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Measuring $^{13}C/^{15}N$ chemical shift anisotropy in [^{13}C,^{15}N] uniformly enriched proteins using CSA amplification

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ABSTRACT

Extended chemical shift anisotropy amplification (xCSA) is applied for measuring $^{13}C/^{15}N$ chemical shift anisotropy (CSA) of uniformly labeled proteins under magic-angle spinning (MAS). The amplification sequence consists of a sequence of π-pulses that repetitively interrupt MAS averaging of the CSA interaction. The timing of the pulses is designed to generate amplified spinning sideband manifolds which can be fitted to extract CSA parameters. The $^{13}C/^{13}C$ homonuclear dipolar interactions are not affected by the π-pulses due to the bilinear nature of the spin operators and are averaged by MAS in the xCSA experiment. These features make the constant evolution-time experiment suitable for measuring CSA of uniformly labeled samples. The incorporation of xCSA with multi-dimensional $^{13}C/^{15}N$ correlation is demonstrated with a GB1 protein sample as a model system for measuring $^{13}C/^{15}N$ CSA of all backbone ^{15}NH, ^{13}CA and ^{13}CO sites.

1. Introduction

Magic-angle spinning (MAS) is an ubiquitous method for obtaining high-resolution nuclear magnetic resonance (NMR) spectra of solids. Rapid sample spinning averages line broadening arising from anisotropic spin interactions, such as chemical shift anisotropy (CSA) and dipolar coupling, yielding high-resolution spectra of powder samples. These anisotropic spin interactions are of great interest because of their direct relation to molecular structure and local electronic environment. In the presence of molecular motion, measurement of the scaled chemical shift anisotropy and dipolar coupling can also yield order parameters from the molecular dynamics. Numerous NMR techniques have been developed to reintroduce/recouple CSA or dipolar coupling under fast MAS, which is particularly useful for uniformly labeled proteins in conjunction with multi-dimensional correlation experiments because it can be used under high-resolution conditions. However, the presence of ^{13}C homonuclear coupling among the directly bonded carbons can interfere with recoupling of the anisotropic interactions of interest. For CSA measurement, only a few recoupling pulse sequences, namely ROCSA [1] and $R18_1^7$ [2–4], have been designed specifically for uniformly labeled samples by minimizing the effects from homonuclear dipolar coupling. In this work, we show that a simple method of CSA amplification can be applied directly to uniformly labeled systems. CSA amplification is a class of pulse sequences that uses π-pulses to repeatedly interrupt the averaging of CSA by MAS. The timing of pulses can be designed in a way such that the effective CSA evolution yields spinning sideband manifolds equivalent to those at a much lower spinning frequency. As shown in early works by Maricq and Waugh [5], and Herzfeld and Berger [6], spinning sidebands are a direct result from modulation of the CSA by MAS. For spinning rates that are several times smaller than the breadth of the anisotropy, the spectral intensity is concentrated into a handful of narrow spinning sidebands that can be fitted to determine the CSA tensor parameters. This 'slow spinning' method has become a method of choice for CSA measurement of dilute spin systems wherein CSA is the only spin interaction present and the line width is usually independent of the spinning frequency. For example, it has been possible to measure the ^{15}N CSA in uniformly ^{15}N labeled GB1 protein samples [7,8], since homonuclear coupling among ^{15}N nuclei is weak. Nevertheless, dipolar coupling among covalently bonded ^{13}C spins requires fast

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MAS to obtain high spectral resolution, making the slow spinning method unsuitable. The CSA amplification method presented here makes the measurement of spinning sideband intensities possible under fast MAS high-resolution conditions.

In the following, a brief theory of our CSA amplification method [9] (dubbed xCSA) is presented, as well as why homonuclear dipolar coupling does not affect it. This key feature, essential for application to uniformly ^{13}C labeled systems, is examined experimentally using an alanine sample. A comparison of ^{13}C sideband intensities of [^{13}C, ^{15}N] and [^{15}N] labeled alanine verifies that the homonuclear couplings have indeed negligible effects on the CSA measurement. For application to uniformly labeled proteins and macromolecules, the xCSA pulse sequence needs to be combined with two-dimensional $^{13}C/^{15}N$ correlation to achieve site resolution. Three-dimensional xCSA experiments with NCA and NCO correlation for $^{13}C/^{15}N$ CSA measurement of the backbone NH, CA, and CO sites are demonstrated with a sample of [U-^{13}C, ^{15}N] labeled GB1 protein.

2. Theory

CSA amplification is a class of pulse sequences for rotating solids pioneered by Griffin et al. [10] and Gullion [11], which utilizes π-pulses to interrupt the averaging of CSA under MAS. The timing of the π-pulses can be designed such that the effective evolution yields sidebands that are exactly the same as if the spinning rate is reduced or the CSA is magnified, hence these methods have been dubbed extended chemical shift modulation (XCS) or CSA amplification. The relative sideband intensities are the same when multiplying the anisotropy or reducing the spinning frequency by a factor κ. A number of CSA amplification pulse sequences have been developed to obtain the largest amplification factor, while using the minimum number of π-pulses and shortest total duration. A recent review on this topic provides a thorough explanation and comparison of these sequences [12]. In the following, we use our recent CSA amplification sequence [9], dubbed extended CSA amplification (xCSA), to describe the theory of CSA amplification and the effect from homonuclear dipolar couplings.

