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Authors

Wu, Chin H
De Angelis, Anna A
Opella, Stanley J

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Magic angle Lee-Goldburg frequency offset irradiation improves the efficiency and selectivity of SPECIFIC-CP in triple-resonance MAS solid-state NMR

C.H. Wu, Anna A. De Angelis, and Stanley J. Opella*

Department of Chemistry and Biochemistry, University of California, San Diego, La Jolla, California 92093 USA

Abstract

The efficiency and selectivity of SPECIFIC-CP, a widely used method for selective double cross-polarization in triple-resonance magic angle spinning solid-state NMR, is improved by performing the tangential-shaped ^{13}C irradiation at an offset frequency that meets the Lee-Goldburg condition (LG-SPECIFIC-CP). This is demonstrated on polycrystalline samples of uniformly ^{13}C , ^{15}N labeled N-acetyl-leucine and N-formyl-Met-Leu-Phe-OH (MLF) at 700 MHz and 900 MHz ^1H resonance frequencies, respectively. For the single $^{13}\text{C}\alpha$ of N-acetyl-leucine, relative to conventional broad band cross-polarization, the SPECIFIC-CP signal has 47% of the intensity. Notably, the LG-SPECIFIC-CP signal has 72% of the intensity, essentially the theoretical maximum. There were no other changes in the experimental parameters. The three $^{13}\text{C}\alpha$ signals in MLF show some variation in intensities, reflecting the relatively narrow bandwidth of a frequency-offset procedure, and pointing to future developments for this class of experiment.

Keywords

SPECIFIC-CP; LG-SPECIFIC-CP; triple-resonance; Lee-Goldburg; magic angle spinning; peptides

Introduction

Cross-polarization with ^1H decoupling during acquisition of ^{13}C or ^{15}N is the basic building block for many multidimensional experiments in high resolution solid-state NMR [1], along with magic angle spinning (MAS). The transfer of magnetization from ^1H to ^{15}N to ^{13}C , referred to as double cross-polarization (DCP) [2] is an essential aspect of triple resonance magic angle spinning (MAS) solid-state NMR experiments, which are required for studies of uniformly ^{13}C , ^{15}N labeled peptides and proteins. The most commonly used approach is “spectrally induced filtering in combination with CP” (SPECIFIC-CP) [3]. In this method

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*Corresponding author: sopella@ucsd.edu.

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spectral filtering of a heteronuclear spin pair, e.g. ^{13}C and ^{15}N , provides spectral simplification in one-dimensional spectra, and establishes the starting point for complex multi-dimensional experiments used for the assignment of resonances and the measurement of inter-nuclear distances [4, 5].

Although the theoretical signal intensity from DCP ^1H to ^{15}N to ^{13}C with SPECIFIC-CP is an impressive 73% compared to the broadband direct cross-polarization ^1H to ^{13}C , this is difficult to achieve in practice. Often, the signals are in the range of 30% - 40% of the 100% baseline from broadband cross polarization. This can be attributed to a number of factors. However, since the theoretical maximum has been achieved by restricting the sample to the middle third of the rotor, at the cost of total sample volume and sensitivity, inhomogeneities in the radiofrequency irradiation clearly play a role [4].

Here we show that by offsetting the ^{13}C irradiation to meet the Lee-Goldburg [6] “magicangle” condition, it is possible to recover the lost signal intensity. This is demonstrated on two different polycrystalline peptides at 700 MHz and 900 MHz ^1H resonance frequencies. We refer to this procedure as LG-SPECIFIC-CP. As in all frequency-dependent pulse sequences, it has a limited bandwidth, which is a subject for future developments. In addition, the loss of intensity in many applications of SPECIFIC-CP to proteins are due to motional effects, which this procedure does not address and this too is a subject for future developments. Nonetheless, the incorporation of Lee-Goldburg irradiation into SPECIFIC-CP results in a substantial increase in both signal intensity and selectivity that is useful in current studies.

Experimental

The experiments were performed on spectrometers with ^1H frequency resonance frequencies of 900 MHz and 700 MHz. The 900 MHz spectrometer was equipped with a Bruker Avance III HD console and a Bruker 3.2 mm E-free $^1\text{H}/^{13}\text{C}/^{15}\text{N}$ triple-resonance MAS probe (www.bruker.com). The 700 MHz spectrometer was equipped with a Bruker Avance console and a home-built 3.2 mm $^1\text{H}/^{13}\text{C}/^{15}\text{N}$ triple-resonance MAS probe with a triple-tuned 3.8 mm ID, 6-turn solenoid coil and utilizing Revolution (<http://www.revolutionnmr.com>) spinning hardware. The spinning rate was controlled at 11.111 kHz.

