Iron(IV)hydroxide pK_a and the Role of Thiolate Ligation in C–H Bond Activation by Cytochrome P450

Cytochrome P450s (P450s) are a family of monooxygenase enzymes that are nearly ubiquitous in nature. P450s are often described as biological blowtorches due to their incredible oxidizing power:[1] They can hydroxylate C–H bonds of about 98-100 kcal/mol. P450s are responsible for the phase I metabolism of 75% of all pharmaceuticals *in vivo* and are thusly referred to as nature's detoxifier.[2] This study focuses on how a protein can perform such difficult, oxidizing reactions without damaging its own fragile protein superstructure. A full understanding of the factors that govern P450 chemistry have wide implications not only for the medical and biological fields but also in synthetic applications.

Briefly, the generally accepted mechanism for C–H bond activation by P450s starts with the ferric enzyme binding a substrate and converting to a high spin species. Next, the enzyme is reduced to its ferrous state in which dioxygen binds, forming a species best described as a ferric superoxide complex. This step is followed by the reduction to a ferric peroxo species. This species, in turn, is protonated to form a ferric hydroperoxo complex. Addition of another proton cleaves the O–O bond, forming compound I – an iron(IV) oxo species with a ligand-based radical – and water. Compound I then abstracts hydrogen from the substrate to yield compound II – an iron(IV) hydroxide species – and a substrate radical which recombine to form the hydroxylated product and ferric enzyme.[3]

One hypothesis of how P450s can perform such difficult reactions without damaging their fragile protein superstructures is their unique thiolate ligation. The thought is that this axial thiolate ligand (as compared to histidine-ligated heme systems) allows for an increase in the pK_a of compound II (an Fe(IV)–OH called the "rebound intermediate"), which correspondingly lowers the reduction potential of compound I (an Fe(IV)=O, the active intermediate in P450 chemistry). This results in a decreased driving force for auto-oxidation (destruction) of fragile residues in the delicate protein.

Through a combination of rapid freeze quench and a variety of spectroscopies including Mössbauer, stopped flow UV/Vis, and x-ray absorption (Beam Line 7-3 at SSRL), the researchers were able to pinpoint the pK_a of the ferryl species in compound II to be 11.9, which is elevated in comparison to the pK_a of compound II in histidine-ligated heme systems that cannot perform the same difficult reactions as P450s.

Mössbauer spectroscopy showed the formation of a new species at elevated pH. The parameters of this species (narrower quadrupole splitting) were consistent with the formation of an authentic Fe(IV) oxo species. Stopped flow UV/Vis spectroscopy also showed reversible spectral changes with multiple isosbestic points indicating that these pH-driven changes were resulting from deprotonation of the hydroxo species with increasing pH. Data from Mössbauer and stopped-flow UV/Vis experiments were used to construct pH titration curves that indicate a pK_a of 11.9, figure 1. Using Marcus theory, it was determined that this elevated pK_a results in a greater than 10,000 fold reduction in the rate constant of nonproductive oxidations (i.e. of protein residues) versus histidine-ligated systems. This eliminates these autoxidation reactions as competition for substrate reactions. It was concluded that these results could explain why there are no histidine-ligated P450s.

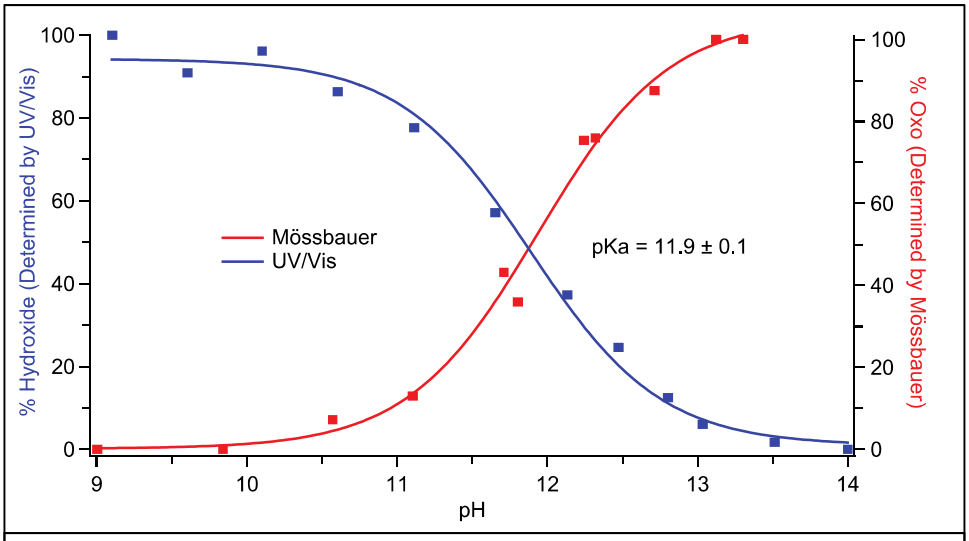

Figure 1. Titration curves made from data collected with Mössbauer (red) and stopped-flow UV/Visible (blue) spectroscopies show the same pK_a value of 11.9 for compound II in cytochrome P450.

XAS was used to confirm that the low pH and high pH species were in fact the hydroxide and oxo forms, respectively. The results are shown in figure 2. Both high and low pH species had Fe K-edges that lie 1.5 eV above the ferric edge, which is consistent with an Fe(IV) species. However, the low pH species has an Fe–O bond distance of 1.84 Å indicative of an Fe(IV)–OH species while the high pH species had and Fe–O bond of 1.68 Å indicative of an Fe(IV)=O species.

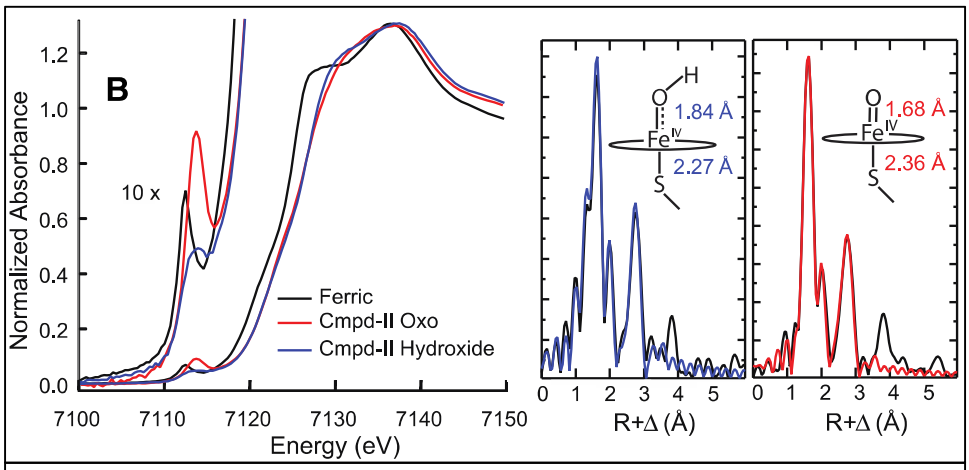

Figure 2. Left: Rising Fe K-edges of ferric compound II in its oxo and hydroxide forms. A 1.5 eV edge shift between ferric and Fe(IV) species can be seen as well as differences in pre-edge features between the Fe(IV) oxo and hydroxide. Right: EXAFS Fourier transforms of the hydroxide (left) and oxo (right) species highlight the difference between Fe(IV)=O and Fe(IV)-OH.

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