Journal of Magnetic Resonance 245 (2014) 98–104

Contents lists available at ScienceDirect

Journal of Magnetic Resonance

journal homepage: www.elsevier.com/locate/jmr

Multiple acquisition/multiple observation separated local field/chemical shift correlation solid-state magic angle spinning NMR spectroscopy

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ARTICLE INFO

Article history:
Received 14 April 2014
Revised 6 June 2014
Available online 28 June 2014

Keywords:
MACSY
Dual acquisition
Dual observation
PELF
Dipolar couplings
Protein NMR
R-INEPT
CXCR1

ABSTRACT

Multiple acquisition spectroscopy (MACSY) experiments that enable multiple free induction decays to be recorded during individual experiments are demonstrated. In particular, the experiments incorporate separated local field spectroscopy into homonuclear and heteronuclear correlation spectroscopy. The measured heteronuclear dipolar couplings are valuable in structure determination as well as in enhancing resolution by providing an additional frequency axis. In one example four different three-dimensional spectra are obtained in a single experiment, demonstrating that substantial potential saving in experimental time is available when multiple multi-dimensional spectra are required as part of solid-state NMR studies.

1. Introduction

Multiple acquisition spectroscopy (MACSY) offers two universal advantages when multiple multi-dimensional spectra are required in NMR studies. First, that the overall amount of time spent signal averaging is greatly reduced, by at least a factor of 3, and potentially even larger factors in combination with non-uniform sampling techniques and further optimization. Second, that by obtaining all of the data on the same sample at the same time, accurate alignment of multiple data sets is assured. MACSY is based on the principle of acquiring multiple multi-dimensional data sets during a single NMR experiment where the relaxation time of at least one of the nuclei is sufficiently long, when properly handled, to maintain coherence for the duration of the experiment.

Dual acquisition was first demonstrated in 1984 for solution NMR of macromolecules by combining two-dimensional correlated spectroscopy (COSY) and nuclear Overhauser enhancement spectroscopy (NOESY) in a single experiment (COCONOSY) [1,2]. In 2008, Fukuchi et al. demonstrated applications of dual acquisition in high resolution solid-state NMR with the COCODARR experiment in which data sets for two different two-dimensional $^{13}C/^{13}C$ homonuclear correlation spectra were acquired during the course of a single experiment [3]. This was followed by the application of dual acquisition to a separated local field (SLF) spectroscopy [4] version of the experiment [5]. More recently, Gopinath et al. and Lamley and Lewandowsky have built on this foundation by employing simultaneous cross-polarization (CP) to ^{13}C and ^{15}N to obtain two multi-dimensional spectra in a single experiment [6–9].

Here we demonstrate that there is a significant advantage to using dipolar INEPT (RINEPT) [10] for cross-polarization in dual acquisition experiments. Several additional spectroscopic enhancements, including non-uniform sampling (NUS) [11,12], culminate in the measurement of four three-dimensional spectra in a single experiment, and multidimensional spectra of a 350-residue membrane protein in phospholipid bilayers under physiological conditions [13]. This family of experiments offers the possibility of simultaneous observation of $^{1}H–^{13}C$ and $^{1}H–^{15}N$ heteronuclear dipolar couplings in addition to various homo- and hetero-nuclear chemical shift correlations.

Heteronuclear $^{1}H–^{13}C$ and $^{1}H–^{15}N$ dipolar couplings are particularly valuable in structural studies of proteins because they provide highly reliable measurements of angles and distances. Additionally, the heteronuclear dipolar couplings can be used to measure order parameters that quantify the local and global dynamics of peptides and proteins. In these experiments the use of proton evolved local field spectroscopy (PELF) [14] has several advantages over the original versions of separated local field spectroscopy. In particular, PELF has better sensitivity compared to

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http://dx.doi.org/10.1016/j.jmr.2014.06.011
1090-7807/

constant time conventional separated local field experiments because of the absence of the signal-depleting extra delay. Also, it gives simple Pake powder pattern spectra for all sites of interest in protein studies, including CH_2, and CH_3, also in contrast to the original version of SLF spectroscopy [15].

