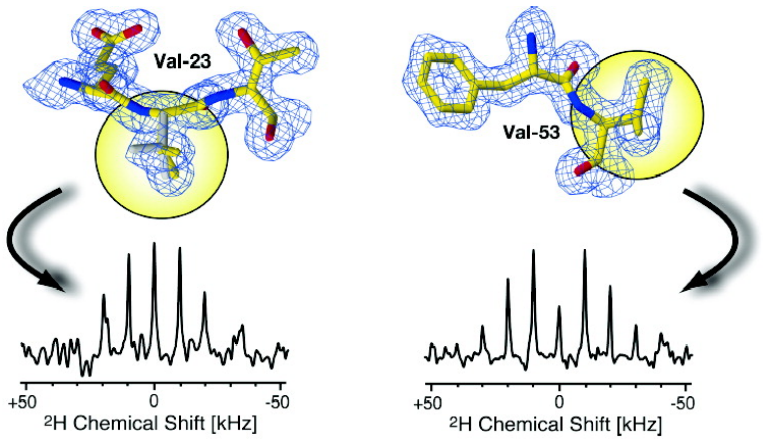
Communication

Characterization of Dynamics of Perdeuterated Proteins by MAS Solid-State NMR

Maggy Hologne, Katja Faelber, Anne Diehl, and Bernd Reif

J. Am. Chem. Soc., **2005**, 127 (32), 11208-11209• DOI: 10.1021/ja051830l • Publication Date (Web): 22 July 2005



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 11 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

J|A|C|S
COMMUNICATIONS

Published on Web 07/22/2005

Characterization of Dynamics of Perdeuterated Proteins by MAS Solid-State NMR

Maggy Hologne,[†,‡] Katja Faelber,[†,⊥] Anne Diehl,[†] and Bernd Reif*[,†,∥]

Forschungsinstitut für Molekulare Pharmakologie (FMP), Robert-Rössle-Str. 10, 13125 Berlin, Germany

Received March 22, 2005; E-mail: reif@fmp-berlin.de

Recently, structure determination of small peptides and proteins became possible using MAS (magic angle spinning) solid-state NMR spectroscopy.[1] So far, dynamic aspects are not considered in uniformly isotopically enriched samples. Information on dynamic processes are, however, important to properly characterize the function of a protein. In the past, deuterium labeling was used successfully to investigate dynamics in the solid-state NMR in various crystalline and amorphous solids, such as liquid crystals,[2] polymers,[3,4] biomembranes,[5] and membrane proteins.[6] The quadrupolar interaction, which dominates the deuterium spectral shape, is very sensitive to the molecular motion over an extremely large kinetic window.[7,8] Anisotropy of the spin−lattice relaxation time, T_1, was used previously in solid-state NMR to study fast molecular motion (10^{-8}−10^{-12} s).[7] In static solids, molecular dynamics in the slow motion regime (10^{2}−10^{-5} s) can be investigated by selective inversion, decay of quadrupolar order, or 2D exchange spectroscopy.[9] Intermediate motions, 10^{-4}−10^{-7} s, are usually studied by interpretations of the line shape distortions due to anisotropic ^{2}H T_2 relaxation.[3,10] We and others could show that deuteration and back-substitution of exchangeable protons efficiently suppress strong ^{1}H,^{1}H interactions and allow sensitive ^{1}H detection,[11] determination of long-range ^{1}H,^{1}H distances,[12] and the localization of mobile water molecules in the protein structure.[13]

