Communication

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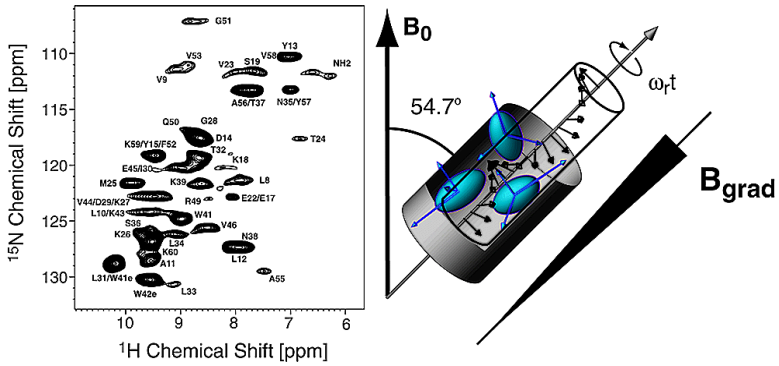

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^{1}H Detection in MAS Solid-State NMR Spectroscopy of Biomacromolecules Employing Pulsed Field Gradients for Residual Solvent Suppression[⊥]

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Sensitivity in MAS solid-state NMR is hampered thus far by detection of low-γ nuclei. ^{1}H detection can provide a gain in sensitivity of $(\gamma_H/\gamma_X)^{3/2}$ corresponding to a factor of 8 and 31 compared to the ^{13}C and ^{15}N detected version of the experiment, respectively, where γ_H and γ_X correspond to the gyromagnetic ratios of protons and the respective heteronuclei. This gain in sensitivity can only be achieved, however, if the two nuclei have comparable efficiency and line width. Inverse detection schemes are used routinely in solution-state multidimensional heteronuclear correlation experiments.[1,2] In the solid state, ^{1}H detection of protonated samples yields an increase in signal-to-noise—compared to the sensitivity that can be achieved upon detection on the heteronucleus—only at MAS rotation frequencies above 30 kHz.[3−5] For many biological samples, which are temperature sensitive, high rotation frequencies are prohibitive. Furthermore, the active volume of high-speed MAS rotors is restricted, reducing the maximum achievable signal-to-noise ratio for a given sample. Therefore, we suggested recently to use perdeuteration together with back-exchange of the amide protons to attenuate the strong proton proton dipolar couplings.[6,7] This concept retains maximum sensitivity in contrast to previously suggested deuteration strategies,[8,9] since all amide sites in the protein backbone contain an NMR active nucleus. At moderate rotation frequencies, perdeuteration yields a significant improvement in sensitivity (×5 vs ^{15}N detection at 13 kHz). This enhancement factor scales approximately inversely proportional to the rotation frequency (×9 at 33 kHz). Many applications, e.g. structure determination of membrane proteins, might not be possible without the use of ^{1}H detection. A major problem in ^{1}H detection of biological samples consists, however, in suppression of the magnetization of the residual solvent necessary to keep samples hydrated.

In this communication, we report for the first time ^{1}H-detected ^{1}H,^{15}N correlation experiments using pulsed field gradients (PFGs) for water suppression, carried out on a uniformly ^{2}H,^{15}N-enriched sample of a SH3 domain from chicken α-spectrin. In contrast to solution-state NMR, where water molecules tumble freely in solution, these are tightly bound to the protein microcrystals in the solid state. Water suppression is therefore impossible using conventional schemes. Presaturation leads to a rapid suppression of protein proton resonances due to saturation transfer. Binomial excitation schemes[10] failed, due to the very broad water resonance line. We show here that pulsed field gradients can successfully be used in solid-state NMR to attenuate the solvent resonance. PFGs

