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Low-Power High-Resolution Solid-State NMR of Peptides and Proteins

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The resolution in solid-state NMR spectra has recently been improved to a level that the structure determination of medium-sized ^{13}C,^{15}N-labeled proteins in microcrystalline and noncrystalline samples becomes feasible.[1,2] To obtain high spectral resolution, the application of magic-angle sample spinning (MAS) and radio frequency (rf) pulse sequences is indispensable. Typically, rf-fields exceeding 100 kHz are required for extended time periods (>50 ms). Such rf irradiation can lead to unwanted intermittent heating and, as a consequence, deterioration of the sample as well as to instrumental artifacts.[3] The heating is especially critical in samples with a high water and salt content like peptides and proteins. It is, therefore, important to develop NMR experiments that can be performed with considerably less rf power.

Broadband, homonuclear two-dimensional ^{13}C chemical-shift correlation spectroscopy[4,5] is a basic experiment used for the resonance assignment in peptides. In this Communication, we describe such an experiment performed with rf-field amplitudes lower than 15 kHz for the proton-decoupling and lower than 40 kHz for the adiabatic dipolar recoupling.[4,5] Higher fields were only applied for the preparation of the initial ^{13}C polarization using conventional adiabatic cross polarization.[6] Due to its short duration, this period is less critical, and we also believe that it will be possible to replace it by a low-power equivalent in the future.

Previously published correlation spectra require high-power rf irradiation during most of the experiment: (i) During the evolution time, t_1, high-power heteronuclear decoupling is used to obtain high-resolution spectra. Typically, the rf-field strength is at least 3 times the MAS frequency to avoid recoupling conditions.[7] (ii) During the mixing time, τ_m, often a high-power multiple-pulse sequence on the ^{13}C spins is required to obtain the desired effective Hamiltonian. At the same time, high-power decoupling has to be applied to the proton spins with a field strength of at least 3 times the one used on the ^{13}C channel.[8,9] The exception to this statement is proton-driven spin-diffusion[4,5] which is therefore quite popular despite the fact that the transfer is considerably less efficient than that with the best coherent or adiabatic recoupling sequences.[10] (iii) During the detection time, t_2, again high-power heteronuclear decoupling has to be applied. In total, several ten's of milliseconds of high-power rf irradiation are required and cause the adverse effects mentioned above.

Recently, we have demonstrated that the fast spinning regime (MAS at >50 kHz[11]) offers new opportunities for the design of low-power decoupling methods[12,13] and efficient polarization-transfer.[10] In the following, we demonstrate that it is possible to implement a broadband low-power chemical-shift correlation experiment. The scheme shown in Figure 1 uses DREAM[14,15] recoupling (without proton-decoupling) and low-power XiX decoupling.[13] The experiment is only efficient under rapid MAS. In a recent study, we have found that the quality of a sample of the 82 amino acid protein Crh is not aversely influenced by rapid MAS.[10] The DREAM experiment has the advantage of being an adiabatic experiment[16] leading to a high polarization-transfer efficiency and that its performance increases with increasing spinning frequency.

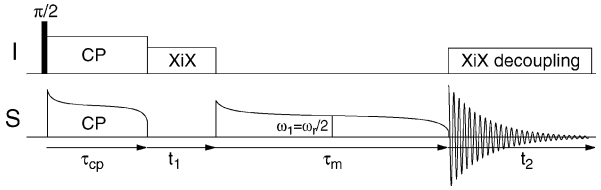

Figure 1. DREAM pulse sequence used for recording the 2D ^{13}C chemical-shift correlation spectra. The cw decoupling during the mixing time τ_m was only applied in the reference spectra. The XiX decoupling during the evolution and detection time was either high-power or low-power XiX decoupling.

Using a home-built experimental 1.3 mm MAS probe on a Bruker 600 MHz wide-bore spectrometer, we have recorded two-dimensional DREAM spectra of the cyclic decapeptide antamanide[17,18] at an MAS frequency of 60 kHz using low-power schemes. The sample contained roughly 1 mg of peptide and was cooled such that the temperature stayed below 30 °C. The XiX decoupling[7,19] during the evolution and detection time was adjusted to a field strength of about 14.5 kHz and a pulse length of 70 μs. During the DREAM recoupling period of 8 ms, no proton decoupling was applied. The rf amplitude during the DREAM period was tangentially swept from 23 to 37 kHz. The cross-polarization time was 1 ms using an rf-field amplitude of about 150 kHz on the ^1H channel avoiding rotary-resonance. The spectral width was 50 kHz in both dimensions, and 1024 complex data points were acquired in t_2. Sixteen scans were added up for each of the 1024 t_1 increments. The recycle delay was set to 3 s, leading to an acquisition time of about 14 h. The spectra were Fourier transformed using a shifted sine-square window function with zero filling to 8 k data points in both dimensions.

