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SPINAL modulated decoupling in high field double- and triple-resonance solid-state NMR experiments on stationary samples

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Abstract

Continuous wave irradiation has limited bandwidth for heteronuclear ^{1}H decoupling at high fields and for ^{13}C decoupling in $^{1}H/^{13}C/^{15}N$ triple-resonance experiments. SPINAL-16 modulation is shown to improve the efficiency of ^{1}H and ^{13}C heteronuclear decoupling on single crystals of peptides and on magnetically aligned samples of membrane proteins in bicelles, which is of particular importance because aqueous samples of biomolecules are lossy at high fields, which limits the strengths of the RF fields that can be applied.

Keywords: Decoupling; SPINAL; 900 MHz; Aligned samples; Single crystals

1. Introduction

The advantages of performing NMR experiments at high fields are realized only when all aspects are fully optimized. Among the principal considerations for high resolution solid-state NMR experiments is the efficacy of the radiofrequency irradiations that provide heteronuclear decoupling [1]. In principle, the effects of the increased spread of chemical shift frequencies at high fields could be overcome with more intense radiofrequency irradiations. However, in practice, decoupling field strengths are limited by the power handling capabilities of the probes, and this is compounded by the detuning and Q-lowering effects of lossy aqueous samples of biopolymers at high fields. At 900 MHz, the resonance frequencies of ^{1}H amide resonances, which are the dominant sources of heteronuclear dipolar couplings to ^{15}N and ^{13}C sites in proteins, are spread over 13 kHz, since the chemical shift tensor spans more than 14 ppm [2,3]. With frequency offsets this large, it is difficult to obtain fully decoupled spectra using CW ^{1}H irradiation at a typical RF field strength of 50 kHz. It is also difficult to perform ^{13}C decoupling in triple-resonance experiments on ^{13}C- and ^{15}N-labeled samples because of the intrinsic frequency differences between carbonyl and aliphatic carbon resonances. As a result, there is a need to implement methods that improve the efficiency of heteronuclear decoupling in high field NMR experiments. Considerable progress has been made in addressing this issue for solution NMR and magic angle sample spinning solid-state NMR experiments. In this article, we demonstrate the benefits of SPINAL-16 decoupling [4] for solid-state NMR experiments on single crystal peptide and magnetically aligned protein samples.

In solution NMR, a wide variety of methods have been implemented that increase the bandwidth of the irradiation used for heteronuclear decoupling of scalar interactions, including pseudorandom noise modulation [5]), MLEV [6], WALTZ [7], GARP [8], frequency switched composite pulses [9], and adiabatic frequency sweep [10]. However, these methods are ineffective in

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solid-state NMR spectroscopy where heteronuclear decoupling of dipolar interactions is required to obtain high resolution spectra.

In solid-state NMR, the development of methods for heteronuclear decoupling is far more advanced for magic angle spinning experiments than for those performed on stationary samples. Two-pulse phase modulation (TPPM) [11] is widely used in magic angle spinning NMR to increase the efficiency of heteronuclear decoupling, for polycrystalline samples. Approaches based on phase inversions [12] and continuous phase shapes [13] and other variations [14] have been implemented with magic angle sample spinning, and this continues to be an active area of research.

In our experience, the decoupling schemes that work well in solution NMR or magic angle sample spinning solid-state NMR generally provide little or no improvement with stationary samples. The improvements in decoupling bandwidth for stationary samples from the initial efforts, such as COMARO [15], are not sufficient for experiments at the currently available field strengths. Other relevant developments have been for selective decoupling [16]. In contrast, SPINAL (small phase incremental alteration) provides substantial improvements in the efficiency of broadband heteronuclear decoupling in double- and triple-resonance experiments. A series of SPINAL sequences [4] have been developed starting from the original TPPM sequence [11]. We have found that the 16-step version works best in applications to single crystals of peptides and aligned samples of immobile proteins in virus particles and phospholipid bilayers. In this article, we demonstrate the advantages of SPINAL-16 over CW irradiation for ^{1}H as well as simultaneous ^{1}H and ^{13}C decoupling of ^{15}N NMR spectra at magnetic field strengths corresponding to ^{1}H resonance frequencies between 500 and 900 MHz.