Under MAS, the chemical shift interaction can be separated into the constant isotropic shift and the modulating components from the CSA,

$$\omega(t) = \omega_{iso} + \omega_{CSA}(t),\ \ \omega_{CSA}(t) = \sum_{m=\pm1,\pm2} \omega_m \exp(im\omega_r t) \tag{1}$$

The signal phase evolution can be obtained by integration of the modulated chemical shift over time $\omega_{CSA}(t)$, in terms of the indefinite integral $\xi(t)$,

$$\varphi_{CSA} = \int_{t_a}^{t_b} \omega_{CSA}(t)dt = \xi(t)\Big|_{t_a}^{t_b} \tag{2}$$

Application of π-pulses alternates the sign of the accumulated signal phase. At the end of a sequence of n π-pulses, the total phase becomes

$$\varphi_{total} = \sum_{q=0}^{n} (-1)^{n+q}\xi(t)\Big|_{t_q}^{t_{q+1}} \tag{3}$$

Fig. 1a shows our recent CSA amplification sequence designed based on the magic-angle turning (MAT) experiment [13,14]. The sequence consists of a basic amplification unit (Fig. 1b) with four π-pulses spanning two rotor periods. The π-pulses are separated by time units of $\tau_r/3$, where $\tau_r\,(=1/\nu_r)$ is the rotor period. The total evolution for the four evolution segments of the basic amplification unit can be written out explicitly

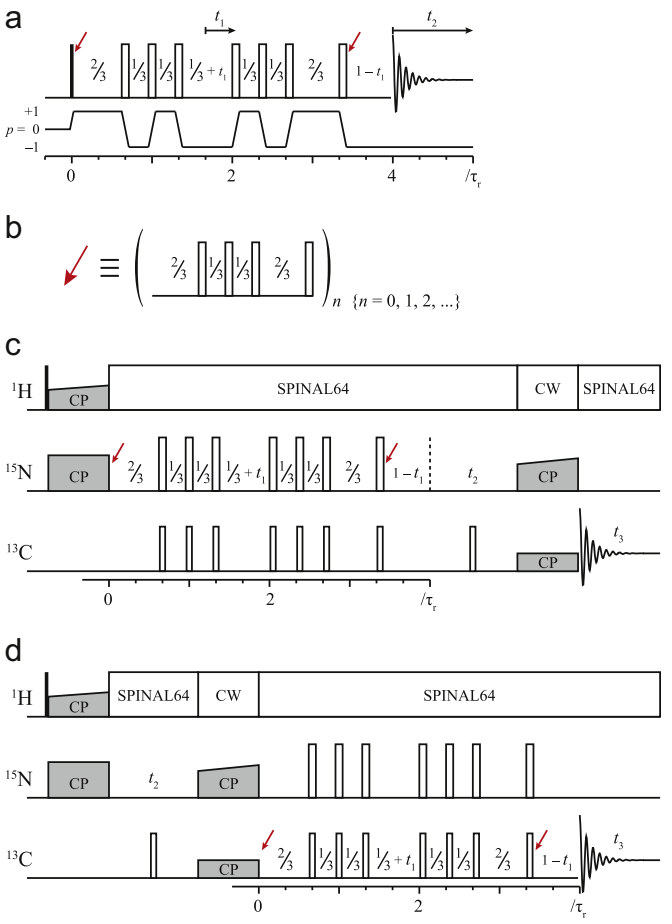

Fig. 1. (a) Pulse sequence and coherence transfer pathway for a κ=6 xCSA experiment. (b) Basic amplification unit that can be inserted at the positions denoted by red arrows if additional CSA amplification is desired. (c, d) 3D xCSA pulse sequences for measurement of (c) ^{15}N and (d) ^{13}C CSA. In (d), the ^{15}N /^{13}C CP and the ^{13}C carrier frequency can be changed to obtain NCA or NCO correlation. Narrow solid and empty rectangles in the pulse sequence denote π/2- and π-pulses, respectively. A cogwheel phase cycle is employed during the CSA amplification sequences to select the desired alternating coherence transfer pathway: 0 for all odd-numbered pulses starting from the excitation or CP pulse, $\{0, 1, 2, \ldots, 2a+1\}\times\pi/(a+1)$ for all even-numbered pulses, and $\{0, \pi\}$ for the receiver phase, where a is the total number of π-pulses. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

$$\begin{aligned}\varphi_{total} &= \left[\xi\left(t_0+\tfrac{2\tau_r}{3}\right)-\xi(t_0)\right]-\left[\xi(t_0)-\xi\left(t_0+\tfrac{2\tau_r}{3}\right)\right]\\ &\quad+\left[\xi\left(t_0+\tfrac{\tau_r}{3}\right)-\xi(t_0)\right]-\left[\xi(t_0)-\xi\left(t_0+\tfrac{\tau_r}{3}\right)\right]\\ &= -6\xi(t_0)+2\xi(t_0)+2\xi\left(t_0+\tfrac{\tau_r}{3}\right)+2\xi\left(t_0+\tfrac{2\tau_r}{3}\right)\end{aligned} \tag{4}$$

Here t_0 is the time at the start of the pulse sequence, noting that $\xi(t_0) = \xi(t_0 + \tau_r)$. Invoking the MAT condition for a second-rank tensor [9,13,14], such as CSA,

$$\xi(t)+\xi\left(t+\tfrac{\tau_r}{3}\right)+\xi\left(t+\tfrac{2\tau_r}{3}\right)=c \tag{5}$$

where c is a constant, Eqn. (4) becomes

$$\varphi_{total} = -6\xi(t_0)+2c \tag{6}$$

The complete CSA amplification sequence consists of two basic amplification units. The second unit is shifted by a t_1 evolution time (Fig. 1a). The last π-pulse of the first basic unit is eliminated such that total phase evolution takes the form

$$\varphi_{xCSA}(t_1) = 6\left[\xi(t_0)-\xi(t_0+t_1)\right] \tag{7}$$