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Accurate CSA measurements from uniformly isotopically labeled biomolecules at high magnetic field

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Abstract

Obtaining chemical shift anisotropy (CSA) principal values from large biomolecular systems is often a laborious process of preparing many singly isotopically labeled samples and performing multiple independent CSA measurements. We present CSA tensor principal values measured in the biomolecular building blocks tyrosine·HCl, histidine·HCl, and all-E-retinal in both isotopically labeled and unlabeled forms at 17.6 T. The measured tensor values are identical for most carbon sites despite significant dipolar couplings between the spins. Quantum mechanical simulations of an arbitrary three spin system were used to evaluate the accuracy of direct CSA measurement as a function of applied magnetic field strength and molecular parameters. It was found that for a CSA asymmetry of 0.2 or more, an accurate measure of the CSA parameters is obtained when the CSA anisotropy is more than six times the largest dipolar coupling in frequency units. If the CSA asymmetry is more than 0.5, this requirement is relaxed, and accurate results are obtained if the anisotropy is more than three times the dipolar coupling. While these limits are insufficient for measurement of CSA's for α-carbons and aliphatic sidechain sites in proteins at current field strengths, they open the way for routine systematic CSA measurements of sites with relatively large CSA tensor values in extensively isotopically labeled biomolecules in widely available magnetic fields.

Keywords: Chemical shift anisotropy; Dipolar coupling; Magic angle spinning; Biological structure; High field

1. Introduction

Solid state NMR is a rapidly developing method for gaining detailed biophysical insight from systems that cannot be studied by solution state NMR or X-ray crystallography. Recent advances have made it possible to determine full 3D structures of uniformly ^{13}C and ^{15}N labeled biological solids through multidimensional methods [1–4]. Other approaches have allowed detailed studies of ligand–protein interactions [5]. Advantages in sensitivity and resolution, as well as advances in magnet design are leading solid state NMR to higher magnetic fields. These new experimental conditions have significant implications for established experimental methods. Herein, we explore the high field limit of CSA tensor measurements in uniformly isotopically labeled biomolecules. The experimental and simulated results indicate that widely available magnetic field strengths are already sufficient for accurate measurements at many biologically interesting molecular sites.

Chemical shift scales with applied field strength, and thus, the anisotropic chemical shift and its cross terms with other quantum mechanical interactions are significantly larger at high magnetic fields. Sideband patterns observed by MAS NMR are distorted by dipolar couplings between adjacent spins in multiply isotopically labeled systems, skewing the results of CSA measurements. However, dipolar couplings do not scale with the applied field, and these distortions are reduced at high field,

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obviating the need for specialized methods of CSA measurement. In this report, we quantify the accuracy of direct, uncompensated CSA measurements in uniformly isotopically labeled biomolecules.

Dipolar recoupling methods are among the most versatile and widely used tools for structure determination by solid state NMR [6]. They employ RF pulses to reintroduce the dipolar couplings, which are otherwise averaged by MAS. In most homonuclear experiments, the results depend on the chemical shifts of the nuclei involved, and sensitivity to higher order chemical shift terms is considered the most important factor in performance variations at moderate field strengths [7]. The importance of these CSA terms increases at higher field, where poorly characterized CSA's become significant sources of error. Structural measurements involving molecular sites with relatively large CSA's are particularly sensitive to these effects. Some knowledge of the CSA is therefore necessary for accurate dipolar recoupling measurements in high magnetic fields.

For dipolar recouping applications in peptide systems, in situ CSA measurements are usually avoided by using canonical values from reference studies of glycine and alanine in small peptides and in amino acid polymers [8,9]; however, the protein environment can influence the CSA, causing significant deviations from these canonical values. This is accepted to be the case for C_α CSA's, which have recently been shown to correlate with the peptide backbone conformation [10], but it is also true for sidechain chemical shifts and for carbonyl CSA's, which reflect the strength of the hydrogen bond. An estimate of the normal CSA variation for any particular site can be obtained from the BioMagRes database [11]. Protein structures deposited in the databank show an isotropic chemical shift range for the backbone C=O of nearly 25 ppm (165–189 ppm). Since the amide bond is planar and invariant, this indicates much larger variations in one or more of the anisotropic CSA parameters. For C=O, this is generally accepted to be primarily due to variations in σ_{22} [12]. Thus, normal variations in CSA can cover a significant frequency range at high magnetic fields, and relying on a single set of canonical values could be misleading. Additionally, for most non-peptide systems, including many biologically important ligands, aggregates, polysaccharides, and modified amino acids, canonical CSA values are not available.

A common, though laborious, approach to this problem has been to prepare a variety of singly isotopically labeled samples and to obtain many independent CSA measurements [13]. With the recent introduction of dipolar compensated CSA measurement methods, CSA's can now be measured in uniformly isotopically labeled systems [14]. For large CSA's, however, these new methods require fast spinning and extremely high RF power levels. This makes them inappropriate for delicate biological samples, including membrane proteins. Herein, we demonstrate that direct high field measurement of larger CSA tensors in uniformly isotopically labeled samples by existing techniques often yields accurate results, even without dipolar compensation. Through simulations, we further explore and quantitate the limits of this high field effect.

CSA measurements on multispin systems involve crowded spectra, and are best measured by multidimensional methods. Many approaches are now available, including magic angle turning approaches such as FIREMAT and its relatives [15], and MAS methods based on TOSS and PASS [16,17]. More recently, techniques for measuring chemical shift powder patterns or enhanced powder patterns under (fast) MAS conditions have been developed [14,18]. In all these methods, the isotropic and anisotropic parts of the chemical shift are separated into two dimensions. Methods that separate the spinning sidebands by order instead of frequency accomplish this separation with a minimum number of t_1 slices, reducing the measurement time. Using uncompensated methods in multiply isotopically labeled systems with significant dipolar couplings requires some consideration of the pulse sequence length and of the nature of the applied RF fields. We have chosen to use 2D PASS because of its short, constant time pulse sequence, and its minimal number of t_1 slices [17].

2. Experimental methods

2.1. Samples

Unlabeled tyrosine, valine, and histidine were obtained from Sigma. U-^{13}C, ^{15}N-tyrosine and U-^{13}C, ^{15}N-histidine were obtained from CIL. Tyrosine and histidine samples were recrystallized from water at pH 7.0 containing equimolar HCl to form the hydrochloride salts. Unlabeled all-E-retinal was obtained from Sigma and used without further purification. [U-^{13}C]all-E-retinal was synthesized by total synthesis [19]. The all-E conformer was isolated by HPLC with 25% diethyl ether in petroleum ether. The total amount isolated was determined by UV–vis spectroscopy assuming an extinction coefficient, $\varepsilon = 48{,}900$ at 368 nm in pentane. To suppress intermolecular dipolar couplings, uniformly labeled retinal was diluted to 6% with natural abundance material before crystallization from pentane at −80 °C. All samples were packed into 4 mm zirconium rotors. The structures and numbering schemes are shown in Fig. 1.

2.2. Solid state NMR

NMR experiments were recorded with an Avance-WB750 spectrometer, equipped with a 4 mm triple reso-