2. Results

The ^{1}H decoupled ^{15}N NMR spectra of a uniformly ^{15}N-labeled magnetically aligned fd bacteriophage in Fig. 1B were obtained with CW ^{1}H irradiation at various frequencies near 900 MHz. The spectra are plotted very compactly in this figure; however, the peak heights reflect the line widths of the overlapping amide resonances, and the relative intensities of signals in the spectra demonstrate that the limited bandwidth of CW ^{1}H irradiation for heteronuclear decoupling affects both resolution and sensitivity. Similar effects are observed for the four ^{15}N resonances obtained from a crystal of a ^{15}N-labeled model peptide in Fig. 2B. Because there are only four signals, it is possible to discern the variability among the resonances resulting from relatively small changes in position of the ^{1}H irradiation frequency.

In a peptide bond, the ^{15}N is strongly coupled to a directly bonded ^{1}H. Since CW irradiation is most effective for heteronuclear decoupling when it is at the resonance frequency of one of the coupled spins (i.e., "on-resonance"), the line widths and intensities of the ^{15}N resonances have a strong dependence on the offset of the ^{1}H irradiation from the "on-resonance" frequency. An increase in peak height corresponds to narrower line widths, therefore the peak heights provide an indication of the efficacy of heteronuclear decoupling. The comparison between the results in Fig. 1A and B (and Figs. 2A and B), demonstrate that SPINAL-16 modulation im-

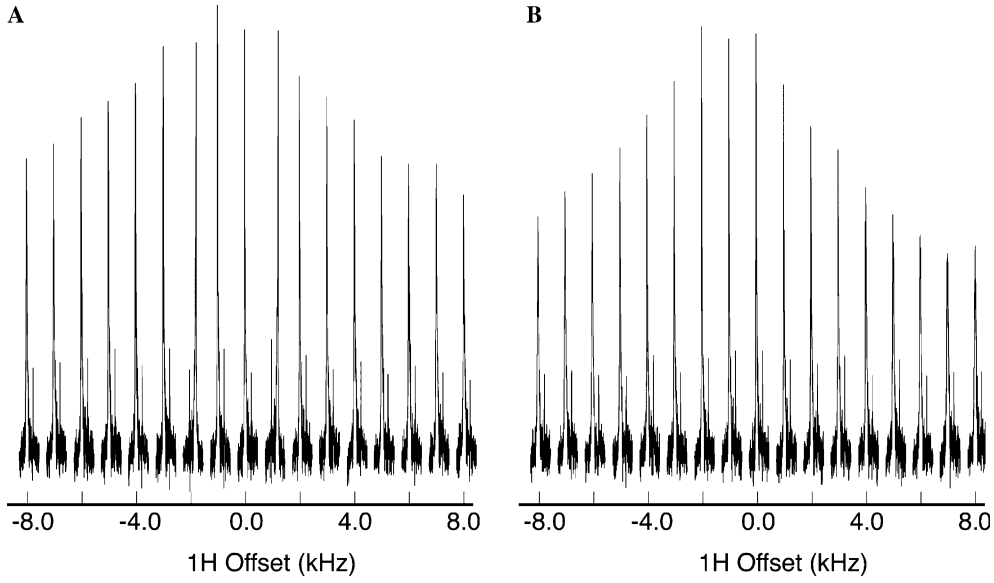

Fig. 1. ^{15}N NMR spectra of a sample of uniformly ^{15}N-labeled magnetically aligned fd bacteriophage. (A) With SPINAL-16 ^{1}H decoupling. (B) With CW ^{1}H decoupling. The ^{1}H decoupling field was 53 kHz.

