Solid-state $^{17}$O NMR as a new probe to study biological structures

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Oxygen is one of the most important elements in organic and biological molecules. Solid-state $^{17}$O (spin-5/2) NMR for organic compounds has, however, remained largely unexplored due to experimental difficulties in detecting $^{17}$O NMR signals. Since 2000, we have developed a comprehensive research program in solid-state $^{17}$O NMR studies of organic and biological compounds [1]. Using the 900 MHz spectrometer at the National Ultrahigh-Field NMR Facility for Solids, we have been able to tackle more challenging problems.

In the past year, we have successfully obtained high quality solid-state $^{17}$O NMR spectra for large biological macromolecules [2]. In particular, we have studied several robust protein-ligand complexes of size ranging from 65 to 240 kDa. The key was to combine several factors that allow the sensitivity to be optimized. First, we discovered that the values of $^{17}$O spin-lattice relaxation time ($T_1$) in solid proteins are on the order of a few milliseconds. Thus we were able to acquire data very rapidly (e.g., a recycle time of 30 ms). Second, we used relatively fast MAS frequencies (ca. 20 kHz) to reduce spinning sideband intensities. Third, we employed hyperbolic scant (HS) pulses [3] to enhance the CT signal. As an example, Figure 1 shows the experimental and simulated $^{17}$O MAS spectra of ovotransferrin-Al-oxalate complex (80 kDa) obtained at 21.14 T, as well as the ligand binding environment. The spectrum was simulated using the following parameters: O1, $\delta_{iso} = 219$ ppm, $\xi = \delta_{33} - \delta_{iso} = -160$ ppm, $\eta = (\delta_{22} - \delta_{11})/\xi = 0.6$, $C_Q = 5.75$ MHz, $\eta_Q = 0.70$; O2, $\delta_{iso} = 237$ ppm, $\xi = -160$ ppm, $\eta = 0.6$, $C_Q = 6.30$ MHz, $\eta_Q = 0.70$; O3, $\delta_{iso} = 274$ ppm, $\xi = -240$ ppm, $\eta = 0.2$, $C_Q = 7.70$ MHz, $\eta_Q = 0.45$; O4, $\delta_{iso} = 282$ ppm, $\xi = -240$ ppm, $\eta = 0.2$, $C_Q = 7.90$ MHz, $\eta_Q = 0.35$. In the spectral simulations, we have used the Euler angles of $\alpha = 0^\circ$, $\beta = 80^\circ$, and $\gamma = 30^\circ$ to describe the relative orientation between the $^{17}$O quadrupole coupling
tensor and the chemical shift tensor for all four oxygen sites, which is based on the computational results for oxalate-metal complexes reported by Wong et al. [4].

To better understand the observed $^{17}$O NMR parameters, we decided to carry out quantum chemical calculations. Because the crystal structure of OTf-Al-oxalate complex is unknown, we used the crystal structure of human serum transferrin-Fe-oxalate (PDB entry 1RYO) [5] as a starting point to build a molecular cluster model to mimic the oxalate binding pocket in OTf-Al-oxalate including all hydrogen bonding interactions. Then we performed a partial geometry optimization for the oxalate ligand and the hydrogen atoms involved in hydrogen bonding to the oxalate at the B3LYP/6-31G(d,p) level while keeping all other heavy atoms in the cluster model fixed in place. After that, we performed ADF calculations on NMR parameters for $^{17}$O, $^{13}$C and $^{27}$Al nuclei. As seen from Figure 2, the agreement between experimental and calculated NMR parameters (quadrupole and shielding) is reasonably good. It does not appear that multinuclear $^{17}$O, $^{27}$Al, and $^{13}$C NMR parameters have previously been used simultaneously to aid structural refinement of a protein-bound ligand molecule. We believe that this aspect of “NMR Crystallography” should be further explored.

In summary, we have continued to make significant progress in this long-term project. The development in the past year represents a breakthrough in this project. Now we are well positioned to tackle real biological problems using this new solid-state $^{17}$O NMR approach. In the next year, we plan to focus on $^{17}$O NMR detection of acyl-enzyme intermediates.

References