In these experiments, the one-bond heteronuclear dipolar couplings are correlated with chemical shift frequencies in a site-specific manner that can be either intra- or inter-residue in polypeptides; this is valuable in the resonance assignment process. Moreover, in rotationally aligned samples of membrane proteins in phospholipid bilayers, the wide range of heteronuclear dipolar coupling frequencies, which have uniform values in static polycrystalline samples, add another frequency dimension for resolution of signals that have the same chemical shift frequencies; this too is valuable in the resonance assignment process [16].

2. Experimental

The experiments were performed on spectrometers with ^{1}H resonance frequencies of 750 MHz and 700 MHz. The 750 MHz spectrometer was equipped with a Bruker Avance console and a Bruker 3.2 mm Efree ^{1}H/^{13}C/^{15}N triple-resonance MAS probe (www.bruker.com). The 700 MHz spectrometer was equipped with a Bruker Avance II console and a home-built 3.2 mm ^{1}H/^{13}C/^{15}N triple-resonance MAS probe incorporating Revolution (http://www.revolutionnmr.com) spinning hardware. The spinning rate was controlled at 10.000 kHz ± 2 Hz. The ^{1}H resonance frequency of water was used to monitor the temperature of the protein-containing phospholipid bilayer sample. It also served as an internal chemical shift reference frequency at 4.8 ppm at 20 °C. The ^{13}C chemical shift frequencies of the polycrystalline samples were referenced externally to solid samples with the methylene ^{13}C resonance of adamantane at 38.48 ppm and the ^{15}N resonance of ammonium sulfate at 26.8 ppm [17–19].

The experimental data were acquired using the pulse sequences diagrammed in Fig. 1. In all of the experiments, swept frequency two-pulse phase modulation (SW_f-TPPM) [20] with 90 kHz radio frequency (RF) field strength was used to provide ^{1}H decoupling. 50 kHz, 62 kHz and 90 kHz RF field strength pulses were applied at the resonance frequencies for the ^{15}N, ^{13}C, and ^{1}H nuclei, respectively. Double cross-polarization (DCP) from ^{15}N to ^{13}C was accomplished using spectrally induced filtering in combination with cross-polarization (SPECIFIC-CP) [21] and proton assisted insensitive nuclei cross-polarization (PAIN-CP) [22,23]. 10% ramped amplitude pulses at the ^{13}C resonance frequencies were optimized for maximum polarization transfer in the applications of SPECIFIC-CP. Typical RF field strengths for SPECIFIC-CP were 27 kHz for ^{15}N, 17 kHz for ^{13}CA and 37 kHz for ^{13}CO. During PAIN-CP ~50 kHz RF fields were applied synchronously to the ^{1}H, ^{13}C and ^{15}N nuclei, and their amplitudes were adjusted for maximum PAIN-CP efficiency. Experiments were optimized with 2 ms and 3 ms heteronuclear mixing for PAIN and SPECIFIC-CP. Homonuclear ^{13}C/^{13}C spin-exchange was effected by proton driven spin diffusion (PDSD) [24], dipolar assisted rotational resonance (DARR) [25], and proton assisted recoupling techniques [23,26,27]. One to three bond correlations among carbon nuclei were optimized using 20 ms mixing under PDSD and DARR. Long-range correlation experiments were carried out using 2 ms PAR and up to 100 ms DARR mixing.

Recoupling of the hetero-nuclear dipolar coupling frequencies and cross-polarization in MAS experiments utilized a symmetry-based $R18_1^7$ scheme [28]. A pair of 180° pulses with 70° phase modulation of ($\pi_{70}\pi_{-70}$) was employed in the $R18_1^7$ scheme. The scaling factors for the pulse sequences were measured experimentally with ^{13}C and ^{15}N detection using a uniformly ^{13}C, ^{15}N labeled sample of polycrystalline N-acetyl leucine (NAL). The measured dipolar splitting of 6.8 kHz for ^{1}H–^{13}C and 3.6 kHz for ^{1}H–^{15}N correspond to a scaling factor of 0.18. Two- and three-dimensional separated local field experiments were performed using direct ^{13}C-detection with or without ^{15}N editing.