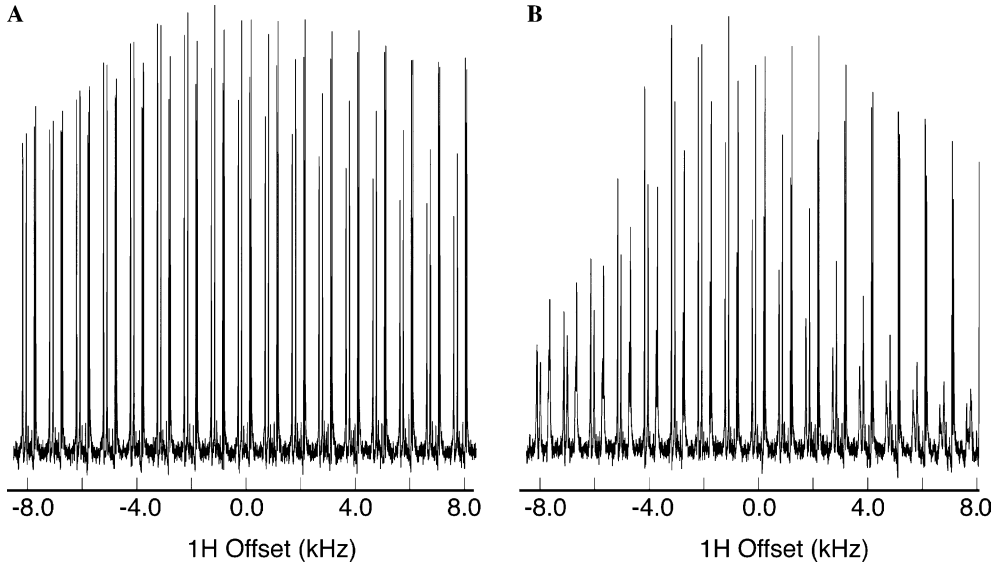

Fig. 2. ^{15}N NMR spectra of a single crystal of *N*-acetyl-valine. (A) With SPINAL-16 ^{1}H decoupling. (B) With CW ^{1}H decoupling. The ^{1}H decoupling field was 53 kHz.

proves the bandwidth of ^{1}H decoupling at 900 MHz where 80 W of radiofrequency power corresponds to a 53 kHz field.

SPINAL-16 decoupling also has important roles in improving the bandwidth of simultaneous ^{1}H and ^{13}C decoupling in triple-resonance ^{1}H/^{13}C/^{15}N NMR experiments on stationary samples of peptides and proteins labeled with both ^{13}C and ^{15}N. The ^{15}N NMR spectra of the single-crystal sample of ^{15}N-, ^{13}C-labeled *N*-acetyl-glycine shown in Fig. 3 are ^{1}H decoupled; however, the spectrum on the right (Fig. 3B) was obtained with both ^{1}H and ^{13}C decoupling. The ^{13}C couplings present

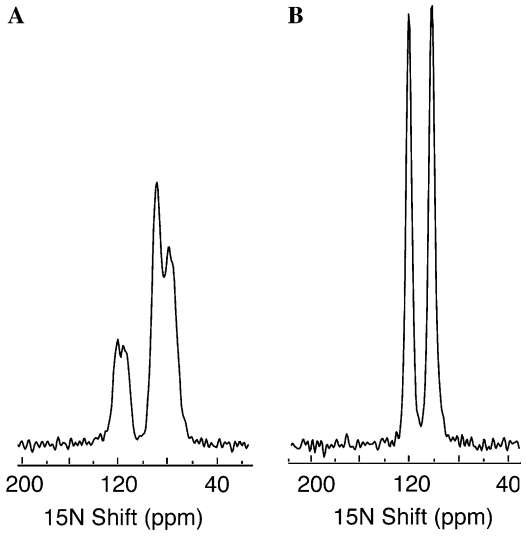

Fig. 3. ^{15}N NMR spectra of a single crystal of ^{13}C- and ^{15}N-labeled *N*-acetyl-glycine obtained with on-resonance CW irradiation corresponding to a ^{1}H field of 50 kHz and a ^{13}C field of 39 kHz. (A) ^{1}H decoupled. The partially resolved splittings are due to couplings to nearby ^{13}C-labeled sites. (B) ^{1}H and ^{13}C decoupled.

in the spectrum on the left (Fig. 3A), which was obtained with only ^{1}H decoupling, result in complex, unresolved splittings that obscure the two single-line resonances present in the fully decoupled spectrum. We use this sample to illustrate several features of SPINAL-16 heteronuclear decoupling in triple-resonance experiments.