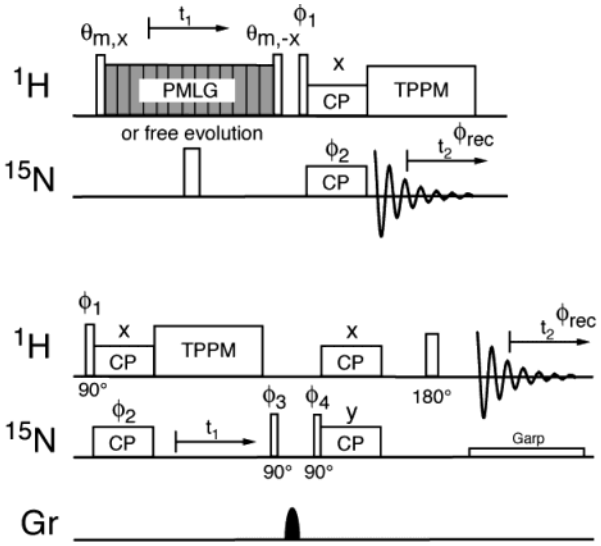

Figure 1. (Top) ^{15}N-detected ^{1}H,^{15}N correlation experiment. Spectra were recorded with and without PMLG for homonuclear ^{1}H,^{1}H decoupling in the indirect dimension. (Bottom) ^{1}H-detected ^{1}H,^{15}N correlation using pulsed field gradients for H_2O suppression. A 16-step phase cycle is employed for optimum water suppression: $\phi_1 = y,-y$; $\phi_2 = 2(y),2(-y)$; $\phi_3 = 4(x),4(-x)$; $\phi_4 = 8(x),8(-x)$; $\phi_{rec} = \phi_1 + \phi_2 + \phi_3 + \phi_4$. ^{1}H CP phases and decoupling rf fields must be along x to avoid interference with H_2O suppression. The total experimental time was 3.5 h in each experiment.

are nowadays commonly employed in solution-state NMR for coherence order selection[11] and water suppression.[12] Gradients have been introduced recently to solid-state NMR for coherence order selection in ^{13}C,^{13}C and ^{13}C,^{15}N correlation experiments.[13] They have been used furthermore to restrict the sample volume to circumvent radio frequency field inhomogeneity problems[14] and to suppress zero-frequency artifacts in natural abundance samples.[15]

Figure 1 represents the pulse sequences that are employed for the ^{15}N- and ^{1}H-detected ^{1}H,^{15}N correlation experiment. In the ^{15}N-detected version of the experiment, spectra were recorded with and without PMLG ^{1}H,^{1}H homonuclear decoupling[16,17] in the indirect evolution period. After CP transfer to nitrogen, TPPM decoupling is used for efficient decoupling of protons.

τ_{CP} was set to 150 μs to restrict magnetization transfer only between directly bonded nuclei. Typical CP transfer efficiencies are in the order of 55%. In the ^{1}H-detected version, ^{15}N magnetization is stored along the z axis after the indirect evolution period. A purge gradient is applied to dephase residual transverse water magnetization. Typically, sine-shaped gradients are employed of 5 ms duration and 30 G/cm of maximum strength. After back-transfer to ^{1}H, a Hahn-echo, comprising two rotor periods, was implemented to eliminate baseline rolling due to probe ring down. GARP[21] was

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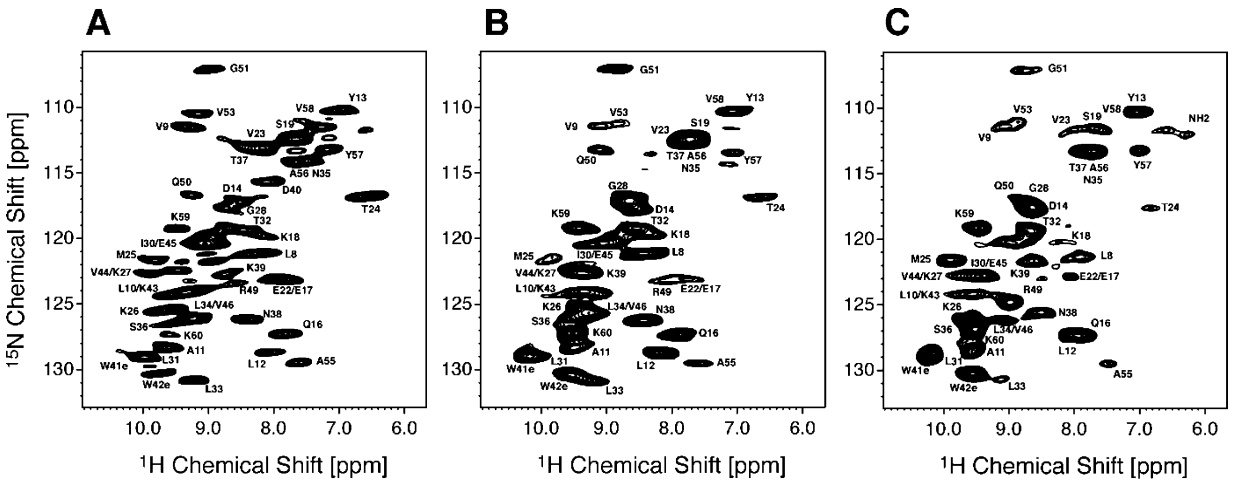

