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# New Limits for Solid-State $^{17}$O NMR Spectroscopy: Complete Resolution of Multiple Oxygen Sites in a Simple Biomolecule

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Nuclear magnetic resonance (NMR) is a key technique in providing atomic scale information on molecular architecture. Solid-state NMR approaches are playing an increasing role in biomolecular science; however, almost all such NMR reports concern spin-1/2 nuclei ($^{1}$H, $^{13}$C, $^{15}$N). Oxygen is one of the most important and abundant elements in biological systems,[1] but it is little studied by NMR. Since oxygen plays a central role in many biological interactions, such as protein-protein, metal-protein, and in nucleic acids, it would be beneficial to be able to study the oxygen directly. From the NMR point of view, spin-1/2 nuclei have been preferred since in complex systems spectral resolution of the many different sites can be obtained and an armory of 2D techniques employed to give information about distances and bonding between neighboring atoms. The only NMR-active oxygen isotope, $^{17}$O, has low natural abundance (0.037%) and spin ($I = {}^{5}/_{2}$). The resulting quadrupole interaction usually significantly broadens the signal. Even under magic-angle spinning (MAS), the line width for $^{17}$O with large quadrupole interactions[2] means that signals from multiple sites are not readily resolved, so that site-specific information is masked, and many solid-state NMR techniques that depend on resolution and narrow lines cannot be applied. Consequently, solid-state $^{17}$O NMR from complex biomolecules presents a significant challenge. Despite these difficulties, with the advance of high-field NMR spectrometers and methodologies, such as multi-quantum (MQ)-MAS[3] and double-rotation (DOR),[4] there has been a significant increase in solid-state $^{17}$O NMR studies of inorganic[5−7] and organic/biomaterials.[8−14]

Here, by using $^{1}$H-decoupled DOR, $^{17}$O NMR signals are resolved from all eight similar, but distinct, oxygen sites in monosodium L-glutamate monohydrate (L-MSG),[16] which is used extensively as a food flavor enhancer. As shown in Figure 1a, the $^{17}$O MAS spectrum (recorded at 14.1 T) shows a broad signal centered at ∼220 ppm with a line width of ∼8 kHz. The broad signal arises from the overlapping second-order quadrupole broadened lines with the presence of multiple sites indicated by the detailed features on the MAS envelope. However, with eight overlapping signals, spectral deconvolution to provide site-specific information is completely impracticable. In comparison, the $^{1}$H-decoupled $^{17}$O DOR spectrum (Figure 1b) exhibits major spectral improvement by successfully removing the second-order quadrupole broadening, producing seven sharp isotropic resonances with line widths less than 1 ppm, whose DOR isotropic positions ($\delta_{DOR}$) are given in Table 1. The intensity for the resonance at 196 ppm is nearly twice that of the other six isotropic lines, suggesting that this resonance is from two oxygen sites. These sharp signals are ∼120 times narrower than those in the MAS spectrum. This is the first time that solid-state $^{17}$O NMR has revealed eight distinct oxygen sites from a biomolecule. Previously, we have reported $^{17}$O DOR spectra for the same MSG sample;[14] however, the spectral resolution was much inferior with line widths ∼300 Hz, so that only five isotropic resonances were resolved. The poorer spectral resolution is due to the residual O−H dipolar interactions in the absence of $^{1}$H decoupling, as well as a lower spinning speed. In addition to the significantly improved spectral resolution in the DOR experiment, the time required for obtaining a good signal-to-noise spectrum compared to MAS experiments is much reduced.