The ability of SPINAL-16 to reduce the power requirements for ^{1}H decoupling is illustrated with the spectra in Fig. 4. Comparison of the resonance intensities as a function of frequency offset in Figs. 4A and B demonstrate the difference between using CW and SPINAL-16 ^{1}H irradiation at a field strength of 50 kHz at a ^{1}H resonance frequency of 550 MHz. At lower decoupling field strengths, for example 31 kHz (Figs. 4C and D), the benefits of SPINAL-16 decoupling are even more dramatic.

In Fig. 5, the ^{15}N NMR spectra of *N*-acetyl-glycine with optimal ^{1}H decoupling are shown as a function of ^{13}C irradiation power and frequency offset. In this situation ^{13}C SPINAL-16 irradiation affords the opportunity to reduce the power required to obtain fully decoupled ^{15}N spectra. The spectra in Figs. 5A and B were obtained in the presence of ^{13}C irradiation with a field strength of 39 kHz. The benefits of SPINAL-16 with lower power irradiation (18 kHz) on the ^{13}C channel (Figs. 5C and D) are readily observed.

The spectra in Fig. 6 demonstrate the simultaneous application of ^{1}H and ^{13}C SPINAL-16 decoupling to a ^{13}C- and ^{15}N-labeled protein. The protein is the same 60-residue membrane protein (MerFt) in magnetically aligned bicelles used to obtain the data in Fig. 1, except that the sample is labeled selectively with ^{13}C′-Tyr (3 sites) and ^{15}N-Leu (13 sites, including the N-terminus). The ^{13}C irradiation frequency was 178 ppm (referenced

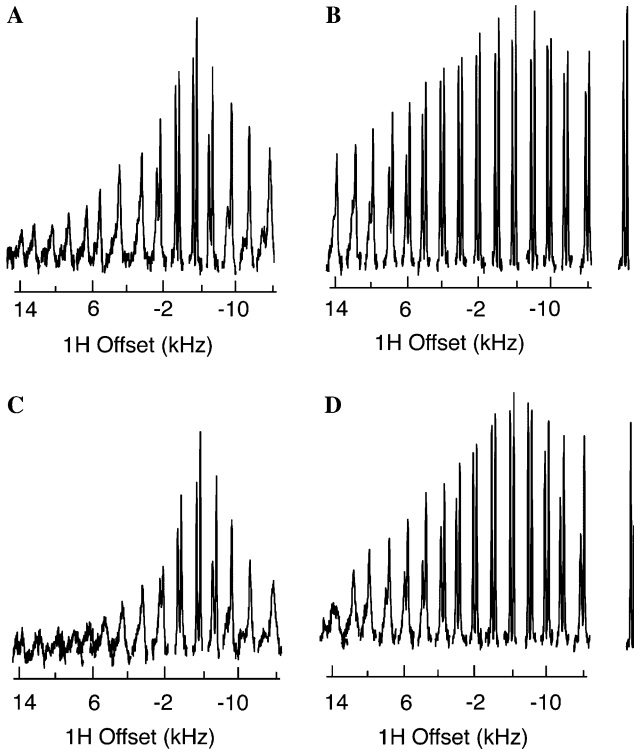

Fig. 4. ^{15}N NMR spectra of *N*-acetyl-glycine with optimal ^{13}C decoupling provided by SPINAL-16 ^{13}C irradiation at 39 kHz field strength as a function of ^{1}H irradiation power levels and offset frequencies. (A) CW ^{1}H irradiation at 50 kHz. (B) SPINAL-16 ^{1}H irradiation at 50 kHz. (C) CW ^{1}H irradiation at 31 kHz. (D) SPINAL-16 ^{1}H irradiation at 31 kHz. The single spectra on the right side have the highest intensities obtained under optimal conditions for both ^{1}H and ^{13}C irradiation.

