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Characterization of $^1H-^1H$ Distances in a Uniformly $^2H,^{15}N$-Labeled SH3 Domain by MAS Solid-State NMR Spectroscopy§

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In the past few years, interest in the application of high-resolution, solid-state magic angle spinning (MAS) NMR experiments for structure determination of uniformly labeled peptides and proteins has grown rapidly. A number of special assignment techniques have been developed and recently have found application to small proteins.[1,2] However, a general method to determine the global fold of a protein is not yet established. Long-range structural information is crucial in determining the three-dimensional fold of a protein. In uniformly labeled systems, long-range distance information (corresponding to a small coupling) is perturbed by strong homonuclear dipolar couplings which make it difficult to use an abundant spin for direct long-range transfers.[3,4] Recently, several approches have been published to tackle these problems. Quantitative analysis of cross-peak intensities in a TEDOR experiment yield multiple restraints in uniformly $^{13}C,^{15}N$-labeled peptide.[5] Rienstra et al. have shown that ramped Hartmann−Hahn cross polarization transfer between side-chain carbon and backbone nitrogen allows the determination of weak heteronuclear couplings.[6] Spin dilution circumvents the problem by retaining only the weak dipolar couplings of interest. A labeling strategy involving selectively labeled glycerol originally proposed by LeMaster[7] for solution-state NMR has been employed by Hong[8] to obtain labeling only in hydrophobic core regions of a protein. This approach has been successfully extended by Oschkinat and co-workers to calculate the structure of a SH3 domain exclusively on the basis of solid-state NMR data.[9]

The so-far presented techniques rely all on correlation between low γ heteronuclei. In principle, however, long-range distance information can be obtained best via correlations among high γ nuclei due to the dependence of the dipolar coupling on the gyromagnetic ratio. We have demonstrated recently that this approach is viable for a small model peptide Nac-Val-Leu-OH.[10,11] In this communication, we exploit this methodology for a uniformly $^2H,^{15}N$-labeled sample of the SH3 domain of chicken α-spectrin to obtain long-range $^1H-^1H$ distances. Altogether, three samples have been prepared using different deuteration strategies: (preparation 1) a sample that was uniformly deuterated on the α carbon position and <30% deuterated on the side-chain carbon by growing cells in a medium containing ^1H glucose and D_2O[12]; (preparation 2) a uniformly deuterated (>90%) sample by growing cells on a medium containing ^2H glycerol and D_2O[13]. The obtained spectra were compared to those from a sample that was ^{15}N-labeled but not deuterated (preparation as described in ref 14) (preparation 3). In the experiment, two ^{15}N evolution periods (t_1 and t_2) are connected by a mixing element. The resolution in the ^{15}N dimension is good enough to assign almost all correlations unambiguously.