In this communication, we present deuterium NMR experiments on a uniformly ^{2}H,^{13}C,^{15}N-labeled, crystalline sample of the α-spectrin SH3 domain. We show that, using this approach, a wealth of dynamic information can be obtained which was not accessible so far in the solid state. The experiments involve the measurement of ^{2}H T_1 relaxation times, as well as the measurement of the tensor parameters by evolution of the ^{2}H chemical shift. The presented experiments are based on 3D pulse programs employing a ^{13}C,^{13}C correlation in order to resolve individual ^{2}H atoms. In the experiment, magnetization originates from ^{2}H, taking advantage of the relatively short spin−lattice relaxation time, T_1, of deuterium (∼ 60 ms for CD_3). After a ^{2}H chemical shift evolution period, magnetization is transferred from ^{2}H to ^{13}C using cross-polarization. Application of an adiabatic CP on ^{2}H, as suggested by Emsley,[14] did not yield an improvement with respect to the observed ^{13}C resonance intensity. After a ^{13}C indirect evolution period, RFDR[15] homonuclear ^{13}C,^{13}C mixing yields magnetization transfer between neighboring ^{13}C nuclei. To obtain T_1 recovery curves, a ^{2}H π-pulse was implemented followed by a delay, Δ, which was varied from 1 to 100 ms prior to the ^{2}H $\pi/2$-read-out pulse. No ^{1}H decoupling is required in all experiments. Instead, deuterium scalar decoupling during detection and the indirect ^{13}C evolution period was found to be beneficial. CP was optimized in order to restrict magnetization transfer between directly bonded ^{2}H and ^{13}C (τ_{CP} = 2.5 ms). All

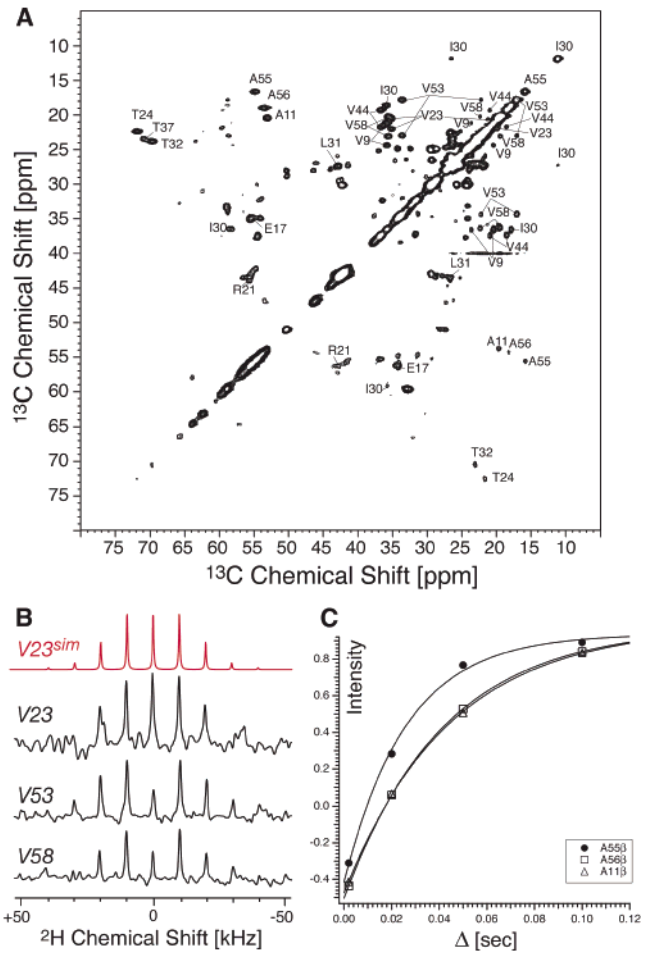


Figure 1. Three-dimensional ^{2}H,^{13}C,^{13}C MAS solid-state NMR correlation experiments for u-^{2}H,^{13}C,^{15}N-labeled SH3 (ω_r = 10 kHz, ^{2}H $\omega_{rf}/2\pi$ = 60 kHz for $\pi/2$, 40 kHz for CP, and 2−3 kHz for ^{2}H GARP[16] decoupling; RFDR mixing time = 3 ms, recycle delay = 250 ms; T = 7 °C. (A) Two-dimensional ^{13}C,^{13}C projection. (B) ^{2}H spectra along F_1 for selected valine-γ resonances. The simulated spectrum on the top assumes a two-site jump with a jump angle of 40°. (C) ^{2}H T_1 recovery curves for the three alanine-β resonances in the SH3 domain.