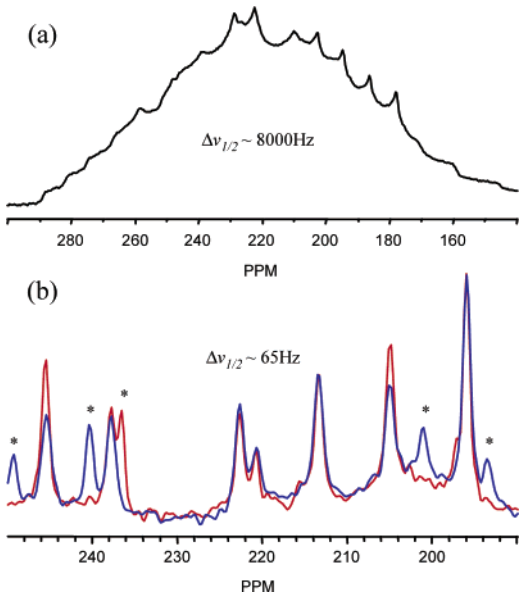


***Figure 1.*** $^{1}$H-decoupled (a) $^{17}$O MAS and (b) $^{17}$O DOR NMR spectra of L-MSG at 14.1 T. The 20% $^{17}$O-enriched sample was prepared according to ref 14. The $^{17}$O spectra were recorded at 81.37 MHz. The $^{17}$O chemical shift was referenced to $H_2O$ at 0 ppm. The MAS spectrum was acquired with a 3.2 mm MAS probe and a rotor synchronized spin−echo sequence with pulses of 0.8 and 1.6 μs, and ∼100 000 transients, with the sample spinning at ∼18 kHz. The $^{17}$O DOR spectrum was recorded using odd-order sideband suppression[15] with 4800 (red) and 6000 (blue) transients, using a repetition rate of 1 s and $^{1}$H decoupling of 35 kHz for 12 ms and processed with 20 Hz line broadening. The isotropic resonances were determined by two different outer rotor speeds of 1800 (blue) and 1650 (red) Hz. The spinning sidebands are marked by asterisks (∗).

To deduce the $^{17}$O NMR interaction parameters (chemical shift and quadrupolar parameters) for each oxygen site in L-MSG, an undecoupled $^{17}$O DOR spectrum at 8.45 T and $^{1}$H-decoupled $^{17}$O 3Q spectra at 14.1 and 18.8 T were recorded. The corresponding isotropic spectra are shown in the projection of the DOR/3Q plot

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***Table 1.*** $^{17}$O NMR Interaction Parameters of the Oxygen Sites in MSG. $P_Q$ and $\delta_{iso}$ are Taken from the DOR/MQ Plot[17] Shown in Figure 2

| possible O site[a] | $\delta_{DOR} \pm 0.5$ ppm (14.1 T) | $\delta_{iso} \pm 2$ ppm | $P_Q \pm 0.05$ MHz[c] |
|---|---|---|---|
| O4 or O14 | 245.5 | 294 | 7.40 |
| O4 or O14 | 237.8 | 286 | 7.35 |
| O1 or O2 | 222.6 | $274 \pm 4^b$ | $7.8 \pm 0.1^b$ |
| O3 or O13 | 220.7 | $272 \pm 4^b$ | $7.8 \pm 0.1^b$ |
| O3 or O13 | 213.1 | $271 \pm 4^b$ | $8.0 \pm 0.1^b$ |
| O1 or O2 | 204.5 | 257 | 7.65 |
| O11 and O12 | 195.6 | 251 | 7.85 |

[a] The assignments are based on the line width observed from DOR spectra at 14.1 T with no high-power $^1$H decoupling. The labeling corresponds to the crystal structure.[16] [b] The larger errors arise from the multiple possibilities of connecting the isotropic positions in Figure 2 for these sites. [c] The 3Q MAS gives $\eta_q = 0.4-0.5$ for all lines.

