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Quadrupole-Central-Transition ^{17}O NMR Spectroscopy of Protein−Ligand Complexes in Solution

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Although oxygen is ubiquitous in biological molecules such as proteins and nucleic acids, its direct detection by NMR spectroscopy is generally difficult. The natural abundance of the only NMR-active oxygen isotope, ^{17}O, is extremely low (0.037%). In addition, ^{17}O has a quadrupolar nucleus ($I = {}^5/_2$), which often gives rise to broad NMR signals. On the other hand, ^{17}O NMR parameters are remarkably sensitive to various molecular interactions such as hydrogen bonding and metal ion binding, making ^{17}O NMR an attractive probe of biological structures and dynamics provided that practical difficulties in recording ^{17}O NMR signals can be overcome. In recent years, solid-state ^{17}O NMR has been considered to be more useful for studying organic and biological molecules than liquid-state NMR.[1] While there are indeed many advantages of obtaining ^{17}O NMR spectra for solid samples, it is still highly desirable to develop ^{17}O NMR methods for studying biological macromolecules under physiologically relevant conditions. In this work, we demonstrate that it is possible to observe narrow ^{17}O NMR signals for large protein−ligand complexes in solution. According to quadrupole relaxation theory, the NMR signal arising from a half-integer quadrupolar nucleus generally consists of ($I + {}^1/_2$) Lorentzian components. Under the nonextreme narrowing condition, i.e., $\omega_0^2\tau_c^2 \gg 1$ where ω_0 is the Larmor (angular) frequency of the observing nucleus and τ_c is the molecular rotational correlation time, only the so-called central transition (CT) may be detected whose line width depends inversely on the applied magnetic field strength. Consequently, it is possible to obtain relatively narrow CT signals at high magnetic fields. Although this quadrupolar relaxation property was recognized several decades ago,[2−6] its application has only been demonstrated for detecting metal ions such as ^{43}Ca ($I = {}^7/_2$), ^{51}V ($I = {}^7/_2$), ^{27}Al ($I = {}^5/_2$), ^{69,71}Ga ($I = {}^3/_2$), and ^{45}Sc ($I = {}^7/_2$) in metalloproteins.[7−10] Following Vogel and co-workers,[10] we use the term of quadrupole-central-transition (QCT) spectroscopy to describe this general approach. It should be pointed out that similar ^{17}O relaxation effects were first experimentally observed 20 years ago in the pioneering work of Lee and Oldfield[11] in which ^{17}O NMR spectra were reported for C^{17}O bound to heme proteins; however, the nuclear quadrupole coupling constant (C_Q) for C^{17}O is particularly small (C_Q < 1 MHz). Here we show that ^{17}O QCT spectroscopy is feasible for studying large protein−ligand complexes where the oxygen sites may have C_Q as large as 12 MHz.

Figure 1 shows the QCT ^{17}O NMR spectra of [^{17}O$_2$]palmitic acid bound to human serum albumin (HSA, 66 kDa) obtained at two magnetic fields. As expected, the position of the ^{17}O QCT signal in ppm (δ_{CT}) is shifted to a lower frequency from the true chemical shift position (δ_{iso}) due to the "dynamic frequency shift".[5] Using $\delta_{CT} = \delta_{iso} - 6000(P_Q/\nu_0)^2$ where P_Q is known as the quadrupole product, $P_Q = C_Q(1 + \eta^2/3)^{1/2}$, we obtained the following ^{17}O NMR parameters for palmitic acid bound to HSA: $\delta_{iso} = 297$ ppm and $P_Q = 9.3$ MHz (see Figure S1). It should be noted that these parameters represent averaged values for at least six palmitic acid ligands bound to HSA.[12] These ^{17}O NMR parameters suggest that all palmitic acid ligands bound to HSA are deprotonated at pH 7.5.