NMR experiments were performed on a Bruker Avance 600 MHz WB spectrometer equipped with a 4 mm triple-resonance MAS probe.

Figure 1A represents the 2D ^{13}C−^{13}C plane from the 3D experiments taken at $\omega_1(^2H)$ = 0 Hz. Assignments were obtained previously.[17] Aliphatic methyl groups display the highest intensity due to their long ^{2}H T_2. Aromatic resonances are mostly missing in the ^{13}C,^{13}C correlation due to ^{2}H line broadening because of dynamic processes. As an example, Figure 1B displays the ^{2}H dimension along the second indirect evolution period for three valine-γ resonances. Simulations show that the full ^{2}H pake pattern

† Forschungsinstitut für Molekulare Pharmakologie (FMP) Berlin, Germany.
‡ Present address: Laboratoire de RMN biomoléculaire, Université Claude Bernard Lyon 1, CNRS UMR 5180, 69622 Villeurbanne, France.
⊥ Institut für Chemie/Kristallographie, Freie Universität Berlin, Takustr. 6, 14195 Berlin, Germany.
∥ Charité Universitätsmedizin, 10098 Berlin, Germany.

10.1021/ja051830l CCC: $30.25

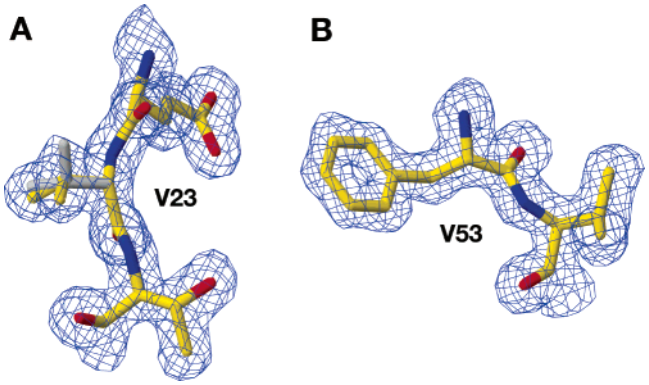


Figure 2. Structural model and experimental electron densities (at 1σ) around residues (A) V23 and (B) V53 in the X-ray structure of the SH3 domain (PDB code: 1U06). The model for side chain V23 was refined assuming two orientations with equal occupancy.

can be represented well, even by using only a radio frequency field of 60 and 40 kHz for the excitation pulse and the CP, respectively. This is due to the fact that crystallite orientations are almost sampled isotropically due to sample rotation. The spectra for V53 and V58 are characteristic for a methyl group, where the effective quadrupolar coupling constants are fitted to $C_q = 54$ and 51 kHz, respectively, and to an asymmetry parameter, $\eta = 0$. The small C_q is a result of fast rotational motion around the pseudo-3-fold spinning axis.[18] The spectrum for V23, in contrast, is characterized by an intense central peak. To fit this spectrum, an asymmetry parameter of $\eta \neq 0$ must be assumed.

The best fit for the quadrupolar parameters in the case of V23γ is obtained if the tensor adopts values of $C_q = 46.6 \pm 2.1$ kHz and $\eta = 0.59 \pm 0.1$. The nonzero asymmetry parameter and the reduced value of the quadrupolar coupling constant indicate additional motion. We simulated the average quadrupolar tensor components $\langle C_q \rangle$ and $\langle \eta \rangle$ in case of V23γ, assuming different motional processes.[19] The best fit is obtained, assuming a two-site jump between two equivalent sites with a jump angle of 40° (Figure 1B). The average quadrupolar parameters are then $\langle C_q \rangle = 43.6$ kHz and $\langle \eta \rangle = 0.21$. Other motional models yield larger root mean square deviation values between experimental and simulated data. In the case of a three-site jump model, the quadrupolar coupling constant for the best fit will be reduced to 18 kHz, with $\eta = 0$. A two-site jump (jump angle = 120°) yields $\langle C_q \rangle = 33$ kHz and $\langle \eta \rangle = 0.58$. An out-of-plane libration model ($\varphi = 15°$) gives a best fit of $\langle C_q \rangle = 47.5$ kHz and $\eta = 0.11$. Analysis of the X-ray structure indicates that the methyl groups of V23 adopt two conformations (Figure 2). Thus, solid-state NMR and X-ray crystallography can provide complementary information on the structure of solid samples since it is possible now to differentiate between ensemble averages and local dynamics. A conventional pake pattern (with $\eta = 0$) would be expected in the case of crystal imperfections.