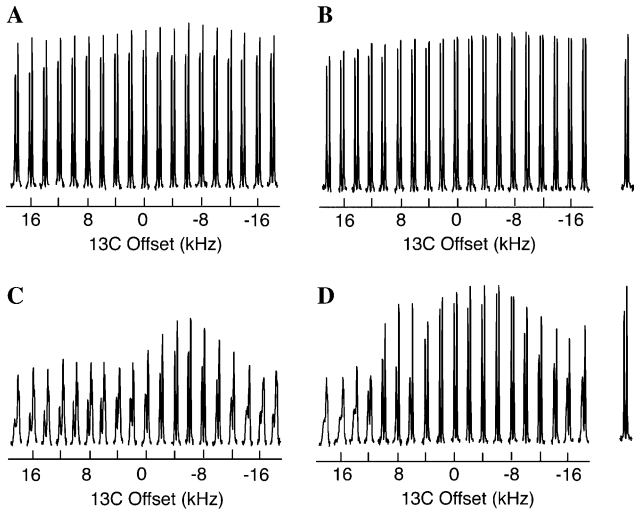

Fig. 5. ^{15}N NMR spectra of ^{13}C- and ^{15}N-labeled *N*-acetyl-glycine with optimal ^{1}H decoupling provided by SPINAL-16 ^{1}H irradiation at 50 kHz field strength as a function of ^{13}C irradiation power levels and offset frequencies. (A) CW ^{13}C irradiation at 18 kHz. (B) SPINAL-16 ^{13}C irradiation at 39 kHz. (C) CW ^{13}C irradiation at 18 kHz. (D) SPINAL-16 ^{13}C irradiation at 18 kHz. The single spectra on the right side have the highest intensities obtained under optimal conditions for both ^{1}H and ^{13}C irradiation.

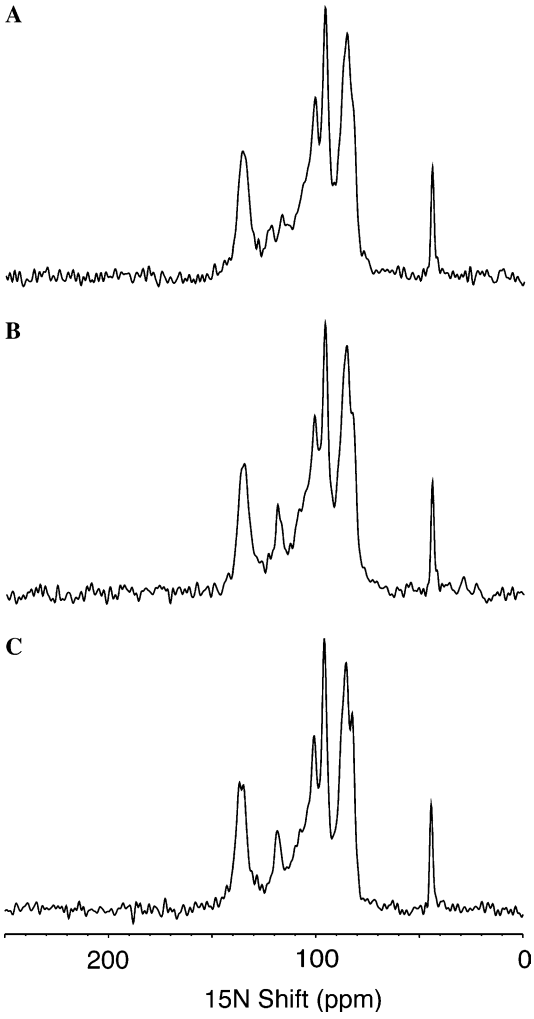

Fig. 6. ^{15}N NMR spectra of selectively $^{13}C'$-Tyr (3 sites) and ^{15}N-Leu (13 sites) labeled MerFt in magnetically aligned bicelles. (A) With ^{1}H CW decoupling. (B) With ^{1}H and ^{13}C CW decoupling. (C) With ^{1}H and ^{13}C SPINAL decoupling. The ^{1}H decoupling field was 60 kHz and the ^{13}C field was 42 kHz.