Figure 2. Experimental ^{15}N-detected (A,B) and ^{1}H-detected (C) ^{1}H,^{15}N correlation spectra using the pulse sequence displayed in Figure 1. A was recorded using PMLG with decoupling of the HN scalar coupling according to ref 18. B was recorded allowing for a free evolution in the indirect dimension without application of PMLG. For clarity, the ^{15}N-detected spectra A,B are displayed mirror imaged. Spectra have been recorded at $B_0 = 500$ MHz and a MAS rotation frequency of 10 kHz, using a commercial 2.5-mm double resonance probe which has been equipped with a gradient coil. The sample has been prepared as described in ref 19. The assignment of exchangeable proton and ^{15}N resonances is based on ref 20.

applied on the ^{15}N channel during detection to achieve heteronuclear (scalar) decoupling which yields a gain in resolution in the order of the size of the $^{1}J_{HN}$ scalar coupling (∼94 Hz). Application of PMLG to achieve ^{1}H,^{1}H homonuclear decoupling yields only a small increase in resolution in the ^{1}H dimension (Figure 2). The loss in sensitivity associated with CRAMPS[22,23]-type multipulse sequences during detection, like w-PMLG,[24] therefore outweighs the possible improvement in resolution. Use of PFGs for coherence order selection as it is done in solution-state NMR[25,26] is not possible, since the currently available gradients are too weak to obtain a significant dephasing of the water magnetization using very short gradient pulses (∼100−200 μs). Furthermore, active shielding would be required to enable reduced gradient recovery delays. Short gradient pulses are necessary to retain high sensitivity due to the short ^{1}H T_2 times (∼1−2 ms). The experimental results for the pulse schemes are represented in Figure 2. The apparent resolution in the ^{1}H dimension in the ^{1}H detected experiment is approximately a factor of 1.5 smaller compared to the resolution that is achievable in the ^{15}N-detected version using PMLG ^{1}H,^{1}H homonuclear decoupling in the indirect ^{1}H dimension (see also Supporting Information). Different intensities of certain cross-peaks (spectrum A compared to B,C in Figure 2) can be attributed to tightly bound water molecules in the protein structure which lead to a differential dipolar broadening of the individual ^{1}H amid resonances in the ^{1}H-detected experiment. Differences between A, B compared to C are assigned to sample heating due to TPPM decoupling during acquistion.

We have shown that it is possible to detect protons in a perdeuterated SH3 sample without the need of CRAMPS-type multiple-pulse homonuclear decoupling sequences. Pulsed field gradients turn out to be essential to achieve efficient solvent suppression. We expect that this labeling concept which achieves high senitivity due to ^{1}H detection, in combination with the possibility to measure long-range ^{1}H−^{1}H distances as we have shown previously,[19] will be a useful tool for the determination of unknown protein structure in the solid state.

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Supporting Information Available: Two figures displaying the impact of pulsed field gradients on solvent suppression, as well as a figure comparing ^{1}H line widths with and without application of PMLG homonuclear decoupling (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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