The results of the ^{2}H T_1 experiments are represented in Figure 1C. For A55-β, a ^{2}H T_1 value is measured which is significantly shorter ($T_1 = 15.5$ ms) compared to those of other alanine methyl resonances, T_1 (A11-β) = 44.0 ms and T_1 (A56-β) = 41.7 ms. We assume that the small value for A55 is due to a water clathrate cluster around this residue, which allows a faster methyl group rotation. A general expression for the T_1 relaxation rate for deuterium quadrupolar relaxation is, for example, given by Spiess:[20]

$$\frac{1}{T_1} = \frac{3\pi^2}{2}\left(\frac{e^2 q_{zz} Q}{h}\right)^2 (J_1(\omega_0) + 4 \times J_2(2\omega_0))$$

$J_1(\omega_0)$ and $J_2(\omega_0)$ are spectral density functions corresponding to single quantum and to two-quantum spin flips, respectively. Both functions are orientation dependent in the solid state. Analytical expressions for T_1 relaxation rates for a variety of coupling mechanisms are derived by Torchia and Szabo.[21] In contrast to static solids or liquid crystalline samples, a concrete motional model cannot easily be fit to the frequency dependent T_1 relaxation rates,[22] due to almost isotropic sampling of all crystallite orientations during MAS.

In conclusion, we could show that deuterium NMR experiments are possible in the solid state to characterize dynamics, using uniformly ^{2}H,^{13}C,^{15}N isotopically enriched proteins. The described experiments are, in particular, conservative with respect to sample heating and are beneficial for sample stability since no proton decoupling is required in neither the ^{2}H nor the ^{13}C dimension. We expect that this approach will find widespread use in the characterization of the dynamics of membrane proteins or of amyloidogenic peptides and proteins.

Acknowledgment. This research was supported by the DFG Grant Re1435. We thank Profs. Hartmut Oschkinat and Udo Heinemann for stimulating discussions and continuous support.

