Solid-state ^{17}O NMR studies of enzyme-inhibitor complexes

Jianfeng Zhu, Joanna Leake, and Gang Wu

Department of Chemistry, Queen's University, Kingston, Ontario

gang.wu@chem.queensu.ca

In recent years, we have successfully demonstrated that it is possible to obtain high-quality solid-state ^{17}O (spin-5/2) NMR spectra for biological macromolecules [1, 2]. To have regular access to the 900 MHz spectrometer at the National Ultrahigh-Field NMR Facility for Solids has played a critical role in these developments. In 2010-11, we have begun to shift our attention to tackle challenging but more biologically relevant problems.

Our immediate interest is to use solid-state ^{17}O NMR to probe intermediates or transition-state analogs in several enzymatic reactions. One recent example from this project involves a solid-state ^{17}O NMR study of the complex between N-tosyl-L-lysine chloromethyl ketone (TLCK) and trypsin (a 24 kDa serine protease). TLCK belongs to a class of compound known as chloromethyl ketones (CMKs) and is an irreversible inhibitor of trypsin. As illustrated in Figure 1, the inactivation of trypsin by TLCK is due to the alkylation of the active His-46 residue by TLCK. Furthermore, TLCK is also covalently connected to Ser-195, forming a hemiketal group. More importantly, the hemiketal hydroxyl group is close to the so-called "oxyanion hole" formed by the backbone N-H groups from Ser-195 and Gly-193 residues. It has been postulated that the "oxyanion hole" provides the key stabilization for the tetrahedral intermediate during the catalytic process. Thus, the TLCK-trypsin complex may be considered as models of enzyme-mediated transition-state stabilization. Previous ^{13}C NMR studies [3] suggest that the value of pKa for the hemiketal hydroxyl ionization is about 8, which is several pKa units lower than that of a normal hemiketal hydroxyl group. This observation has long been used

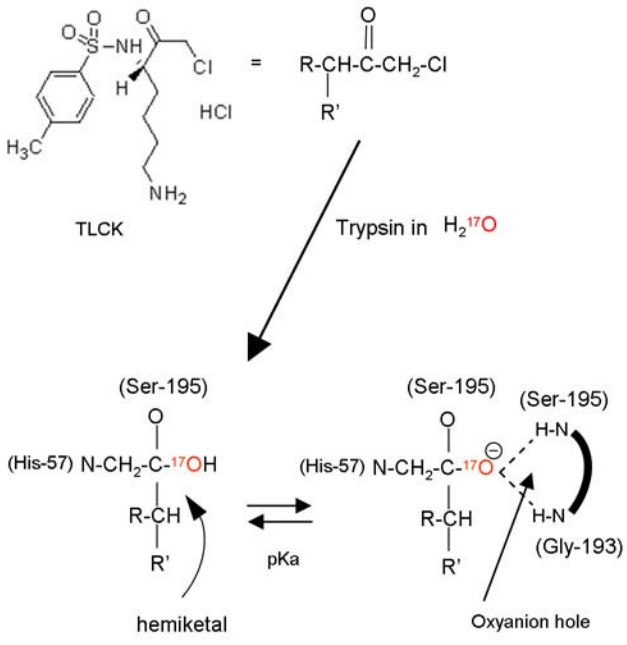

Figure 1: Molecular structure of TLCK and its complex with trypsin. Also shown are the ionization (acid/base) equilibrium of the hemiketal hydroxyl group and the location of the "oxyanion hole".

as strong evidence supporting the hypothesis regarding the role that the "oxyanion hole" plays in enzymatic reactions. As seen from Figure 1, as the oxygen atom is at the centre of action, ^{17}O NMR would be an ideal technique for probing this ionization equilibrium.

Figure 2 shows the experimental and simulated ^{17}O MAS spectra of TLCK-trypsin obtained at 21.14 T. Two groups of signals were observed. The signal at δ_{iso} = 278 ppm can be assigned to the carboxyl groups on the protein sidechains (either Asp and Glu residues). It is quite surprising that these carboxyl groups can undergo oxygen exchange under the mild condition used in the protein sample preparation. This process needs to be further investigated. Most interestingly, the signal having δ_{iso} = 67 ppm, C_Q = 7.8 MHz, and η_Q = 0.8 is clearly due to the hemiketal hydroxyl group. This is the first time that this type of functional group is detected by solid-state ^{17}O NMR [1]. This preliminary result is quite encouraging. Our next step is to obtain solid-state ^{17}O NMR spectra for the TLCK-trypsin complex prepared at various pH values.

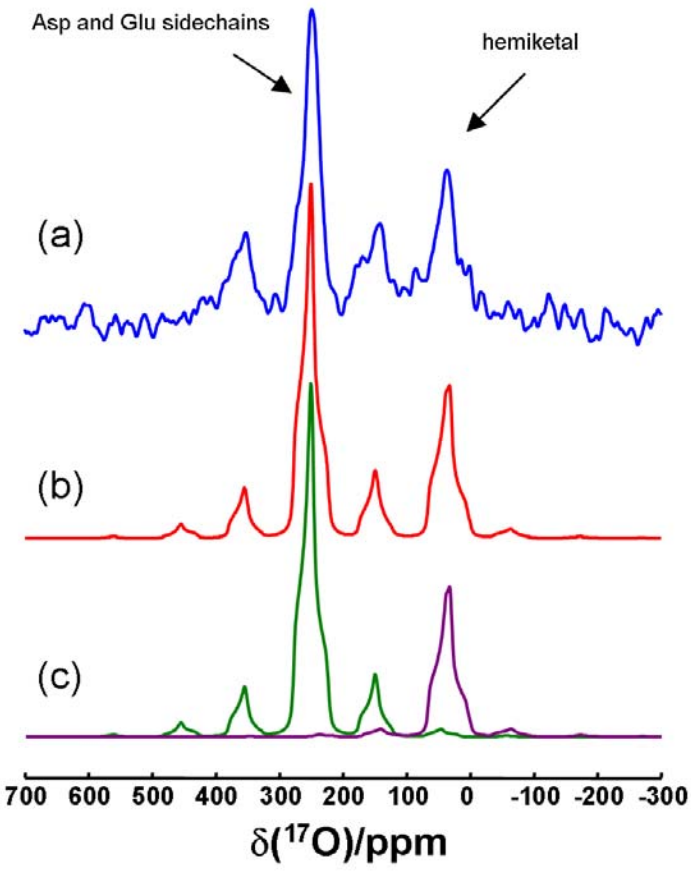

Figure 2: (a) Experimental and **(b, c)** simulated ^{17}O MAS spectra of TCLK-trypsin at 21.14 T. Solid protein was packed into a Si_3N_4 rotor. The sample spinning frequency was 12.5 kHz. A total of 2,092,000 transients were recorded with a recycle delay of 30 ms (the total experimental time was 21 hrs). The spectra were obtained with assistance from Drs. Eric Ye and Victor Terskikh at the National Ultrahigh-Field NMR Facility for Solids.

In summary, we have continued to make progress in expanding the realm of solid-state ^{17}O NMR applications in the study of biological systems. Our next step is to focus on preparation and solid-state ^{17}O NMR detection of acyl-enzyme intermediates.

References

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[3] J.P.G. Malthouse, N.E. Mackenzie, A.S.F. Boyd, and A.I. Scott, *J. Am. Chem. Soc.* **105** (1983) 1685.