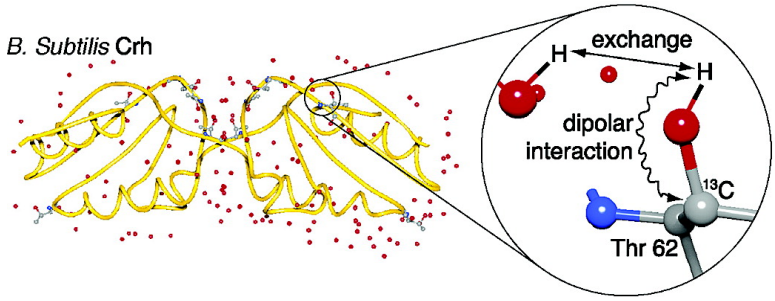
Communication

Water–Protein Interactions in Microcrystalline Crh Measured by H–C Solid-State NMR Spectroscopy

Anne Lesage, and Anja Bckmann

J. Am. Chem. Soc., **2003**, 125 (44), 13336-13337• DOI: 10.1021/ja036720y • Publication Date (Web): 10 October 2003



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 10/10/2003

Water−Protein Interactions in Microcrystalline Crh Measured by $^1H-^{13}C$ Solid-State NMR Spectroscopy

Anne Lesage*,† and Anja Böckmann*,‡

Laboratoire de Chimie, UMR 5532 CNRS-ENS, Ecole Normale Supérieure de Lyon, 46, allée d'Italie, 69364 Lyon, France, and Institut de Biologie et Chimie des Protéines, UMR 5086 CNRS, 7, passage du Vercors, 69367 Lyon, France

Received June 17, 2003; E-mail: anne.lesage@ens-lyon.fr; a.bockmann@ibcp.fr

Water, which is ubiquitously present in biological systems, plays a major role in determining the structure, dynamics, and function of biomolecules such as proteins or DNA. NMR has proven to be a powerful tool to study protein/water interactions in solution.[1] While slowly exchangeable protons can be directly observed by $^1H/^2H$ isotope exchange, the detection of fast exchangeable protein protons and hydration water usually relies on the observation of correlation peaks arising from the nuclear Overhauser effect (NOE) in two-dimensional (2D)[2,3] or selective one-dimensional (1D) experiments.[4] With regard to biomolecules in the solid state, site-resolved observation of hydration water is feasible by X-ray crystallography.[5] Solid-state NMR has also been applied as early as 1988 by Harbison and co-workers[6] to demonstrate, in a 1D approach based on the long transverse dephasing time of solvent protons, chemical exchange between water and sites of the membrane protein bacteriorhodopsin. Despite this pioneering study, very little is still known about the dynamics of water interactions in immobilized protein samples.

Over the past few years, solid-state NMR spectroscopy has been rapidly evolving as an alternative tool to liquid-state NMR spectroscopy and X-ray crystallography for the structural studies of insoluble biomolecules such as membrane-integrated proteins.[7] In parallel, the recent development of efficient proton−proton homonuclear decoupling techniques[8,9] has led to a considerable improvement in the resolution of proton spectra of complex solid compounds.[10,11] This now allows one to probe water−protein interactions in the solid state through the direct observation of solvent proton chemical shift, as is commonly done in liquid-state NMR, rather than through the dynamic properties potentially shared by various protons (of different solvents, for example).

In this Communication, we report the observation of hydrogen exchange with water in a solid protein as correlation signals at the ω_1 water proton frequency in a 2D $^1H-^{13}C$ dipolar correlation spectrum. These measurements were done on the microcrystalline protein Crh whose sample preparation and sequential assignments in the solid state have been reported recently.[12] In this study, a perdeuterated variant of the protein was used, in which we reexchanged labile deuterons with protons.

The 1D proton spectrum of the sample (data not shown) shows two dominant narrow lines (about 10 Hz width at half-height) at 4.7 and 3.6 ppm corresponding, respectively, to water and poly(ethylene glycol) that was used for microcrystallization. Figure 1 shows the pulse sequence used to record the $^1H-^{13}C$ correlation spectrum. Despite the fact that the protein is in large part deuterated, we found that homonuclear decoupling was required to get optimal proton resolution and avoid overlap with water resonance. Figure 2A shows an extract of the ^{13}C aliphatic region of the resulting 2D map. As expected, this spectrum displays cross-peaks between the reexchanged amide protons and their neighboring Cα spins. Correlations with the carbonyl resonances are also observed (data not shown). In addition, several cross-peaks involving aliphatic protons are visible which arise from the noncomplete deuteration of the side chains, involving mainly CH_3 groups. In contrast, complete deuteration of the α-protons is indicated by the absence of Hα−Cα cross signals.