The resulting spectrum is presented in Figure 2a. For comparison, a spectrum under high-power conditions (Figure 2b) was recorded with XiX decoupling at 200 kHz rf-field amplitude and a pulse length of 52 μs. Continuous-wave decoupling also at 200 kHz was used during the mixing time. At a first glance, the two spectra are quite similar, despite the fact the applied average proton rf power during evolution, mixing, and detection is lower by a factor of more than 190 for the low-power experiment. In particular, no significant line broadening can be observed in the low-power XiX decoupled spectrum as compared to the high-power decoupled spectrum (Figure 2b). The overall intensity of the cross-peaks is also quite similar, although a closer comparison of the two spectra shows that some of the cross-peak intensities are different. Some of the cross-peaks change their sign, for example, the proline δ-α cross-peaks. Others are only visible in one of the spectra, for example, the proline β-carbonyl cross-peaks.

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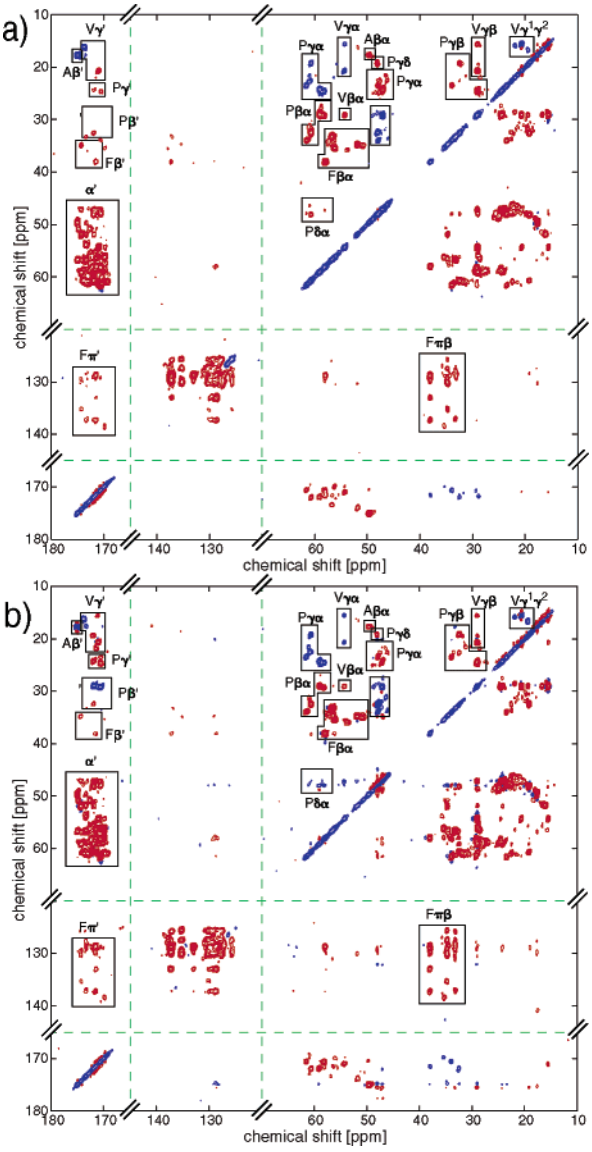

Figure 2. Comparison of DREAM spectra at 60 kHz MAS (a) with low-power XiX decoupling during t_1 and t_2 and no decoupling during the mixing time τ_m and (b) with high-power XiX decoupling during t_1 and t_2 and high-power cw decoupling during the mixing time τ_m.

It should be pointed out that the sample available for our experiments[18] was of lower quality than the one used for earlier experiments and showed some structural inhomogeneity. This can be seen for example in the valine γ^1-γ^2 cross-peaks which are triplicated. The observation of the structural inhomogeneity does, however, not influence the experimental results reported here.

To test the range of spinning frequencies where the DREAM experiment can be carried out without proton decoupling, we compared spectra with and without decoupling during the mixing time at MAS frequencies of 30, 40, 50, and 60 kHz (data shown as Supporting Information). To simplify the comparison of the spectra, high-power XiX decoupling was used during the free evolution times of those experiments. At 30 kHz MAS frequency, the spectrum without proton decoupling during the mixing time shows, as expected, very little intensity because the ^{13}C polarization decays under the influence of the residual coupling to the protons. The spectra at 40 kHz MAS still show strong attenuation of the peak intensities, but at 50 kHz MAS the differences become much smaller with further improvements at 60 kHz spinning frequency. The higher MAS frequency has the additional benefit that the bandwidth of the DREAM experiment becomes large enough to cover the full ^{13}C chemical-shift range on a 14 T (600 MHz ^{1}H frequency) magnet. Obviously, the necessary MAS frequencies can only be achieved by small rotors which contain small sample volumes.

We have shown that, for MAS frequencies exceeding 50 kHz, chemical-shift correlation experiments using the DREAM scheme without proton irradiation during the dipolar recoupling period and low-power XiX decoupling during the free evolution periods become feasible. In our sample of the decapeptide antamanide, only an insignificant increase in line width as compared to the high-power implementation of the experiment could be observed. There are small differences in polarization-transfer efficiency between the experiments with and without proton decoupling during the mixing time which are not yet fully understood. The overall intensity of the two spectra, however, is comparable, and there is no general loss in polarization or in information contents. It is expected that low-power experiments will allow the study of sensitive and salt-containing samples using high-resolution solid-state NMR methods.

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Supporting Information Available: Additional contour plots of two-dimensional DREAM spectra at MAS frequencies of 30, 40, 50, and 60 kHz with and without decoupling during the mixing time τ_m (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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