimize quadrature images in the direct dimension. POST-C7 was chosen for the excitation and reconversion of DQ coherences because of its inherent offset compensation and its γ-encoding property.[10] This latter gives an enhanced overall sensitivity and yields DQ spectra free of rotor-encoded spinning sidebands.[11] As no synchronization is required between the MAS and the t_1 increment (which has to correspond to an integral number of the decoupling cycle), interference conditions between the MAS and the homonuclear decoupling scheme can easily be avoided.

Experiments were performed on a Bruker Avance 500 MHz spectrometer using a single-channel 2.5 mm CRAMPS probe. The dipeptide β-AspAla was obtained from Bachem (Bubendorf, Switzerland) and used without further purification.

A ^{1}H DQ CRAMPS spectrum (recorded in 170 min) of the dipeptide (10 mg) obtained at $\nu_R = 12.5$ kHz using the pulse sequence of Figure 1 is shown in Figure 2a. For comparison, a rotor-synchronized ^{1}H DQ MAS spectrum obtained using the BABA[13] recoupling sequence at $\nu_R = 30$ kHz is shown in Figure 2b. The DQ CRAMPS experiment delivers a resolution enhancement of at least a factor of 5 in both the single- and double-quantum dimensions. This drastic improvement in resolution is especially visible in the aliphatic region of the spectra. Thus, the CH_3, the two diastereotopic CH_2 and the two CH resonances are clearly resolved in the horizontal projection of Figure 2a, whereas there are only two resolved peaks in the alkyl region of the F_2 projection of Figure 2b. Note that proton line widths of less than 0.5 ppm were measured for the aliphatic correlations in the DQ dimension and that these line widths are sometimes much less than twice the SQ widths (which are themselves all under 0.3 ppm).

A close inspection of the ^{1}H−^{1}H proximities extracted from the known crystal structure of the dipeptide β-AspAla reveals that we observe all the correlations corresponding to close (<3.5 Å) proton−proton proximities. This allows the complete assignment of the ^{1}H spectrum (detailed in Supporting Information). In particular, the high-resolution DQ spectrum provides a clear assignment of the two diastereotopic CH_2 protons and two CH resonances. This is illustrated from the traces of Figure 3, which displays traces parallel to F_1 extracted from the 2D DQ CRAMPS spectrum at (a) 2.2, (b) 2.7, (c) 4.1, and (d) 5.0 ppm. For the traces a and b corresponding to the two CH_2 protons, the strongest DQ peak is that involving the two CH_2 protons themselves (at 4.9 ppm).

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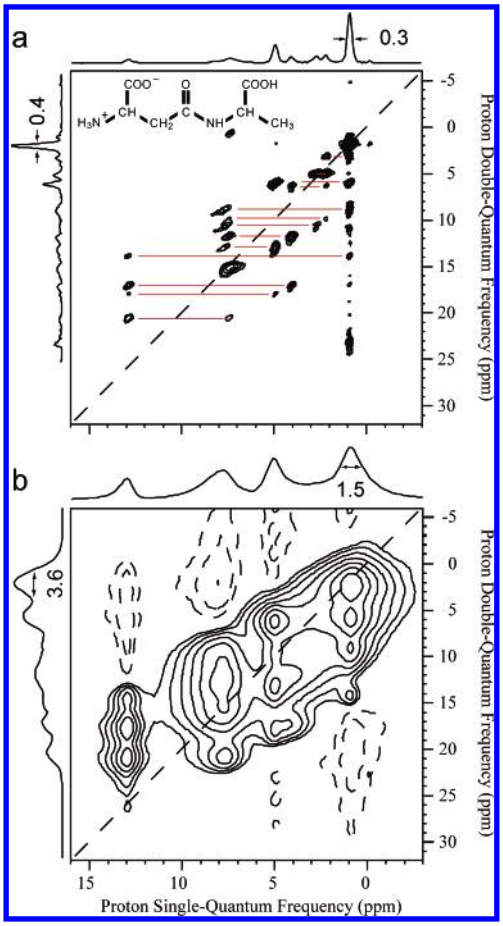