All of the experimental spectra were acquired with a 20 millisecond acquisition time, signal averaging of 16 scans, and 10 s recycle delay following 4 dummy scans. In all cases, swept-frequency TPPM ($\text{SW}_f\text{-TPPM}$) [7] heteronuclear decoupling sequence was applied to the ^1H channel during data acquisition. The spectra of polycrystalline uniformly ^{13}C , ^{15}N labeled N-acetyl-leucine (NAL) were obtained at a ^1H resonance frequency of 700 MHz. The spectra of polycrystalline uniformly ^{13}C , ^{15}N labeled N-formyl-Met-Leu-Phe-OH (MLF) were obtained at a ^1H resonance frequency of 900 MHz.

Prior to Fourier transformation, line broadening of 25 Hz was applied. The ^{13}C chemical shift frequencies of the polycrystalline samples were referenced externally to a solid sample of adamantane with the methylene ^{13}C resonance at 38.47 ppm (at 25 °C), which is equivalent to external tetramethylsilane (TMS) at 0 ppm. The experimental data were

processed with the program TopSpin (<http://www.bruker.com/>) and visualized using the program Igor Pro (<http://www.wavemetrics.com/>).

Results and Discussion

The pulse sequences utilized in this study are diagrammed in Figure 1. The baseline performance used in the comparisons of SPECIFIC-CP and LG-SPECIFIC-CP resulted from application of the spin-lock cross-polarization pulse sequence diagrammed in Figure 1A. The ^1H $\pi/2$ pulse was followed by 90° phase-shifted continuous wave (CW) irradiation where the bandwidth of the Hartmann-Hahn matched spin-lock cross polarization was broadened somewhat by the application of a linear amplitude ramp on the ^1H irradiation during ^1H to ^{13}C CP [8]. Constant amplitude CW irradiation was applied to the ^{13}C channel. We have also applied ramped irradiation to the ^{13}C or ^{15}N channels with constant amplitude irradiation on the ^1H channel, and found negligible difference in transfer efficiency.

The pulse sequences for double cross-polarization (DCP) from ^1H to ^{15}N to ^{13}C are diagrammed in Figure 1B and 1C. Following the initial ^1H to ^{15}N CP period, an adiabatic amplitude-modulated tangential shape was applied to irradiate the ^{13}C channel [9]. Constant-power irradiation was applied to the ^{15}N channel and SW_f -TPPM heteronuclear decoupling was applied to the ^1H channel. In the pulse sequence in Figure 1B, a frequency jump to an offset satisfying the Lee-Goldburg homonuclear decoupling condition was applied to the ^{13}C channel, in addition to the amplitude modulation during the ^{15}N to ^{13}C CP. This was followed by a 35.3° pulse applied on-resonance.

The pulse sequence diagrammed in Figure 1C is the widely applied SPECIFIC-CP experiment [3]. The pulse sequence in Figure 1B is introduced here, and differs from the sequence in Figure 1C by the Lee-Goldburg frequency shift of the ^{13}C irradiation. The offset frequency irradiation is denoted by 'LG' in the Figure. As described above, the ^{13}C irradiation is shaped by a tangent function in the pulse sequences in both Figure 1B and 1C; the ^{13}C irradiation in Figure 1B differs in that it is applied at a specific offset frequency, while that in Figure 1C is applied on-resonance for the ^{13}C signals of interest. In the examples shown here these are the $^{13}\text{C}\alpha$ signals from two different polycrystalline model peptides.

The experimental data demonstrating the benefits of incorporating the Lee-Goldburg frequency offset in the SPECIFIC-CP experiment are shown in Figure 2. The data from NAL, with one $^{13}\text{C}\alpha$ signal, are in the left column, and the data from MLF, with three $^{13}\text{C}\alpha$ signals, are in the right column. For the spectrum of MLF, the $^{13}\text{C}\alpha$ signals were previously assigned from left to right to be from the Leu, Phe, and Met residues. The top spectra (Figure 2A and 2D) were obtained with conventional cross-polarization, as diagrammed in Figure 1A. They contain resolved signals from all carbon sites in the peptides, although the intensities of the strong aliphatic resonances are truncated to facilitate the display of the $^{13}\text{C}\alpha$ resonances.

The spectra in Figure 2C and 2F were obtained with SPECIFIC-CP as diagrammed in Figure 1C. The $^{13}\text{C}\alpha$ resonance(s) have substantial amplitude, one for NAL and three for MLF, as expected, and the other aliphatic signals are substantially suppressed. The equivalent spectra in Figure 2B and 2D were obtained with LG-SPECIFIC-CP as diagrammed in Figure 1B.

