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EXAFS Studies of Non-Heme Iron Enzymes and Zn- Wilson Proteins

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ABSTRACT: X-ray absorption spectroscopy has been very useful for characterizing the structures of metalloenzymes, their reaction intermediates, and metastable inorganic species that serve as models thereof. In the past year at NSLS, we have obtained EXAFS data on (a) a fatty acid Δ^9 -desaturase in its reduced, peroxo, and peroxo-cycled states, (b) the peroxo intermediate obtained at the initial stage of ferritin core assembly in *E. coli*, (c) synthetic high valent iron-peroxo and iron-oxo complexes relevant to the oxygen activation mechanism of the nonheme iron enzymes, and (d) Zn bound to the copper transporting ATPase associated with Wilson disease with different metal-protein ratios. Our XAS data analysis shows that there is a significant change in the Fe-Fe distance as the Δ^9 -desaturase goes through its redox cycle. Specifically, the Fe-Fe distance goes from 4.2 Å in the reduced enzyme to 3.8 Å in the peroxo intermediate and to 3.1 Å in the peroxo-cycled form. These distances respectively correspond to dicarboxylate-bridged diiron core structures with no other bridge, a 1,2-peroxo bridge, and an oxo bridge. Interestingly the EXAFS spectrum of the Δ^9 -desaturase peroxo intermediate is quite different from that of the frog ferritin peroxo intermediate, despite the fact that the two intermediates have similar Mössbauer and Raman spectra. The frog ferritin intermediate has been reported by Penner-Hahn and co-workers to have an 2.5 Å Fe-Fe distance, which requires a very different diiron core structure. We have also carried out a preliminary analysis of the peroxo intermediate of *E. Coli* ferritin, which does not show a short Fe-Fe distance as well. Clearly the inconsistency of the EXAFS results for these three peroxo intermediates, which may be expected to have similar core structures, should be resolved in future experiments.

We have obtained EXAFS data from three metastable iron species that may serve as models for putative intermediates in the catalytic cycles of nonheme iron enzymes. The first is a low-spin Fe(III)-OOH complex, the second is a side-on peroxo-iron (III) complex, and the third is an Fe(III)-O-Fe(IV)=O complex. These three complexes represent the first of each type to be studied by EXAFS. The EXAFS analysis provides the first metrical data into the metal coordination spheres of these complexes and confirms the structures proposed for these complexes.

In our study of the copper transporting ATPase associated with Wilson disease, we have found that Zn(II) binds to a different site from Cu(I). Cu(I) binds to two cysteines in all Cu: protein stoichiometries, but Zn binds mostly to sites that have O/N ligands. Only at high Zn: protein ratios is some binding to cysteine observed.