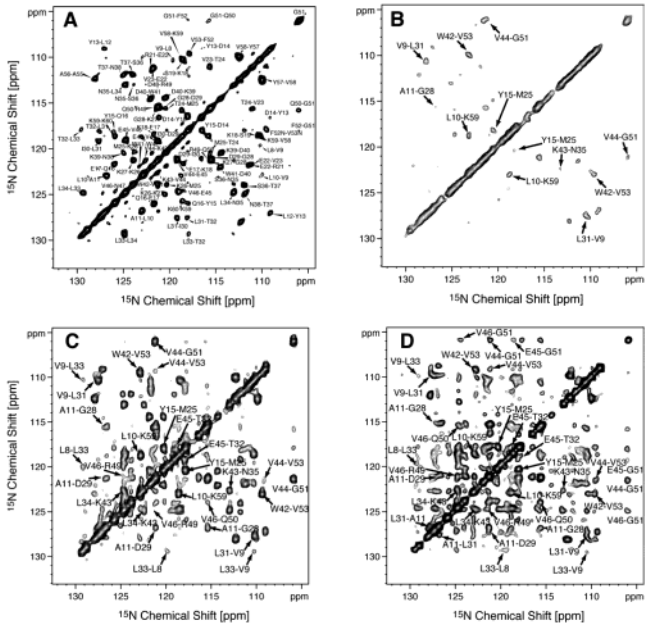

Figure 1. $^{15}N,^{15}N$ correlation spectra for a $^2H,^{15}N$-labeled sample of α-spectrin SH3. Magnetization transfer between ^{15}N spins is achieved via ^1H driven spin diffusion (A) and Post-C7 $^1H,^1H$ dipolar recoupling (t_3 = 200 μs = 2 τ_R) (B). Correlation spectra using $^1H,^1H$ spin diffusion for mixing (C: t_{mix} = 450 μs, D: t_{mix} = 2.4 ms. Long-range correlations are indicated with arrows. Total experimental time for each experiment was 13.6 h in all cases (sample quantity: ca. 8 mg of protein in a 4-mm rotor).

For reference, a ^1H-driven $^{15}N,^{15}N$ spin diffusion experiment[15] was recorded (Figure 1). This experiment yields mostly sequential $^{15}N,^{15}N$ correlations in the protein backbone.[16] To obtain long-range $^1H-^1H$ distance information, magnetization was transferred back to ^1H after t_1 to allow for direct $^1H-^1H$ mixing.

First,$^1H-^1H$ mixing was achieved by DQF-Post-C7[17] for direct $^1H-^1H$ recoupling. A mixing time of two rotor periods for double quantum excitation yields maximum cross-peak intensities (Figure 1B). In a second experiment, $^1H-^1H$ spin diffusion was used for mixing. Spectra recorded with mixing times of 450 μs and 2.4 ms are shown in Figure 1C,D. Cross-peak intensities are comparable using the partially deuterated (preparation 1) and fully deuterated sample (preparation 2) (data not shown). Magnetization transfer into the side chain is truncated, due to quantitative deuteration of the H^α position in both cases. Initial rate fitting (Figure 2A) was done as described in ref 10. The extracted values for the (unscaled) $^1H,^1H$ dipolar couplings are in qualitative agreement with the values for distances as found in an energy-minimized X-ray structure[18] of the SH3 domain: e.g., D(G51-H^N, V44-H^N) = 5540.0 Hz = 2.79 Å, (3531.6 Hz, d = 3.24 Å); D(G51-H^N, F52-H^N) = 3330.0 Hz = 3.30 Å, (1354.0 Hz, d = 4.46 Å); D(G51-H^N, Q50-H^N) = 2750.0

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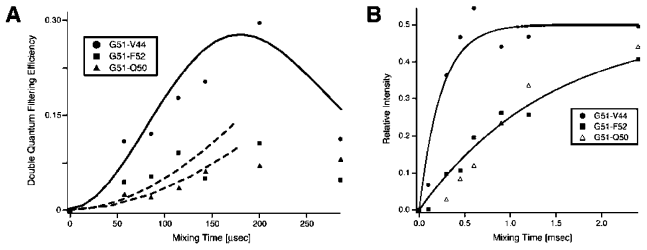

Figure 2. Cross-peak buildup curves for the correlations G51-V44, G51-F52, and G51-Q50, using (A) Post-C7 and (B) ^{1}H spin diffusion mixing.

Hz = 3.52 Å, (1327.0 Hz, d = 4.49 Å). Expected values for ^{1}H−^{1}H dipolar couplings and distances are indicated in parentheses. The effective relaxation time during the mixing process was estimated from the values of the ^{1}H line widths (1.8 kHz). The experimental correlations display systematically shorter distances which might be due to indirect correlation via water molecules.