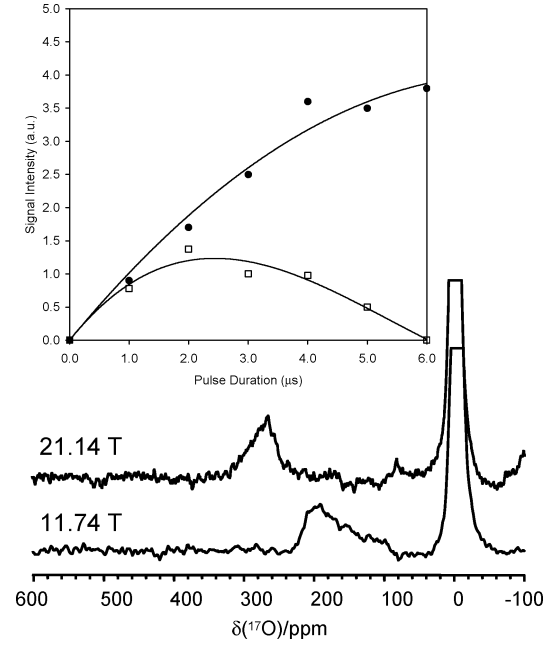

Figure 1. Experimental ^{17}O QCT NMR spectra of [^{17}O$_2$]palmitic acid (20% ^{17}O atom) bound to HSA (3 mM HSA in phosphate buffer pH 7.5). The ratio of protein/ligand was 1:6. Other experimental parameters are: 21.14 T, 500 000 transients; 11.74 T, 411 178 transients. A recycle time of 0.1 s was used in all experiments. The inset illustrates the distinct nutation response from the ^{17}O CT signal (□) as compared with that of water (●).

Figure 2 shows the ^{17}O QCT spectra of [^{17}O$_4$]oxalate bound to chicken ovotransferrin (OTf, 80 kDa). In addition to the signal from free oxalate ligand at $\delta_{iso} = 263$ ppm, three separate signals are generally observed whose positions (in ppm) change linearly with ν_0^{-2}, as expected for CT signals. More importantly, the line widths of the ^{17}O CT signals decrease drastically on going from 11.74 to 21.14 T. In comparison, the free ligand signal shows no field dependence in both its position and line width. Analysis of the field-dependence of the CT signals yields δ_{iso} and P_Q for the [^{17}O$_4$]oxalate ligand bound to OTf: O$_1$, 222 ppm, 6.2 MHz; O$_2$, 236 ppm, 6.8 MHz; O$_{3,4}$: 271 ppm, 7.3 MHz (see Figure S2). Remarkably, different oxygen atoms from the oxalate ligand exhibit quite different ^{17}O NMR parameters, reflecting different chemical environments at individual oxygen atoms of the oxalate ligand. On comparing to the ^{17}O NMR spectrum for an Al-oxalate complex shown in Figure 2b, we can assign O$_1$ and O$_2$ to the oxygen atoms coordinated to Al^{3+} (the "coordina-

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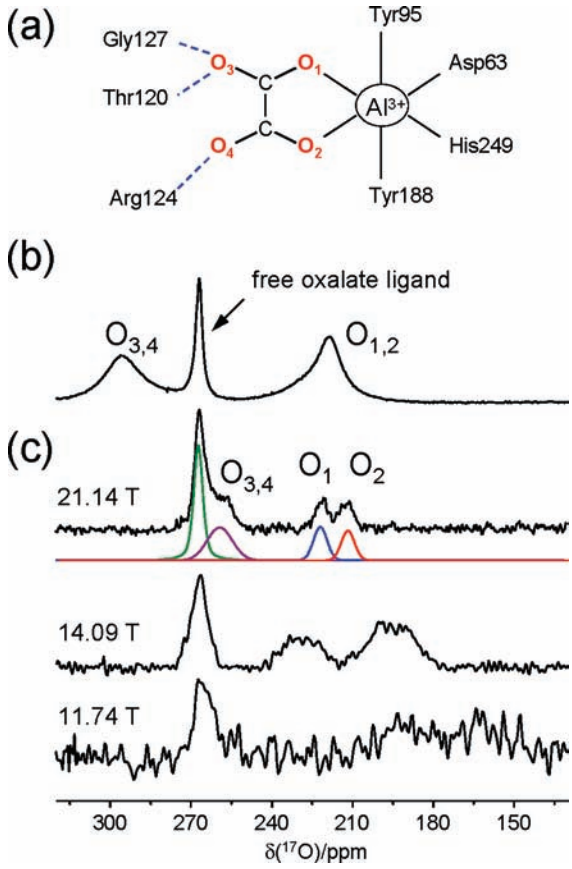