Figure 2. (a) Two-dimensional ^{1}H DQ CRAMPS spectrum of the dipeptide β-AspAla recorded using the pulse sequence of Figure 1 with $\omega_r = 12.5$ kHz. The length for the excitation and reconversion periods was 68.6 μs (corresponding to 3 basic POST-C7 elements). For each of 320 t_1 slices, 16 scans were recorded with a recycle delay of 2 s. The proton RF field was set to $\nu_1 = 87$ kHz during the POST-C7 blocks and to 100 kHz for decoupling during t_1 and t_2. In t_2, one complex data point was acquired in each acquisition windows (5 μs length). The DUMBO-1 blocks were 96 μs long (corresponding to three basic DUMBO-1 cycles). Including the prepulses, the effective dwell time in F_2 was 102.8 μs, (maximum $t_2 =$ 16.4 ms). The t_1 increment was set to 96 μs (corresponding to three basic e-DUMBO-1_{22} cycles). The θ_1 and θ_2 pulses were 1.5 and 0.2 μs, respectively. The scaling factors for eDUMBO-1_{22} (during t_1) and w-DUMBO-1 (during t_2) were determined experimentally as 0.58 and 0.50, respectively. (b) Two-dimensional ^{1}H DQ MAS spectrum of the dipeptide β-AspAla with $\omega_r = 30$ kHz using the BABA pulse scheme to excite and reconvert the DQ coherences (one rotor period). For each of 48 t_1 slices, 16 transients were coadded. The recycle delay was 1.5 s. A ^{1}H $\pi/2$ pulse length of 2.5 μs was used for all pulses. The t_1 increment was set to 33.3 μs, corresponding to a rotor-synchronized F_1 spectral width.

The other DQ peak in Figure 3b at 2.7 + 8.0 = 10.7 ppm indicates that the 2.7 ppm CH_2 proton is pointing toward the NH of the amide linkage, while the DQ peaks in Figure 3a at 2.2 + 4.1 = 6.3 ppm and 2.2 + 7.5 = 9.7 ppm indicate that the 2.2 ppm CH_2 proton is pointing toward the Asp CH and NH_3 protons. In traces c and d corresponding to the two CH resonances, the most intense DQ peaks are with the NH_3 (at 11.6 ppm) and the CH_3 (at 5.9 ppm) resonances, such that they correspond to the Asp and Ala CH protons, respectively.

In conclusion, we have shown on a model medium-sized molecule that, using current methodology, the proton DQ CRAMPS experiment yields high-quality high-resolution spectra as compared to the reference fast MAS technique. Particularly, this yields access to the crowded alkyl region of DQ spectra, which was until now inexploitable. In the example given here, we observed all 22 of the short-range inter- and intramolecular contacts expected from the crystal structure. The dramatic improvement in resolution opens new perspectives for the structural investigation of more complex systems in supramolecular chemistry and materials. Additionally, extensions of this technique to a third ^{13}C or ^{15}N chemical shift dimension would be practicable and allow the determination of multiple structural constraints in the form of ^{1}H−^{1}H proximities for larger labeled biosystems such as membrane proteins.

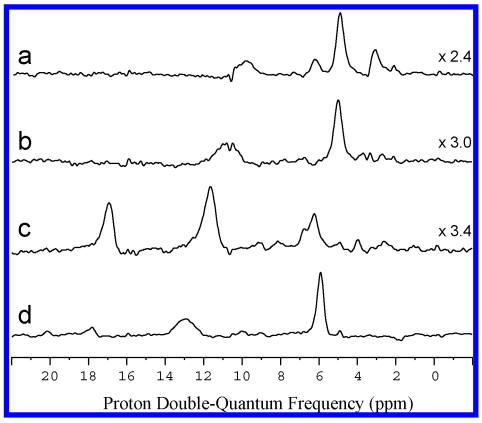

Figure 3. Traces parallel to F_1 extracted from the 2D ^{1}H DQ CRAMPS spectrum in Figure 2a at $F_2 = 2.2$ (a), 2.7 (b), 4.1 (c), and 5.0 ppm (d). Note that the peaks at 3.1 and 17.0 ppm in traces a and c correspond to *intermolecular* $CH_2^a-CH_3$ and $CH^{asp}-OH$ proximities, respectively.

