Significantly Shorter Fe–S Bond in Cytochrome P450-I is Consistent with Greater Reactivity Relative to Chloroperoxidase

C-H bond activation is often considered the "holy grail" of inorganic chemists, as the ability to specifically activate C-H bonds would be one of the most used transformations in all of chemistry. Cytochrome P450s (P450s) are thiolate ligated heme proteins that are often referred to as nature's detoxifiers and can do this difficult C-H bond activation with ease. P450s are known to hydroxylate C-H bonds on the order of about 98-100 kcal/mol. However, similar thiolate-ligated heme proteins, such as chloroperoxidase (CPO), cannot do this same chemistry. CPO can only hydroxylate already activated (weaker) C-H bonds with strengths less than about 91 kcal/mol. Until compound I in P450s (the active intermediate), was captured and characterized in high yield in 2010, chloroperoxidase had long been used as a model for P450 chemistry because of its similar function and because the reaction intermediates were easier to trap and study in high yields and concentrations.[1,2,3] The current study now details differences between CPO and P450 using variable temperature Mössbauer and x-ray absorption spectroscopy at Beam Line 7-3 at SSRL.

Briefly, the accepted mechanism for C-H bond activation in P450s begins with ferric enzyme binding substrate. Next, the enzyme is reduced to ferrous and binds dioxygen, forming a ferric superoxide complex, followed by reduction to a ferric peroxo species. This species is protonated to make the ferric hydroperoxo complex. Adding another proton cleaves the O-O bond, forming compound I and water. Compound I is an iron(IV) oxo species with a ligand-based radical which then abstracts hydrogen from the substrate to yield compound II and a substrate radical, which recombine to form hydroxylated product and ferric enzyme. Compound II is an iron(IV) hydroxide species. The strength of the O-H bond formed in compound II is directly related to the strength of the C-H bond that can be broken.[4]

In the past, one hypothesis was that CPO could not perform the same chemistry as P450 simply because the same substrates could not access both active sites. However, in this study the authors have shown that CPO can react with 5-hexenoic acid but cannot react with 5-haxanoic acid while P450 can react with both. This implies that there is an inherent difference in reactivity between compound I in P450 (P450-I) and CPO (CPO-I) and that it is not based on steric hindrances in the active site.

Using differences in how the Mössbauer spectrum of each compound changed with temperature this study determined that the exchange coupling (|J|) is 30% larger in P450-I than in CPO-I. This increase could be due to a shorter Fe-S bond or increased spin density of the radical on the thiolate ligand in comparison to CPO-I. To determine if this difference was from the Fe-S bond length, samples of P450-I and CPO-I were analyzed at Beam Line 7-3 at SSRL.

Extended x-ray absorption fine structure (EXAFS) studies on multiple sets of samples revealed that the Fe-S bond in P450-I was in fact 0.09 Å shorter than that in CPO-I, figure 1. This shorter Fe-S bond increases the electron donation into the ferryl π* orbital. This added donation weakens and slightly lengthens the Fe=O bond. This elongation stabilized the O-H bond formed in compound II (the next intermediate in the P450 cycle). This can be thought of as lowering the activation barrier for C-H bond activation in P450s, figure 2. One explanation for why the Fe-S bond is shorter in P450 than is CPO is that there are fewer hydrogen bonds to the sulfur in P450 than in CPO allowing for the shortening of the Fe-S bond.

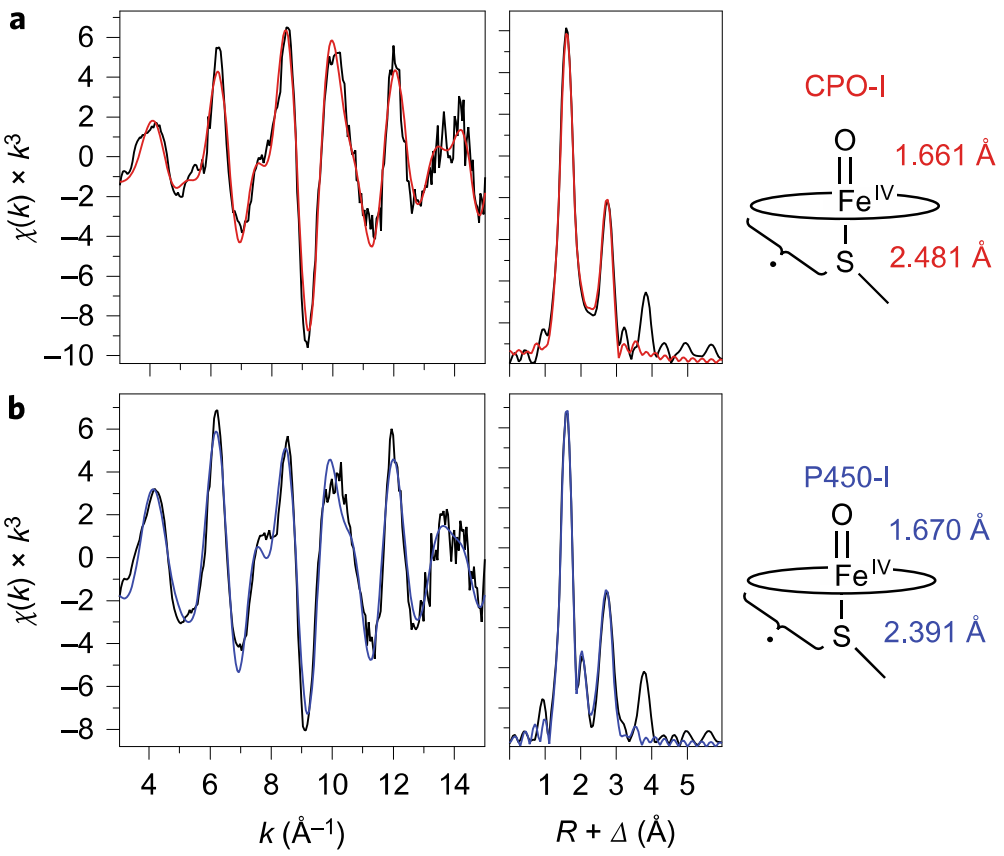

Figure 1. Top (a) CPO-I, bottom (b) P450-I. Left is raw EXAFS data and Fourier transforms in black and best fits in red and blue respectively. Distances on the right represent the average of multiple sets of samples for each compound. This shows a shorter Fe-S bond in P450-I as well as a slightly longer Fe=O bond compared to CPO-I.

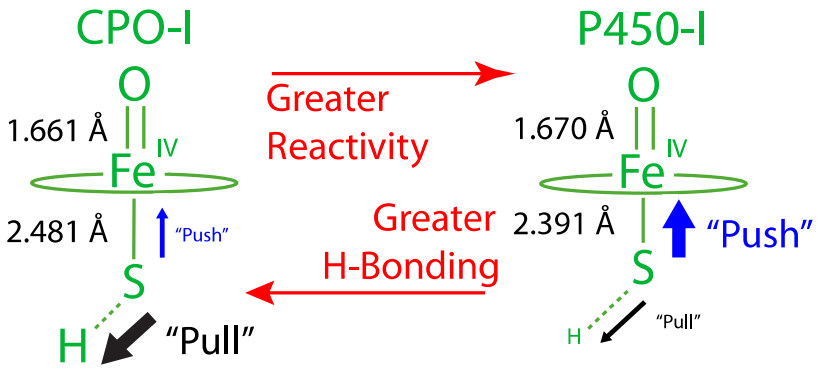

Figure 2. Stick figures illustrating the differences in hydrogen bonding, bond distances, and reactivity between CPO-I and P450-I.

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