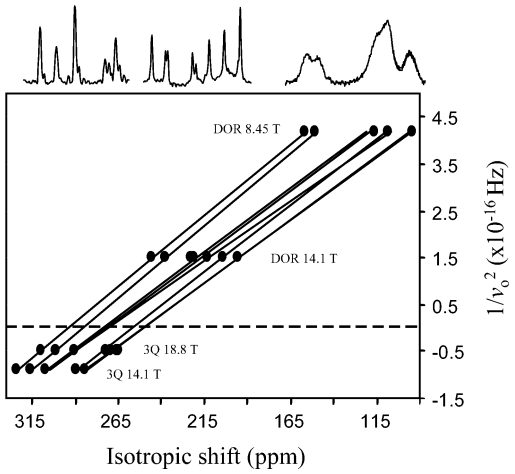


***Figure 2.*** Field dependence of the isotropic positions from the $^{17}$O DOR and 3Q data. The projections are the DOR spectra and the isotropic 3Q spectrum at 18.8T.

in Figure 2. Plotting the isotropic positions from the corresponding spectra with the inverse of the Larmor frequencies allows estimation of the combined quadrupole parameter, $P_Q = \chi_q(1 + \eta_q^2/3)^{1/2}$, and the isotropic chemical shift, $\delta_{iso}$, for each resonance.[17] The results are summarized in Table 1. It should be noted that despite nearly 4 days acquisition at 18.8 T, 3Q MAS has a much lower spectral resolution and produces a lower signal-to-noise than the DOR spectra shown in Figure 1b. (Despite 3Q MAS data providing, in principle, $\chi_q$ and $\eta_q$ for each line, problems with excitation and the number of spinning sidebands severely limit their accuracy with $\eta_q = 0.4-0.5$ for all lines.)

The X-ray crystal structure of MSG reveals that all oxygen sites have one delocalized C−O bond of 1.25−1.27 Å, and all have broadly similar electronic environments with either a hydrogen bond and/or a Na−O interaction.[16] A relatively large $^{17}$O $\delta_{iso}$ shift range, ∼45 ppm (Table 1), is found for these similar oxygen sites, suggesting that the shifts are highly sensitive to the local environment. Although the shift range is large, without the high spectral resolution obtained from DOR (Figure 1b), the assignment of the individual $\delta_{iso}$ even over a shift range of 45 ppm would be difficult.

On the basis of the individual resonance line widths (190−320 Hz) for the $^1$H-undecoupled DOR spectra together with the structural data given from X-ray crystal structure,[16] a tentative assignment of the isotropic $^{17}$O signals can be made (Table 1). The ambiguity of NMR assignments is partly due to the uncertainty in hydrogen positions from the X-ray structural data. Ab initio calculations, such as those by Yates et al.,[18] are likely to provide additional information for a more accurate assignment. Nonetheless, the $^{17}$O shifts for O14 and O4, where the oxygens experience no O−Na interactions, suggest that the oxygen−metal interactions induce a shift of $\delta_{iso}$. Similar metal induction of the shift was also observed for carboxylic oxygens.[13] Furthermore the quadrupole parameter, $P_Q$, for O4 and O14 is ∼0.4 MHz smaller than that for the other oxygen sites, suggesting that the existence of O−Na interactions also increases the $P_Q$ values.

Here, a novel NMR approach has characterized a multiple oxygen site system by using a combination of high-resolution $^{17}$O DOR and 3Q MAS NMR experiments. The large shift range suggests that $^{17}$O $\delta_{iso}$ is highly sensitive to local environment around the different oxygen sites, for example, hydrogen bonds and ion interactions. The excellent spectral resolution (<1 ppm) from a DOR experiment with line widths comparable to spin-1/2 nuclei ($^1$H, $^{13}$C, $^{15}$N) in biological molecules demonstrates that solid-state $^{17}$O NMR has tremendous potential to be a highly favorable probe for investigating molecular structure and functionality in many complex biomolecules.

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**Supporting Information Available:** Both $^1$H-undecoupled and -decoupled $^{17}$O DOR spectra at 14.1 T. Two-dimensional 3Q MAS spectra at 18.8 and 14.1 T. This material is available free of charge via the Internet at http://pubs.acs.org.

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