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Solid-state ^{17}O NMR in carbohydrates

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Abstract

Solid-state ^{17}O magic-angle spinning nuclear magnetic resonance measurements at 19.5 Tesla were performed on ^{17}O-enriched methyl α-D-galactopyranoside (4-^{17}O), methyl β-D-glucopyranoside (2-^{17}O), methyl α-D-glucopyranoside (4-^{17}O), methyl α-D-glucopyranoside (6-^{17}O), and α-D-glucopyranosyl (1 → 6) α-D-glucopyranoside (6-^{17}O). The ^{17}O quadrupolar coupling constants and asymmetry parameters measured can be predicted with a model based entirely on the first-coordination sphere around oxygen. For the hydroxyl sites observed in the methyl glucosides, the quadrupolar coupling parameters are nearly identical, within 10% as predicted, given their nearly identical first-coordination sphere structures.

It is difficult to overstate the potential of oxygen nuclear magnetic resonance (NMR) spectroscopy as a structural probe in biological systems. Oxygen occurs at least once in nearly every monomer within a variety of biopolymers, and is involved in hydrogen bonding, a major contributor to the structure of biomolecules. Generally, there are two main obstacles to widespread use of oxygen NMR. One is the low natural abundance of 0.037% for ^{17}O, the only NMR active isotope. Thus, samples must be enriched in ^{17}O, requiring synthetic strategies which vary in difficulty depending on the sample. The second is that ^{17}O is a quadrupole nucleus and in nearly all covalent bonding environments experiences such strong nuclear quadrupolar couplings [1,2] that conventional NMR approaches have been of limited utility. While the chemical shift range of ^{17}O spans almost 600 ppm for organic molecules [3], its large quadrupolar couplings of the order of 7–13 MHz lead to such severe homogeneous line broadening [4] in solution state NMR that resonances are often indistinguishable from the baseline. One approach for improving resolution in solution is to collect spectra at elevated temperatures (60–100 °C) where molecular correlations times are shorter than the NMR time scale and line broadening due to quadrupolar relaxation is diminished [3,4]. Such temperatures present a serious limitation for conformational studies of oligosaccharides where most will be present as a rapidly equilibrating mixture of conformers.

Solid-state ^{17}O NMR, in contrast, does not suffer from short relaxation times and large homogeneous line widths. Instead, it suffers from a large anisotropy from the first-order quadrupolar interaction, which leads to inhomogeneous line broadenings of the order of megahertz, often exceeding NMR spectrometer bandwidths. A simplification for half-integer nuclei like ^{17}O, however, is that the central ($m = 1/2 \rightarrow -1/2$) transition only experiences second-order quadrupolar broadening. Unfortunately, this broadening for ^{17}O in organic solids can be of the order of 50–100 kHz at common field strengths such as 9.4 Tesla. Thus, attempting to narrow the central transition with magic-angle spinning (MAS) is difficult due to significant intensity loss into spinning sidebands. Additionally, when

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MAS is applied to this central transition, an isotropic site-resolved spectrum is not obtained because MAS lacks the proper symmetry to eliminate second-order broadenings [5].

In recent years, developments in NMR methodology have made solid-state ^{17}O NMR a more practical probe of structure in organic systems [6]. These include (1) field strengths over 20 Tesla that reduce second-order broadenings, (2) higher speed (>30 kHz) MAS probes that minimize spinning sidebands and simplify spectra, (3) pulse sequences that enhance sensitivity [7] and resolution [5], (4) state-of-the-art computer hardware and ab initio quantum chemistry software packages for predicting NMR parameters, and (5) new synthetic approaches for obtaining ^{17}O labeled compounds [8].

Although several carbonyl or carboxylic acid oxygen sites have been examined by solid-state ^{17}O NMR [6] and by ^{17}O nuclear quadrupole double resonance [10,11], hydroxyl groups and ether linkages in organic compounds have received little or no attention [12]. Here, we examine oxygen in a glycosidic linkage and hydroxyl groups in a series of ^{17}O labeled carbohydrates. The methyl glycosides, which are often used as intermediates in organic syntheses with applications in pharmacology and biochemistry [13], have also been used as model compounds for studying the influence of structure on chemical shift tensors [14].

The experimental ^{17}O MAS NMR spectra obtained at 19.6 Tesla for glycosidic oxygen in the disaccharide and for the hydroxyl oxygen sites in the methyl glycosides are shown in Fig. 1. An MAS spectrum of methyl α-D-Glucopyranoside (6-^{17}O) collected at 9.4 Tesla with an identical number of transients is shown for sensitivity comparison. One cause of low sensitivity with ^{17}O is its low Larmor frequency, ω_0, (approximately 1/8th of ^{1}H). With all other factors equal, NMR sensitivity varies as $(\omega_0)^{7/4}$. This effect alone, would yield a factor of 3.6 sensitivity improvement on increasing from 9.4 to 19.6 Tesla. There is however, a more significant gain for ^{17}O with increasing field due to decreasing second-order quadrupolar broadenings, which are inversely proportional to field strength. Combining these two effects with faster MAS yields the observed sensitivity increase by a factor of 20, or by a factor of 400 in time savings.

