

COMMUNICATIONS

Sensitivity Enhancement in Multiple-Quantum NMR Experiments with CPMG Detection

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We present a modified multiple-quantum (MQ) experiment, which implements the Carr–Purcell–Meiboom–Gill (CPMG) detection scheme in the static MQ NMR experiment proposed by W. S. Warren *et al.* (1980, *J. Chem. Phys.* 73, 2084–2099) and exploited further by O. N. Antzutkin and R. Tycko (1999, *J. Chem. Phys.* 110, 2749–2752). It is demonstrated that a significant enhancement in the sensitivity can be achieved by acquiring echo trains in the MQ experiments for static powder samples. The modified scheme employing the CPMG detection was superior to the original MQ experiment, in particular for the carbonyl carbon with a very large chemical shift anisotropy. © 2002 Elsevier Science (USA)

INTRODUCTION

Multiple-quantum (MQ) NMR spectroscopy has been a useful tool in fundamental studies of spin physics for many body systems (3–5). It has also been used to probe dynamics of spin-clusters (3–8) and to simplify ¹H NMR spectra in a strongly coupled system (1). Recently, higher-order ¹³C multiple quantum (MQ) NMR experiments (2) have been utilized to address one of the fundamental issues in amyloid fibrils, the alignment of individual strands as parallel or anti-parallel in the β amyloid (A β) fibrils associated with Alzheimer's disease (9, 10). Three MQ experiments were, however, performed on *static* powder samples instead of utilizing MAS, since dipolar-recoupling techniques suffer from a scaled dipolar coupling (20–30% of that for static sample), requiring long excitation times for MQ excitation. The static ¹³C MQ experiments proposed by Antzutkin and Tycko demonstrated that higher-order MQ coherences can be generated with a reasonable excitation time (<20 ms) for ¹³C multispin systems with internuclear distances of ~ 5 Å (2), and were successfully exploited in the study of the amyloid fibril

systems (9, 10). However, the low sensitivity in the *static* MQ experiment caused by the inhomogeneous line broadening might limit its application to large biological molecules.

In this short communication, we demonstrate that a significant sensitivity enhancement can be achieved by employing the CPMG detection scheme (11, 12) in the static MQ experiments. The pulse sequence used in this study is shown in Fig. 1, which employs a multiple pulse sequence (eight $\pi/2$ pulses) for the creation of the double quantum Hamiltonian (1). A series of π pulses are inserted between the $\pi/2$ pulses in order to remove the effect of the CSA as proposed by Antzutkin and Tycko (2). The CPMG scheme is implemented during the detection period.

EXPERIMENTAL

All NMR spectra were acquired with a Chemagnetics 3.2-mm probe, on a Varian CMX Infinity 500 spectrometer for which the resonance frequencies of ¹H and ¹³C are 500 and 125 MHz, respectively. An amount of ~ 10 mg of 99% L-alanine-3-¹³C and phenylalanine-1-¹³C (Cambridge Isotopes) was used without further purification for the MQ experiments. RF field strengths of 50 and 110 kHz were used for the ¹³C multiple pulses and ¹H decoupling, respectively.

RESULTS AND DISCUSSION

Figure 2 shows ¹³C double-quantum (DQ) filtered NMR spectra of 99% L-alanine-3-¹³C (a) and phenylalanine-1-¹³C (b) acquired with and without using the CPMG detection scheme. The DQ NMR spectra clearly demonstrate that the CPMG scheme improves sensitivity of the MQ experiments significantly by narrowing the inhomogeneously broadened ¹³C NMR resonance. In particular, a more dramatic enhancement (a factor of ~ 10)

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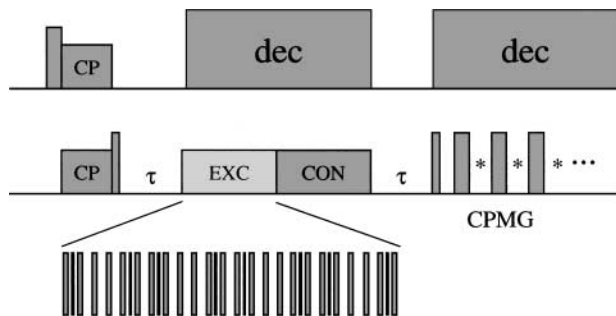


FIG. 1. Pulse sequence for multiple-quantum NMR experiments. Thick and thin pulses correspond to the π and $\pi/2$ pulse, respectively. The phases of $\{x, x, x, x, -x, -x, -x, -x\}$ and $\{x, -x, -x, x\}_6$ were used for the $8\pi/2$ and 24π pulses, respectively.

was observed for the carbonyl carbon which has a very large chemical shift anisotropy (CSA) value. This suggests that the carbonyl carbons, which have not previously been used for the MQ experiments due to the much broader lineshape, may also be utilized in the MQ experiments for amyloid fibrils since they are not directly bonded to hydrogen, less demanding high-power ^1H decoupling.

