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Glimpsing the Critical Intermediate in Cytochrome P450 Oxidations

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Almost all living organisms make use of atmospheric oxygen O₂ for respiration. Another critical role of oxygen is in the partial oxidation of organic compounds, which can remove toxins or create compounds for specialized tasks, such as signaling. The enzymes that accomplish this task, the oxygenases, were discovered by Hayaishi in 1955, and include the dioxygenases, which insert both O atoms into one or more substrates, and the monooxygenases, which incorporate only one of the O atoms into a substrate and reduce the other to water. The largest subset of monooxygenases are the cytochrome P450s (P450s)(1), which catalyze a difficult transformation, the oxidation of aliphatic carbon-hydrogen bonds. The structure of a key intermediate in this reaction, an iron heme group bearing a single oxygen atom has eluded chemists for three decades. On p. xxx of this issue, Rittle and Green (2) used Mossbauer and electron paramagnetic resonance spectroscopy, in concert with optical absorption spectroscopy, to define the “Compound I” in P450 catalysis as a ferryl oxygen atom bound to Fe(IV)-porphyrin cation radical.

These heme-containing enzymes were discovered in the 1960s where even in crude tissue preparations, their optical spectroscopic signature—strong absorption at wavelengths of ~450 nm—stood out. The P450s are now known to be one of the largest super-families of proteins, with more than 14,000 genes identified in diverse organisms from all life forms (3). They serve in two broad functional roles. In catabolic pathways, they initiate the breakdown of environmental compounds, either for use as food or as a means of detoxification. They also serve as the critical steps in the synthesis of secondary metabolites that are often used in signaling. In humans, these roles are represented by the liver and kidney enzymes involved in drug metabolism and in the biosynthesis of steroid hormones in the ovaries, testes and adrenals. Thus, the P450s have received considerable attention from pharmacologists, toxicologists, biochemists and chemists since their discovery.

The active site of the P450 protein contains a heme prosthetic group, the same entity as found in the oxygen transport and storage proteins hemoglobin and myoglobin. Like these common heme proteins, P450s bind atmospheric O₂ when the heme iron is in the reduced, or ferrous, state. Although the globins and P450s share a common reactivity with O₂ to form an end-on O₂-iron bound state, the distinctive chemical reactivity of the P450 arises from their ability to accept two electrons from a redox partner and catalytically break the O-O bond, using the extra reducing equivalents to produce a single water molecule. Oxidizing a recalcitrant hydrocarbon compound via incorporating the remaining second oxygen atom

into the substrate is a difficult chemical transformation that poses several questions. What is the electronic structure of the remaining oxygen atom bound to heme, and how does it catalyze the difficult task of transferring this oxygen atom to a substrate molecule? There must be an extremely “hot flame” at the heme active site to be able to oxidize unactivated hydrocarbons (see the figure).

A major breakthrough in understanding P450 chemistry was made by Groves and co-workers in the mid-1970s (4, 5). By determining the stereochemistry of oxygenated products, they inferred a stepwise process whereby a P450 heme intermediate containing a single oxygen atom initiated a hydrogen abstraction event. This reaction generated a transient substrate radical that would then recombine quickly to form the oxidized product. This two-step mechanism suggested that the reactive intermediate in P450 catalysis was an iron-oxygen adduct similar to that of the so-called “Compound I” intermediate found in the peroxidase class of enzymes. Peroxidases also cycle through various heme iron – oxygen states, but use as a substrate the two electron reduced form hydrogen peroxide. Detailed studies of peroxidases, most notably by Debrunner and colleagues (6-8), defined the electronic structure of this Compound I intermediate.

Could this Compound I state be the P450 flame and would it be possible to form it by adding peroxide or peracid to the ferric enzyme? Many investigators, beginning in the mid-1970s, used rapid reaction methods to try and generate the Compound I state by this approach, yet only fleeting glimpses of the P450 Compound I state were observed (9). A breakthrough in mechanistic investigations of P450 monooxygenases was the crystallographic determination of the ferrous oxygenated intermediate of cytochrome P450 (10). Via reduction by the X-ray beam at 100 K, it was hoped that a stabilized Compound I could be seen, but again only a hint of this critical intermediate was observed.

Using the logic that enzymes from organisms that normally function at elevated temperatures may have altered reaction rates that would favor observing this intermediate, our laboratory was able to observe an optical signature that suggested Compound I formation in the thermophilic P450 CYP119 (11). However, only a relatively small fraction of the heme protein could be caught in this state, perhaps due to the presence of an endogenous substrate at the active site that quickly reacted with the hot oxidant. The optical absorption spectra of this intermediate, while indicative of a Compound I state, was not sufficient proof of the detailed electronic structure or reactivity.

By careful purification of the P450 CYP119 protein, Rittle and Green were able to isolate the Compound I state in high yield. By characterizing its electronic structure using a variety of spectroscopies, they found that the P450 Compound I was analogous to that previously seen in the peroxidases. Importantly, these authors not only evaluated the detailed electronic structure of this iron-oxygen adduct, but also demonstrated the chemical competence and high activity of this P450 state in converting substrates to oxygenated products.

Now that Compound I has been definitely observed in the cytochrome P450s and its immense reactivity in oxidizing carbon substrates quantified, many doors are opened that will lead to an understanding as to how Nature uses the structure of heme proteins to utilize

and control the reactivity of O₂. Not only are these enzymes important targets for therapeutic intervention, understanding the chemical process by which a recalcitrant substrate can be functionalized would provide key insight into harnessing these biotransformations for such useful purposes as generating alternate energy sources.

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Ready to oxidize

The cytochrome P450 enzymes can oxidize normally unreactive carbon-hydrogen bonds. Their iron heme site binds atmospheric dioxygen (O_2), catalytically breaking the O-O bond and reducing one O atom to form water: The other O atom (shown schematically as the oxidizing flame in the P450 active site) inserts into carbon-hydrogen bonds. This key intermediate resembles “Compound 1” first seen in peroxidases.