There are two notable features in a comparison to the SPECIFIC-CP spectra in Figure 1C and 1F. The single $^{13}\text{C}\alpha$ resonance in Figure 2A, 2B, and 2C show particularly clearly the advantages of LG-SPECIFIC-CP. With the intensity of the $^{13}\text{C}\alpha$ signal at 53 ppm in Figure 2A defined as “100%”, the intensity for the signal in Figure 2C is 47%, a typical value for SPECIFIC-CP applied to a crystalline sample. The intensity for the signal in Figure 2B, which was obtained with LG-SPECIFIC-CP was 72% of that obtained by conventional CP. This value is essentially the same as the theoretical maximum possible signal obtained with SPECIFIC-CP. In addition, the spectrum shows greater suppression of other signals, and for all practical purposes shows only the $^{13}\text{C}\alpha$ signal. The small signal to the right of the $^{13}\text{C}\alpha$ peak is a spinning sideband of the $^{13}\text{C}'$ signal.

Both DCPs use the same constant-amplitude ^{15}N pulse with radiofrequency amplitude of 28 kHz ($11.111 \text{ kHz} * 2.5$). In Figure 2B DCP uses the same ^{13}C tangential shaped pulse as in Figure 2C except that the ^{13}C irradiation frequency is offset -22 kHz from 53 ppm and the radiofrequency amplitude is lowered to 80% ($\sin(54.7^\circ)$). The offset satisfies the Lee-Goldburg condition ($11.111 \text{ kHz} * 3.5 * \cos(54.7^\circ)$). A 35.3° pulse with ^{13}C frequency on-resonance to the $^{13}\text{C}\alpha$ signal improves the overall DCP efficiency of LG-SPECIFIC-CP by 20%. LG-SPECIFIC-CP has also been applied to $^{13}\text{C}'$ sites and similar efficiency improvement can be obtained. Using the method outlined above but with $^{13}\text{C}'$ resonance frequency of 176 ppm, the efficiency is improved from 53% to 72%.

MLF spectra in Figure 2D - 2F were obtained using the pulse sequences in Figure 1A to 1C, respectively. The $^{13}\text{C}\alpha$ signals were assigned from left to right to be Leu, Phe, and Met residues with relative intensities for the comparisons of 100%/100%/100%; 56%/70%/51%; and 45%/55%/45%. The experiment was optimized for the middle (Phe) signals, and nearly the theoretical intensity was achieved from LG-SPECIFIC-CP. In all cases, the incorporation of Lee-Goldburg irradiation improved the efficiency of the cross-polarization and its selectivity. However, this result does not depend on the application of a tangential amplitude modulation on ^{13}C . We find similar results in comparisons of LG-SPECIFIC-CP to SPECIFIC-CP with ramped irradiation on the ^{13}C or ^{15}N . As in all frequency-offset dependent sequences, such as those that utilize Lee-Goldburg irradiation, the bandwidth limitation needs to be taken into account. This is a subject for future development of these pulse sequences.

In summary, offset of the shape-modulated ^{13}C irradiation that meets the Lee-Goldburg condition increases the amplitude in the SPECIFIC-CP experiment, which is widely used in triple-resonance solid-state NMR. In the case of a single signal the experimental amplitude is essentially the same as the theoretical maximum. Notably, this was done with full rotors, not restricting the sample to the most homogeneous part of the radiofrequency irradiation, and on both home-built and commercial probes. The experiments are demonstrated on both mid-field (700 MHz) and high-field (900 MHz) spectrometers. It is likely the use of a high field spectrometer contributes to the bandwidth limitations of the frequency-offset dependent pulse sequence. In any case, a substantial gain in both efficiency and selectivity can be obtained by simply offsetting the ^{13}C irradiation during SPECIFIC-CP in a procedure that we refer to as LG-SPECIFIC-CP.

Acknowledgments

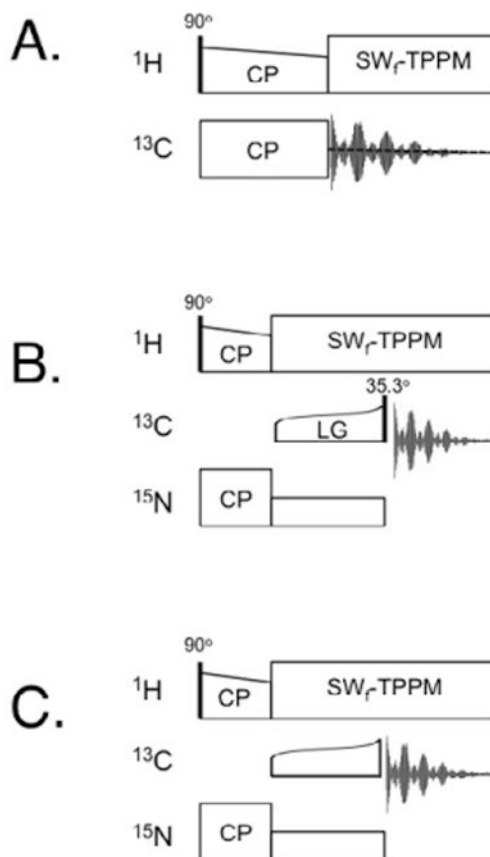
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Highlights

- Lee-Goldburg irradiation improves efficiency of SPECIFIC-CP.
- Lee-Goldburg irradiation improves selectivity of SPECIFIC-CP.
- LG-SPECIFIC-CP is an alternative method for selective DCP.