Changes in the ^{13}C signals as a function of the phase of the excitation pulses are also shown in Fig. 3, which allow us to determine intensities of higher-order MQ coherences simultaneously (see Fig. 3c). A factor of ~ 3 enhancement for the C_β in alanine was achieved using the modified MQ experiment. This sensitivity enhancement with the CPMG detection scheme will be extremely helpful for the application of the MQ experiments to other amyloid fibril systems, in order to investigate the alignment of the fibrils.

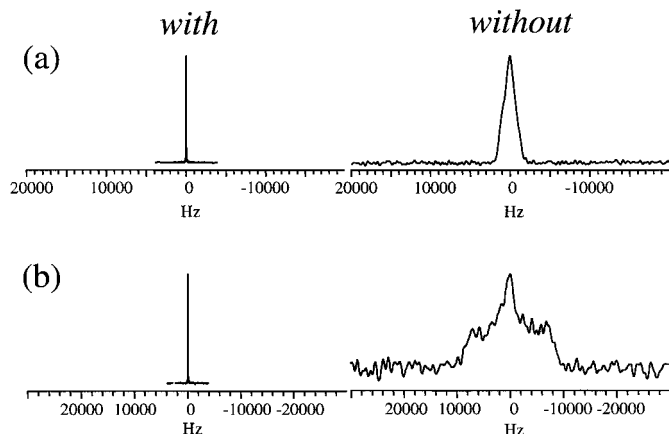


FIG. 2. Double-quantum filtered spectra of 99% L-alanine-3- ^{13}C (a) and phenylalanine-1- ^{13}C (b) obtained with and without the CPMG detection scheme. Excitation time of 2 and 3.6 ms were used for the carbonyl and C_β carbons, respectively.

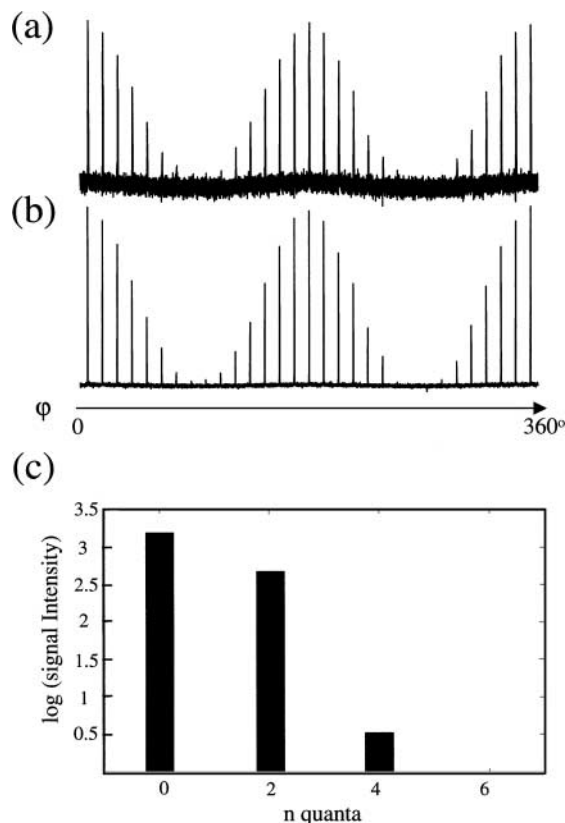


FIG. 3. ^{13}C NMR spectra of L-alanine-3- ^{13}C as a function of the phase of the preparation pulses (φ) without (a) and with CPMG detection (b). (c) Calculated intensities of the multiple quantum coherences obtained by the Fourier transformation of the ^{13}C NMR spectra shown in (b) acquired with excitation time of 4.8 ms. Note that the signal intensities are drawn on a logarithmic scale.

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