Three-dimensional data were collected with 2 ms dipolar evolution, 3 ms to 5 ms ^{13}C and ^{15}N chemical shift evolution in indirect dimensions, and 10 ms direct acquisition. All of the experiments were performed with a 2 s recycle delay. A total number of 16 scans were co-added for the MLF sample, 4 scans for the NAL sample, and 512–1024 scans for the protein sample. The experimental data were processed in NMRPipe [29] and visualized using SPARKY (University of California, San Francisco). Equal numbers of data points were linear predicted for the indirect dimensions prior to Fourier transformation. Sine bell window functions shifted by 30° or 60° were used in the direct and indirect dimensions to process the multidimensional datasets, except for the NUS data. The NUS protein data in Fig. 5 were processed with 0.5 ppm exponential line broadening in the direct dimension and sine bell functions shifted by 30° in the indirect dimensions. The NUS scheduling was optimized using parameters from Bruker's TOPSPIN 3.1 program. A J coupling of 55 Hz and a T_2 relaxation time of 30 ms were used to determine the optimal selection of 50% of the complete set of data points. The NUS data were processed and visualized using the same program.

3. Results

The pulse sequences utilized in this study are diagrammed in Fig. 1. They are named following their coherence transfer pathways. The pulse sequence in Fig. 1A is referred to as single acquisition, dual observation (SADO) in which ^{1}H–^{13}C and ^{1}H–^{15}N dipolar frequencies are encoded in the indirect dimensions followed by simultaneous coherence transfer from ^{1}H to ^{13}C and ^{15}N. Spin diffusion among ^{13}C nuclei and heteronuclear mixing of ^{13}C and ^{15}N magnetization is carried out using PAIN [22] and PAR cross-polarization [27]. This class of experiments correlates polarization transfer between nuclei separated by relatively large distances. The pulse sequence in Fig. 1B is referred to as dual acquisition, dual observation (DADO); it is the same as the pulse sequence shown in Fig. 1A except that the amide and aliphatic ^{1}H resonance frequencies are evolved simultaneously followed by the selective ^{15}N magnetization transfer to either ^{13}Cα (^{13}CA) or ^{13}C′ (^{13}CO) resonances within the same or preceding residue in a polypeptide, respectively. Additionally, amide ^{1}HN chemical shift frequencies are correlated with the ^{13}CA resonances. The pulse sequence in Fig. 1C is referred to as dual acquisition, multiple observation (DAMO); here ^{1}H–^{13}C and ^{1}H–^{15}N dipolar frequencies are correlated with the ^{13}C and ^{15}N chemical shift frequencies of the same or preceding residues. The experiments are either carried out with the same dwell time for ^{13}C (t_1) and ^{15}N evolution (t_1') or by increasing the ^{15}N dwell time. The acquisition of ^{15}N edited data with a longer dwell time was carried out using the method described by Gopinath et al. [7,8]. ^{1}HA–^{13}CA dipolar frequencies in the backbone of a peptide plane are correlated to the side chain chemical shifts separated by multiple bonds within the same amino acid; the same is true for correlation of ^{1}H–^{13}C dipolar frequencies in side chains to the backbone nuclei (^{13}CA and ^{13}CO) and can potentially be extended to long-range correlation depending on the details of the spin diffusion mixing. In addition, ^{1}H–^{15}N dipolar frequencies are correlated to the ^{13}C shifts of backbone and side chain sites. The pulse sequence in Fig. 2D is referred to as triple acquisition, multiple observations (TAMO). Triple acquisition provides the simplest method for transfer of magnetization among homo nuclei or from ^{15}N to ^{13}C. Here, ^{15}N magnetization is transferred to ^{13}CA chemical shift frequencies prior to the second acqui-