The most remarkable feature in this spectrum is that at the ω_1 proton water frequency (4.7 ppm), we clearly observe cross-peaks

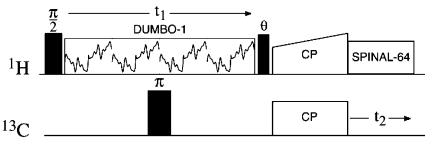


Figure 1. Pulse sequence used for the $^1H-^{13}C$ dipolar correlation experiment. A 180° carbon pulse was inserted in the middle of t_1 to refocus the evolution under the $^{13}C-^1H$ scalar couplings.[13] DUMBO-1 decoupling[9] was applied during t_1 for optimal proton resolution.[11] SPINAL-64 heteronuclear decoupling was applied during acquisition.[14]

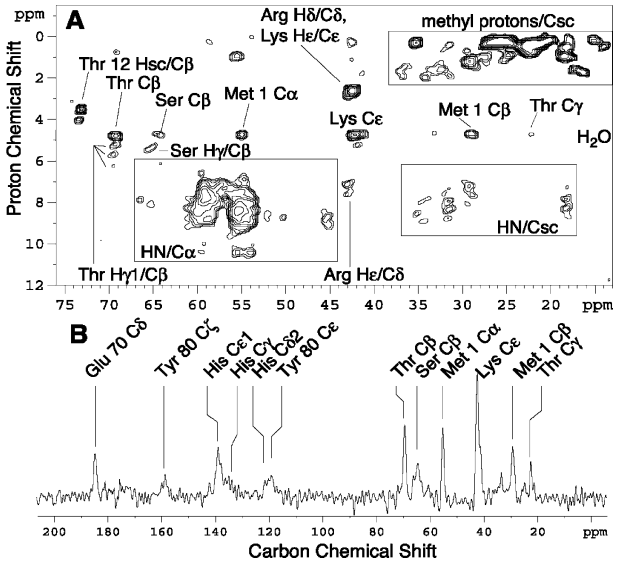


Figure 2. (A) 2D $^1H-^{13}C$ dipolar correlation spectrum recorded on the perdeuterated microcrystalline protein Crh (8 mg of sample in a restricted 4 mm rotor, ^1H frequency 500 MHz, time domain data size in t_1 and t_2, 116 and 528 points, respectively, $t_{1max} = 8.12$ ms, $t_{2max} = 10$ ms, number of scans per increment = 80, 12.5 kHz MAS, 7 μs ^{13}C π pulse, 2 ms ramped CP on ^1H with 56 kHz on ^{13}C, 83 kHz ^1H decoupling during t_1, 66 kHz ^1H during acquisition, recycle delay 3 s, sample temperature = 5 °C). ^1H chemical shifts were corrected with a scaling factor of 0.46 (as determined experimentally by recording the ^1H spectrum of L-Alanine under similar conditions[11]). Proton line widths at half-height of about 200 Hz (0.4 ppm) were measured. Water−protein cross-peaks were identified by analogy with the ^{13}C assignments reported for the protonated protein.[12] (B) ω_1 row extracted from the 2D spectrum of Figure 2A at the water resonance frequency (4.7 ppm). The abbreviation sc represents side chain.

† Ecole Normale Supérieure de Lyon.
‡ Institut de Biologie et Chimie des Protéines.

10.1021/ja036720y CCC: $25.00

between water protons and several protein carbon spins. Figure 2B shows the corresponding ω_1 row extracted from the 2D $^1H-^{13}C$ correlation spectrum. These cross-peaks can arise (i) from nonlabile protein protons whose resonance coincides with the water frequency, (ii) from a direct magnetization transfer from bound water protons to protein carbons, (iii) from dipolar spin diffusion from water to protein protons followed by cross-polarization (CP) to carbon spins, and (iv) from chemical exchange between a water proton and a labile protein proton during the spin-lock period, which then transfers its magnetization to a nearby carbon nucleus by Hartmann−Hahn transfer.[6] ^{13}C chemical shift analysis using the assignments reported recently for the nondeuterated microcrystalline form of Crh[12] reveals that the observed carbon-13 signals in t_2 belong mainly to Tyr, Thr, Ser, Lys, and His residues with labile hydroxyl, imidazole ring, or side-chain amine protons. It is well known that these protons usually display fast exchange rates in a protein.[15] Hence, we conclude that the observed cross-peaks at the water frequency arise most likely from chemical exchange between water and these labile protons, and subsequent magnetization transfer to their neighboring ^{13}C spins, all during the CP step. This implies that the observed hydrogen exchange occurs on the millisecond time scale, which is the same order of magnitude as the intrinsic exchange rates measured for hydroxyl, αNH_3^+, ϵNH_3^+, and imidazole protons in solution at pH 7,[16] pH used for Crh microcrystallization. No cross-peak involving backbone amide protons or side-chain $CONH_2$ (Asn, Gln) were observed at the water frequency in $^1H-^{15}N$ correlation spectra (data not shown); this is consistent with the about 2 orders of magnitude slower intrinsic exchange rate of these protons.[16]