to TMS, with the adamatane low field resonance at 38.4 ppm) and the ^{1}H irradiation frequency was at 9 ppm (referenced to TMS, with H_2O at 4.8 ppm). The only Tyr–Leu linkage in the protein is for residues 42 and 43; therefore the comparison of the undecoupled (Fig. 6A) and ^{13}C decoupled spectra (Figs. 6B and C) enables the assignment of the ^{15}N resonance near 120 ppm to residue Leu43. More detailed comparisons of resolution between the spectra in Figs. 6A and C demonstrate the benefits of SPINAL-16 ^{1}H decoupling even at the relatively low resonance frequency of 500 MHz.

3. Discussion

CW irradiation has been used for heteronuclear decoupling in the vast majority of solid-state NMR

experiments performed on stationary powder, single crystals, and mechanically and magnetically aligned samples. Most of these experiments have been performed on spectrometers with magnets with field strengths corresponding to relatively low resonance frequencies (<500 MHz). However, for experiments in high magnetic fields and for $^{1}H/^{13}C/^{15}N$ triple-resonance spectroscopy, it is essential to utilize more efficient heteronuclear decoupling schemes. Substantial improvements have been made in the bandwidth of heteronuclear decoupling in solution NMR and magic angle sample spinning solid-state NMR; however, these methods generally do not provide much improvement in solid-state NMR of stationary samples. In this article, we demonstrate that SPINAL-16, which was originally developed for liquid crystals, is also highly effective at improving the efficiency of heteronuclear decoupling in single crystals of model peptides and aligned samples of proteins in high fields, and in triple-resonance experiments.

4. Experimental

The spectra in Figs. 1 and 2 were obtained on a Bruker Avance spectrometer with a Magnex 900/51 magnet. A home-built lumped-element probe with a 5 mm ID solenoid coil double-tuned to the ^{1}H and ^{15}N resonance frequencies was utilized. The spectra in Figs. 3–5 were obtained on a home-built spectrometer controlled by a Tecmag Apollo with a Magnex 550/89 wide-bore magnet. A home-built lumped-element probe with a 5 mm ID solenoid coil triple-tuned to the ^{1}H, ^{13}C, and ^{15}N resonance frequencies was utilized. The spectra in Fig. 6 were obtained on a Varian Inova spectrometer with a Magnex 500/89 AS magnet. A Varian T3 probe with a 5 mm coil triple-tuned to the ^{1}H, ^{13}C, and ^{15}N resonance frequencies was utilized.

The single crystal sample of *N*-acetyl-glycine used in these experiments has the dimensions of $3.0 \times 2.5 \times 1.0$ mm. It was placed at an arbitrary orientation relative to the direction of the applied magnetic field, and maintained at room temperature.

The fd bacteriophage sample was prepared as previously described [17]. It was a solution containing 50 mg/mL of uniformly ^{15}N labeled fd bacteriophage.

The samples of MerFt were prepared as previously described [18]. The samples consisted of 2.8 mg of protein and 28% (w/v) lipids, which were the ether-linked analogs of DMPC/DHPC with $q = 3.2$, in aqueous solution. The sample was maintained at 40 °C where the protein containing bicelles align magnetically with their normals perpendicular to the direction of the magnetic field.

All of the spectra in the Figures were obtained by single-contact spin-lock cross-polarization under Hartmann–Hahn matching conditions [1]. Only the decoupling conditions were changed in the course of the experiments.

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