**Figure 1.**

Pulse sequences used to obtain the MAS solid-state NMR spectra of the polycrystalline peptides shown in Figure 2. A. Broadband cross-polarization from ^1H to ^{13}C with ^1H decoupling during acquisition. B. Double cross-polarization ^1H to ^{15}N to ^{13}C with LG-SPECIFIC-CP, where the ^{13}C frequency irradiation meets the Lee-Goldburg offset condition, with 35.3° ^{13}C flip-back pulse. C. Double cross-polarization ^1H to ^{15}N to ^{13}C with SPECIFIC-CP, where the ^{13}C frequency irradiation is on-resonance.

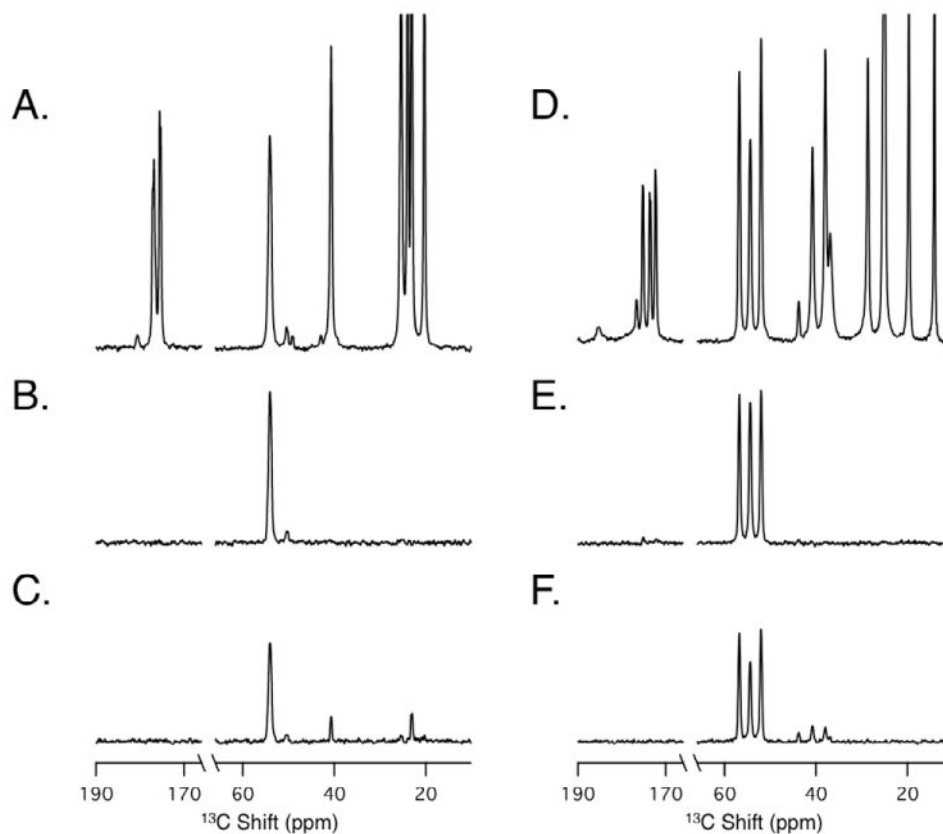


Figure 2.

The split plots showing the carbonyl resonance regions and the aliphatic resonance regions of the one-dimensional MAS solid-state NMR spectra of two different polycrystalline peptides. A. – C. ^{13}C NMR spectra of polycrystalline uniformly ^{13}C , ^{15}N labeled N-acetyl-leucine obtained using the pulse sequences in Figure 1A - 1C, respectively. The spectra were acquired at a ^1H resonance frequency of 700 MHz. D. – F. ^{13}C NMR spectra of polycrystalline uniformly ^{13}C , ^{15}N labeled f-Met-Leu-Phe obtained using the pulse sequences in Figure 1A - 1C, respectively. The spectra were acquired at a ^1H resonance frequency of 900 MHz. The $^{13}\text{C}'$ and $^{13}\text{C}\alpha$ signals were assigned from left to right to be Leu, Phe, and Met.