References

(1) (a) 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. (b) Castellani, F.; van Rossum, B.-J.; Diehl, A.; Schubert, M.; Rehbein, K.; Oschkinat, H. *Nature* **2002**, *420*, 98−102. (c) Zech, S. G.; Wand, A. J.; McDermott, A. E. *J. Am. Chem. Soc.* **2005**, *127*, 8618−8626.
(2) Sandstrom, D.; Zimmermann, H. *J. Phys. Chem. B* **2000**, *104*, 1490−1493.
(3) Spiess, H. *Adv. Polym. Sci.* **1985**, *66*, 23−58.
(4) Hirschinger, J.; Miura, H.; Gardner, K. H.; English, A. D. *Macromolecules* **1990**, *23*, 2153−2169.
(5) (a) Seelig, J. *Q. Rev. Biophys.* **1977**, *10*, 353−418. (b) Davis, J. *Biochim. Biophys. Acta* **1983**, *737*, 117−171.
(6) (a) Copié, V.; McDermott, A. E.; Beshah, K.; Williams, J. C.; Spijker-Assink, M.; Gebhard, R.; Lugtenburg, J.; Herzfeld, J.; Griffin, R. G. *Biochemistry* **1994**, *33*, 3280−3286. (b) Williamson, P. T. F.; Watts, J. A.; Addona, G. H.; Miller, K. W.; Watts, A. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 2346−2351. (c) Howard, K. P.; Liu, W.; Crocker, E.; Nanda, V.; Lear, J.; Degrado, W. F.; Smith, S. O. *Protein Sci.* **2005**, *14*, 1019−1024.
(7) Hoatson, G. L.; Vold, R. L. *NMR Basic Principles and Progress*; Springer-Verlag: Berlin, Heidelberg, 1994; Vol. 32, pp 3−61.
(8) Schmidt-Rohr, K.; Spiess, H. W. *Multidimensional Solid-State NMR and Polymers*; Academic Press: London, 1994.
(9) Reichert, D.; Olender, Z.; Poupko, R.; Zimmermann, H.; Luz, Z. *J. Chem. Phys.* **1993**, *98*, 7699−7710.
(10) (a) Wittebort, R. J.; Olejniczak, E. T.; Griffin, R. G. *J. Chem. Phys.* **1987**, *86*, 5411−5420. (b) Hologne, M.; Hirschinger, J. *Solid State NMR* **2004**, *26*, 1−10.
(11) (a) Reif, B.; Griffin, R. G. *J. Magn. Reson.* **2003**, *160*, 78−83. (b) 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. (c) Paulson, E. K.; Morcombe, C. R.; Gaponenko, V.; Dancheck, B.; Byrd, R. A.; Zilm, K. W. *J. Am. Chem. Soc.* **2003**, *125*, 15831−15836.
(12) (a) Reif, B.; Jaroniec, C. P.; Rienstra, C. M.; Hohwy, M.; Griffin, R. G. *J. Magn. Reson.* **2001**, *151*, 320−327. (b) Reif, B.; van Rossum, B. J.; Castellani, F.; Rehbein, K.; Diehl, A.; Oschkinat, H. *J. Am. Chem. Soc.* **2003**, *125*, 1488−1489. (c) Paulson, E. K.; Morcombe, C. R.; Gaponenko, V.; Dancheck, B.; Byrd, R. A.; Zilm, K. W. *J. Am. Chem. Soc.* **2003**, *125*, 14222−14223.
(13) Chevelkov, V.; Faelber, K.; Diehl, A.; Heinemann, U.; Oschkinat, H.; Reif, B. *J. Biomol. NMR* **2005**, *31*, 295−310.
(14) Auger, C.; Lesage, A.; Caldarelli, S.; Hodgkinson, P.; Emsley, L. *J. Am. Chem. Soc.* **1997**, *119*, 12000.
(15) Bennett, A. E.; Rienstra, C. M.; Griffiths, J. M.; Zhen, W.; Lansbury, P. T., Jr.; Griffin, R. G. *J. Chem. Phys.* **1998**, *108*, 9463−9479.
(16) Shaka, A. J.; Barker, P. B.; Freeman, R. *J. Magn. Reson.* **1985**, *64*, 547−552.
(17) Pauli, J.; Baldus, M.; van Rossum, B. J.; De Groot, H.; Oschkinat, H. *ChemBioChem* **2001**, *2*, 272−281.
(18) Weintraub, O.; Vega, S. *Solid State Nucl. Magn. Reson.* **1995**, *4*, 341−351.
(19) (a) Batchelder, L. S.; Niu, C. H.; Torchia, D. A. *J. Am. Chem. Soc.* **1983**, *105*, 2228−2231. (b) Brouwer, E. B.; Enright, G. D.; Ratcliffe, C. I.; Facey, G. A.; Ripmeester, J. A. *J. Phys. Chem. B* **1999**, *103*, 10604−10616.
(20) Spiess, H. W. *NMR Basic Principles and Progress*; Springer: Berlin, 1978.
(21) Torchia, D. A.; Szabo, A. *J. Magn. Reson.* **1982**, *49*, 107−121.
(22) Trouard, T. P.; Alam, T. M.; Zajicek, J.; Brown, M. F. *Chem. Phys. Lett.* **1992**, *189*, 67−75.

JA051830L