The measured NMR parameters [15] are given in Table 1. In the mid-1980s Oldfield and coworkers [16] established from experimental measurements that the electronegativity of the atoms coordinated to a bridging oxygen atom is a primary factor influencing the electric field gradient (efg) at the bridging oxygen. More recently, Clark and Grandinetti [17] showed that a combination of coordinating atom group number, coordinating atom–oxygen distance, and bridging oxygen angle serve as a better predictor of the efg. They also noted that the majority of the efg generated at the oxygen nucleus, particularly in environments like a sugar hydroxyl or glycosidic linkage, is determined by the nature of the covalently coordinated atoms. For example, the quadrupolar coupling constant, C_q, for the glycosidic

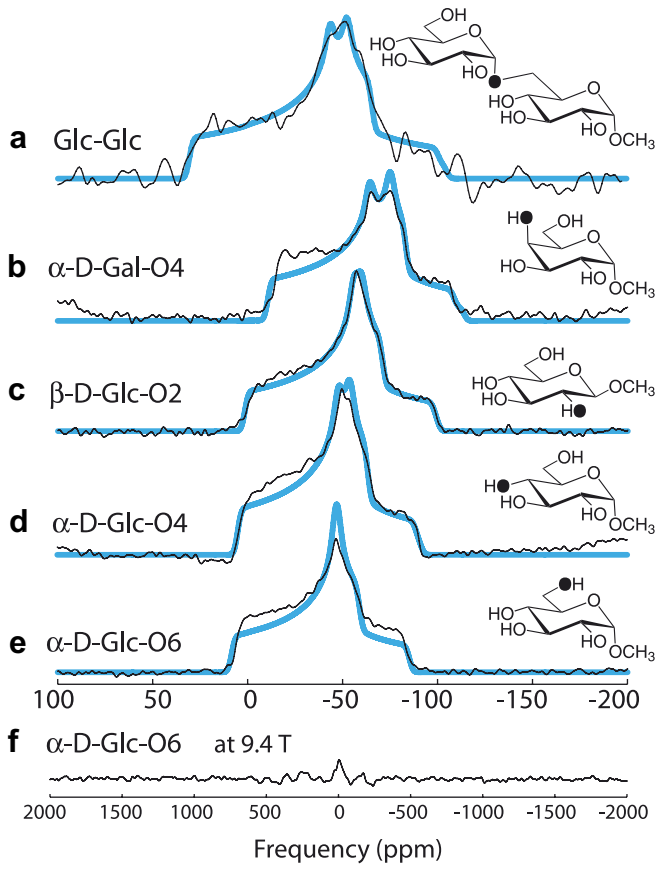

Fig. 1. Experimental and simulated (blue) ^{17}O MAS spectra acquired at 19.6 Tesla (112.76 MHz) of (a) methyl α-D-glucopyranosyl (1 → 6) α-D-glucopyranoside (6-^{17}O), (b) methyl α-D-galactopyranoside (4-^{17}O), (c) methyl β-D-glucopyranoside (2-^{17}O), (d) methyl α-D-glucopyranoside (4-^{17}O), (e) methyl α-D-glucopyranoside (6-^{17}O), (f) MAS spectrum of methyl α-D-glucopyranoside (6-^{17}O) acquired at 9.4 Tesla (54.23 MHz) at a spinning rate of 12 kHz. 19.6 Tesla spectra were collected at a spinning rate of 20 kHz in a 2.5 mm MAS probe using whole-echo acquistion [9], an echo shift of 1 ms and a recycle delay of 0.5 s. Spectrum (a) was acquired with 160000 scans, and (b)–(f) with 40000 scans. All compounds were enriched to 25% at indicated site [8]. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this Letter.)

Table 1
Experimental solution and solid-state ^{17}O NMR results [15]

Sample	δ_{cs}^{soln}/ppm	δ_{cs}^{solid}/ppm	$\lvert C_q\rvert$/MHz	η_q	χ_r^2
α-Gal-O4	−7.6	−8.9	9.51	0.83	7.08
β-Glc-O2	7.9	2.9	9.25	0.93	1.94
α-Glc-O4	9.4	6.4	9.08	0.90	20.48
α-Glc-O6	−10.8	9.5	8.76	1.00	20.22
Glc–Glc	21	32.2	10.75	0.89	3.96

Chemical shift data are referenced to tap water.

oxygen in Fig. 1a, is nearly a factor of two larger than what is found for a typical Si–O–Si linkage, as predicted [17]. The remaining variations in the efg can be explained by the other degrees of freedom in the first and higher coordination spheres. The orientation of the glycosidic oxygen efg principal axis system (PAS) will be similar to the Si–O–Si linkage [18], with the x- and z-axis lying in the C–O–C

plane and the x-axis bisecting the C–O–C angle. Unfortunately, there is no known crystal structure for Glc–Glc so further analysis is not possible at this time. As with the Si–O–Si linkage [18,19], however, it should also be possible to use efg measurements from glycosidic oxygen in known structures to develop a model for predicting C–O–C angle and C–O distance from C_q and asymmetry parameter, η_q.