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Supporting Information Available: (i) Further description of the DQ experiments, (ii) full assignment of the ^{1}H DQ CRAMPS spectrum, and (iii) the dependence upon the DQ recoupling time (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Brown, S. P.; Spiess, H. W. *Chem. Rev.* **2001**, *101*, 4125.
(2) (a) Reif, B.; Jaroniec, C. P.; Rienstra, C. M.; Hohwy, M.; Griffin, R. G. *J. Magn. Reson.* **2001**, *151*, 320. (b) Sakellariou, D.; Lesage, A.; Emsley, L. *J. Am. Chem. Soc.* **2001**, *123*, 5604. (c) Lange, A.; Seidel, K.; Verdier, L.; Luca, S.; Baldus, M. *J. Am. Chem. Soc.* **2003**, *125*, 12640. (d) Reif, B.; van Rossum, B. J.; Castellani, F.; Rehbein, K.; Diehl, A.; Oschkinat, H. *J. Am. Chem. Soc.* **2003**, *125*, 1488. (e) Paulson, E. K.; Morcombe, C. R.; Gaponenko, V.; Dancheck, B.; Bryd, R. A.; Zilm, K. W. *J. Am. Chem. Soc.* **2003**, *125*, 14222. (f) Matsuki, Y.; Akutsu, H.; Fujiwara, T. *Magn. Res. Chem.* **2004**, *42*, 291.
(3) Vinogradov, E.; Madhu, P. K.; Vega, S. *Chem. Phys. Lett.* **2002**, *354*, 193.
(4) (a) Brown, S. P.; Schnell, I.; Brand, J. D.; Müllen, K.; Spiess, H. W. *J. Am. Chem. Soc.* **1999**, *121*, 6712. (b) Brown, S. P.; Zhu, X. X.; Saalwächter, K.; Spiess, H. W. *J. Am. Chem. Soc.* **2001**, *123*, 4275.
(5) (a) Gerstein, B. C. In *The Encyclopedia of NMR*; J. Wiley & Sons: London, 1997; p 1501. (b) Bosman, L.; Madhu, P. K.; Vega, S.; Vinogradov, E. *J. Magn. Reson.* **2004**, *169*, 39.
(6) Lesage, A.; Sakellariou, D.; Hediger, S.; Elena, B.; Charmont, P.; Steuernagel, S.; Emsley, L. *J. Magn. Reson.* **2003**, *163*, 105.
(7) Schnell, I.; Lupulescu, A.; Hafner, S.; Demco, D. E.; Spiess, H. W. *J. Magn. Reson.* **1998**, *133*, 61.
(8) Madhu, P. K.; Vinogradov, E.; Vega, S. *Chem. Phys. Lett.* **2004**, *394*, 423.
(9) Elena, B.; De Paëpe, G.; Emsley, L. Submitted.
(10) Hohwy, M.; Jakobsen, H. J.; Eden, M.; Levitt, M. H.; Nielsen, N. C. *J. Chem. Phys.* **1998**, *108*, 2686.
(11) Geen, H.; Titman, J. J.; Gottwald, J.; Spiess, H. *Chem. Phys. Lett.* **1994**, *227*, 79.
(12) http://www.ens-lyon.fr/CHIMIE; accessed September 2004.
(13) Schnell, I.; Spiess, H. W. *J. Magn. Reson.* **2001**, *151*, 153.

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