Cross-peak buildup using the spin diffusion mixing scheme is very fast, yielding an equilibration of magnetization after around 500 μs for strongly coupled protons (Figure 2B). At short mixing times, only cross-peaks originating from magnetization transfer to the next neighbor proton can be observed. Cross-peak intensities, in general, reached up to 60% (with respect to diagonal peak intensity) indicating only negligible magnetization loss into the H_2O reservoir. At increasing mixing times, longer-range interactions can also be observed (3−5 Å) (e.g., G51-Q50/F52 [4.45 Å], G51-V46 [4.80 Å], G51-E45 [5.05 Å]). Interestingly, weak ^{1}H−^{1}H interactions, as for example in the case of the sequential H^N−H^N correlations between G51 and Q50/F52, respectively, displayed a maximum transfer at equilibrium of about 50% which is similar to the maximum transfer observed for the correlation G51-V44 (3.24 Å). This is unexpected, since the maximum transfer amplitude in a three-spin system is given by $(D_{12}/D_{13})^2$, where D_{12} and D_{13} correspond to the size of the small and large dipolar coupling constant $D_{ij} = (\mu_0/4\pi)\gamma_i\gamma_j h/(2\pi r_{ij}^3)$, between spins 1−2 and 1−3, respectively. The cross-peak buildup is determined by the zero-quantum spectral-density at the chemical shift difference[19,20] and can be described as a $n = 0$ rotational resonance condition.[21] Exact simulations of the cross-peak intensities in the experiment above are difficult, since the effect depends strongly on the ^{1}H isotropic chemical shift difference and on the differential CSA of the two nuclei involved, in addition to the ^{1}H−^{1}H dipolar coupling. We use here an initial rate approximation to describe the cross-peak buildup without geometrical assumptions on the size and orientation of the ^{1}H CSA tensor. As pointed out by Levitt et al.,[21] the relative cross-peak intensity can be estimated as $\tanh[0.5*T_2^{ZQ}*(\omega_B^{(0)})^2*t_{mix}]$ in the case of fast dephasing $[1/T_2^{ZQ} > \omega_B^{(0)}]$, with $\omega_B^{(0)}$ being proportional to the size of the ^{1}H,^{1}H dipolar coupling. The accumulated phase induced by the anisotropic difference chemical shift is small compared to the size of the dipolar coupling. It is noted that differential ^{1}H CSA is necessary to drive ^{1}H spin diffusion. Scaled dipolar couplings for the cross-peak G51-V44, G51-F52, and G51-Q50 are obtained as follows: D(G51-H^N, V44−H^N) = 1740.0 Hz; D(G51-H^N, F52−H^N) = 540.0 Hz, D(G51-H^N, Q50-H^N) = 560.0 Hz, using $1/T_2^{ZQ}$ = 2600.0 Hz in all cases. The ratios of these couplings are in very good agreement with respect to the distances as found in the X-ray structure. ^{1}H isotropic chemical shift differences are thus far not taken into account in the analysis. Largely reduced cross-peak intensities could be observed for direct ^{1}H−^{1}H transfer using a protonated sample (preparation 3) (data not shown). We explain this finding that after back-transfer to protons, magnetization equilibrates on all protons yielding a loss of magnetization after the second transfer to nitrogen. This experiment has been carried out before.[22,23] There, a quantitative distance analysis is impossible, since the transfer dynamics is strongly dependent on the spin density, and thus on local torsional angles. In summary, all of the expected 21 short-range H^N−H^N distances (<3.5 Å) involved in hydrogen bonds, are observed. Furthermore, eight longer-range correlations could be detected that are assigned to be in the range of 3.5−5.5 Å and which are nontrivial (i.e., nonsequential). Incorporation of an additional ^{13}C dimension should allow for the characterization and unambiguous assignment of even longer-range ^{1}H,^{1}H correlations. We expect this approach to become a useful tool to restrain the global fold of a protein in the solid-state. The observed long-range ^{1}H−^{1}H contacts are all involved in inter β-strand connectivites and, thus, allow theorientation of secondary structure elements with respect to one another. These restraints turned out to be important for structure calculations of porines by solution-state NMR.[24,25] Similar connectivities are expected for α-helices.

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Supporting Information Available: Information on the applied pulse sequences and experimental parameters and an error estimation of the measurements (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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