Using a methanol model in ab initio calculations [20] we have examined the variation in oxygen efg in the hydroxyl environment over a range of local structures commonly found in the first-coordination sphere of methyl glucopyranoside hydroxyls. In this model, the relevant structural parameters are the C–O–H angle, C–O and O–H distance, and the presence (or absence) of hydrogen bond donors and acceptors. In glycosides the C–O–H angles range from 105–115°, and the C–O and O–H distance from 1.39–1.43 Å and 0.80–1.00 Å, respectively [21–23]. Over this range, the largest variation in ^{17}O efg occurs with changes in C–O–H angle and O–H distance. Variations due to changing C–O distance account for less than 1%. In Fig. 2 are the variation in the efg components with C–O–H angle and O–H distance for the methanol model. Remarkably, this model predicts no significant variation in ^{17}O efg over the range of C–O–H angles and O–H distances found in the methyl glucosides sites measured here. This prediction is confirmed in our experimental data. In Fig. 3 is a plot of the principal components of the quadrupolar coupling tensor for each hydroxyl measured. Because the sign of C_q is unknown, the principal components were calculated assuming both signs for each site. Experimental variations are less than 10%. For the four sites, β-D-Glc-O2 and α-D-Glc-O6 are acting as both donor and acceptor, α-D-Gal-O4 acts only as donor, and α-D-Glc-O4 acts as neither donor nor acceptor [21–23]. No simple correlation, however, is found between these minor efg variations and first or second-coordination sphere structures. Additionally, motion of the hydrogen could also play a role in the remaining variation of the efg [24].

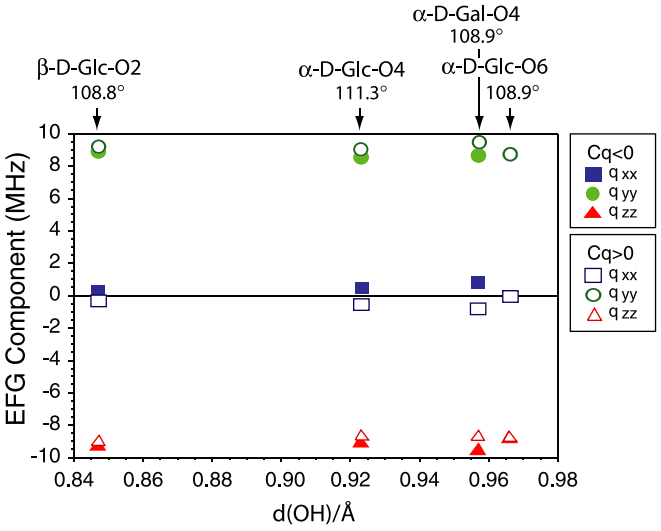

Fig. 3. Principal components of ^{17}O efg (multiplied by e^{2}Q/h) calculated from the measured hydroxyl C_q and η_q values in methyl glucosides of Table 1. The C–O–H angle and d(O–H) distance information were obtained from references [21–23].

Overall, the structural invariance of the ^{17}O quadrupolar couplings observed here for the hydroxyl group will be advantageous in future NMR studies of oriented samples (e.g., membranes) [2], and in correlation experiments with other NMR interactions such as ^{1}H–^{17}O or ^{13}C–^{17}O dipolar and J couplings [6].

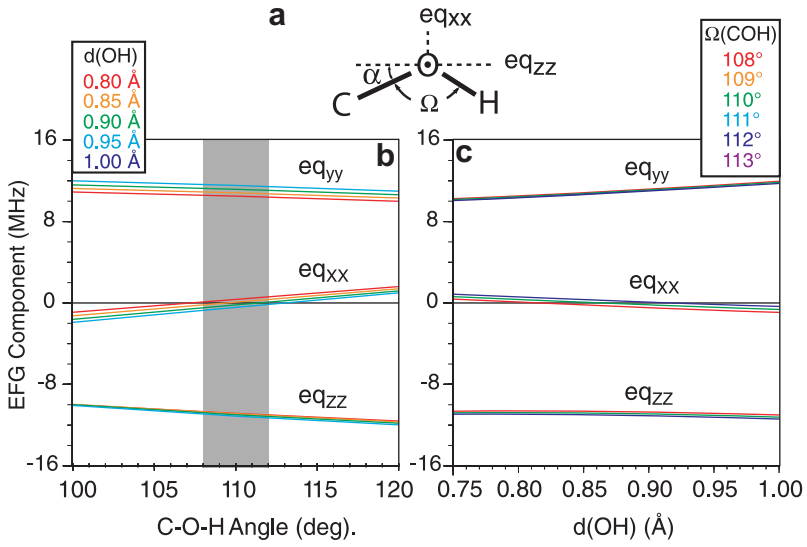

Fig. 2. (a) Molecular frame where efg tensor is diagonal. Variation in efg components (multiplied by e^{2}Q/h) with (b) C–O–H angle and (c) O–H distance. Because efg PAS components are assigned based on increasing magnitude, efg components are left in the molecular frame. GAUSSIAN 03 at the RHF/6-311++G** level was used. Gray area indicates the range of C–O–H angles present in sites measured.

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