More specifically, we observe clear cross-peaks between water and Cϵ resonances of lysine residues, indicating that the Lys Hζ protons are in fast exchange with water protons. In the same way, the hydroxyl proton of the only tyrosine (80) appears to be in fast exchange as suggested by the cross-peaks at the water frequency involving its Cζ and Cϵ resonances. All histidine residues in Crh are located in the C-terminal 6xHis tag added to the protein for overproduction and purification purposes. Histidine imidazole ring protons are known to experience very fast exchange in the liquid state[16] and are likely to give rise to the observed cross-peaks between water protons and His Cγ, Cδ2, and Cϵ1.

Very interestingly, we clearly remark that some residues having exchangeable protons do not display cross-peaks with water. This is, for example, the case for Thr 12, which has an isolated Cβ chemical shift (73.4 ppm)[12] allowing direct identification, and which does not display interactions with water. From X-ray crystallographic studies,[17] Crh is found to be a domain-swapped dimer which is also the structure identified in the microcrystalline state.[12] Thr 12 is located in the hinge region of this dimeric structure. In particular, its hydroxyl group was shown to be part of an intermonomer hydrogen-bonding network involving Thr 12 and Thr 57 of the second monomer. The hydroxyl proton of this residue is thus stabilized, which is consistent with our observations that it is not in fast exchange with the solvent. The remaining Thr residues (30, 57, 59, and 62) display cross-peaks at the proton water frequency, as well as between 5 and 6 ppm. However, their almost degenerate Cβ chemical shifts around 69 ppm make an individual assignment difficult.

Similarly, only two of the four Ser Cβ resonances show cross-peaks at the water proton frequency. From their Cβ chemical shift,[12] they are tentatively assigned to Ser 46 and Ser 52. This is consistent with the fact that the two other serine residues (31 and 56) are involved in hydrogen bonding and therefore do not show exchange with water protons.

Glutamic acid protons are known to experience very fast exchange (<1 ns) in solution and do not generate cross signals at the water frequency.[18] In the same way, for the microcrystalline protein Crh, the glutamic acid residues do not display interactions with water, with a noteworthy exception for Glu 70, easily identified by its distinctive Cδ chemical shift (184.5 ppm, Figure 2B).[12] This can possibly be explained by chemical exchange-relayed magnetization transfer via the fast exchanging N-terminal Met 1 αNH_3^+ protons, which are known to be involved in an intermonomer salt bridge with Glu 70.[17] From this observation, we deduce that the only observed Cα/water cross-peak belongs to Met 1, which could not be observed previously.

In conclusion, we have demonstrated here that heteronuclear correlation spectroscopy is a simple method to detect water−protein interactions in the solid state. In addition, we have shown that, as in solution, these interactions can be correlated to important structural features of the protein, such as hydrogen bonding. As in liquid-state NMR, a variety of 3D or selective 2D techniques can be derived to assign in a more reliable way the water−protein cross-peaks observed here, and to better quantitatively determine the corresponding exchange rates. The observations described here should open the way to studies of solvent accessibility of proteins in the solid state, which is of crucial importance notably for membrane proteins.

Acknowledgment. We thank Michel Juy, Lyndon Emsley, and François Penin for helpful discussions.