Figure 2. (a) Illustration of the oxalate binding site in Al−OTf. (b) Conventional ^{17}O NMR spectrum of an aqueous solution containing $Al(NO_3)_3$ and sodium [$^{17}O_4$]oxalate obtained at 14.09 T. (c) Experimental ^{17}O QCT NMR spectra of [$^{17}O_4$]oxalate (70% ^{17}O atom) bound to OTf (1.2 mM OTf containing 2 equiv of Al^{3+}) at different magnetic fields. Other experimental details: 21.14 T, 600 000 transients; 14.09 T, 1 471 161 transients; 11.74 T, 1 380 322 transients. A recycle delay of 0.1 s was used in all experiments. Note that signal loss was observed for $O_{3,4}$ in the low-field spectra due to its larger line width than that of $O_{1,2}$.

tion end") and $O_{3,4}$ to the "open end" of the oxalate ligand. The fact that the two oxygen atoms directly bonded to the Al^{3+} center

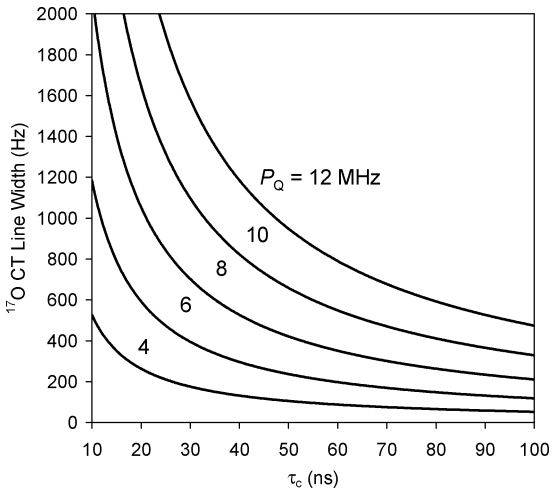

Figure 3. Dependence of the ^{17}O CT line width at 21.14 T on the molecular rotational correlation time (τ_C) for different values of P_Q.

exhibit different ^{17}O NMR parameters is consistent with the recent crystal structure of Fe^{3+}−OTf−oxalate, where two distinct Fe−O distances are observed, 1.96 and 2.03 Å.[13] On the basis of the trend reported by Wong et al.[14] for other oxalate−metal systems, we can conclude that the Al−O_2 bond is shorter than the Al−O_1 bond in the Al−OTf−oxalate complex. Interestingly, the δ_{iso} values for O_1 and O_2 of the OTf-bound oxalate are larger than that found for the corresponding oxygen atoms in the Al−oxalate complex, 215 ppm, suggesting that the Al−O bond length in the Al−oxalate complex is even shorter. On the other hand, $O_{3,4}$ of the protein-bound oxalate exhibit considerably smaller δ_{iso} values than those in the "open end" of the Al−oxalate complex. This indicates that the "open-end" of the protein-bound oxalate must be involved in strong hydrogen bonding. All these features are perfectly in agreement with the crystal structure of Fe−OTf−oxalate.[13]

To assess the general feasibility of ^{17}O QCT spectroscopy in studying proteins, we show in Figure 3 the calculated line width of the ^{17}O CT signal at 21.14 T as a function of τ_C using $\Delta\nu_{1/2}(CT) = 4.9 \times 10^{-3} P_Q^2/(\nu_0^2\tau_C)$. If a line width of 2 kHz is conservatively chosen as the practical detection limit, the ^{17}O QCT approach is applicable to proteins where the value of P_Q can be as large as 12 MHz. This suggests that ^{17}O QCT is suitable for studying most of the oxygen-containing functional groups in large protein−ligand complexes (>30 kDa).

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Supporting Information Available: Graphs showing the field dependence of ^{17}O CT signals. This material is available free of charge via the Internet at http://pubs.acs.org.

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