References

(1) (a) Englander, S. W.; Sosnick, T. R.; Englander, J. J.; Mayne, L. *Curr. Opin. Struct. Biol.* **1996**, *6*, 18−23. (b) Otting, G. *Prog. Nucl. Magn. Reson. Spectrosc.* **1997**, *31*, 259−285.
(2) (a) Dobson, C. M.; Lian, L.-Y.; Redfield, C.; Topping, K. D. *J. Magn. Reson.* **1986**, *69*, 201−209. (b) Guéron, M.; Leroy, J.-L. *Methods Enzymol.* **1995**, *261*, 383−413. (c) Jeener, J.; Meier, B. H.; Bachmann, P.; Ernst, R. R. *J. Chem. Phys.* **1979**, *71*, 4546−4553. (d) Spera, S.; Ikura, M.; Bax, A. *J. Biomol. NMR* **1991**, *1*, 155−165.
(3) Otting, G.; Liepinsh, E.; Wuthrich, K. *Science* **1991**, *254*, 974−980.
(4) (a) Böckmann, A.; Guittet, E. *J. Biomol. NMR* **1996**, *8*, 87−92. (b) Dalvit, C.; Hommel, U. *J. Biomol. NMR* **1995**, *5*, 306−310. (c) Otting, G.; Liepinsh, E. *J. Magn. Reson., Ser. B* **1995**, *107*, 192−196. (d) Böckmann, A.; Penin, F.; Guittet, E. *FEBS Lett.* **1996**, *383*, 191−195. (e) Böckmann, A.; Guittet, E. *FEBS Lett.* **1997**, *418*, 127−130. (f) Böckmann, A.; Guittet, E. *J. Chim. Phys.* **1995**, *92*, 1923−1928.
(5) Baker, E. N. In *Protein−Solvent Interactions*; Gregory, R. G., Ed.; M. Dekker: New York, 1995; pp 143−189.
(6) Harbison, G. S.; Roberts, J. E.; Herzfeld, J.; Griffin, R. G. *J. Am. Chem. Soc.* **1988**, *110*, 7221−7223.
(7) Böckmann, A.; McDermott, A. E. In *The Encyclopedia of NMR*; Grant, D. M., Harris, R. K., Eds.; J. Wiley and Sons: London, 2002.
(8) (a) Levitt, M. H.; Kolbert, A. C.; Bielecki, A.; Ruben, D. J. *Solid State Nucl. Magn. Reson.* **1993**, *2*, 151−163. (b) Hafner, S.; Spiess, H. W. *Solid State Nucl. Magn. Reson.* **1997**, *8*, 17−24. (c) Hohwy, M.; Nielsen, N. C. *J. Chem. Phys.* **1997**, *106*, 7571−7586. (d) Madhu, P. K.; Zhao, X.; Levitt, M. H. *Chem. Phys. Lett.* **2001**, *346*, 142−148. (e) Vinogradov, E.; Madhu, P. H.; Vega, S. *Chem. Phys. Lett.* **1999**, *314*, 443−450.
(9) Sakellariou, D.; Lesage, A.; Hodgkinson, P.; Emsley, L. *Chem. Phys. Lett.* **2000**, *319*, 253−260.
(10) (a) van Rossum, B. J.; Castellani, F.; Rehbein, K.; Pauli, J.; Oschkinat, H. *ChemBioChem* **2001**, *2*, 906−914. (b) Sakellariou, D.; Lesage, A.; Emsley, L. *J. Am. Chem. Soc.* **2001**, *123*, 5604−5605.
(11) Lesage, A.; Sakellariou, D.; Hediger, S.; Eléna, B.; Charmont, P.; Steuernagel, S.; Emsley, L. *J. Magn. Reson.* **2003**, *163*, 105−113.
(12) Böckmann, A.; Lange, A.; Galinier, A.; Luca, S.; Giraud, N.; Juy, M.; Heise, H.; Montserret, R.; Penin, F.; Baldus, M. *J. Biomol. NMR* **2003**, *27*, 323−339.
(13) Lesage, A.; Emsley, L. *J. Magn. Reson.* **2001**, *148*, 449.
(14) (a) Fung, B. M.; Khitrin, A. K.; Ermolaev, K. *J. Magn. Reson.* **2000**, *142*, 97−101. (b) DePaepe, G.; Lesage, A.; Emsley, L. *J. Chem. Phys.* **2003**, *119*, 4833−4841.
(15) Liepinsh, E.; Otting, G.; Wüthrich, K. *J. Biomol. NMR* **1992**, *2*, 447−465.
(16) Wüthrich, K. *NMR of proteins and nucleic acids*; Wiley: New York, 1986.
(17) Juy, M.; Penin, F.; Favier, A.; Galinier, A.; Montserret, R.; Haser, R.; Deutscher, J.; Böckmann, A. *J. Mol. Biol.* **2003**, *332*, 767−776.
(18) Liepinsh, E.; Rink, H.; Otting, G.; Wüthrich, K. *J. Biomol. NMR* **1993**, *3*, 253−257.

JA036720Y