Reactivity of Transient Cytochrome P450 Oxygen Intermediates

John H. Dawson
Department of Chemistry and Biochemistry and the School of Medicine
University of South Carolina, Columbia, SC 29208 USA

Cytochrome P450 is a versatile heme-containing oxygenase that transfers oxygen atoms from dioxygen to a wide range of organic substrates. The P450 reaction cycle involves substrate binding to the ferric enzyme followed by reduction and oxygen binding to give the oxyferrous state. Second electron addition yields a peroxoferric intermediate, protonation of which generates a hydroperoxoferric state. Finally, protonation and loss of water yields an oxo-iron(IV) porphyrin radical (P450 Compound I).

We have investigated the reactivity of these four oxygen-containing intermediates using rapid kinetics and mechanistic enzymology. With David P. Ballou, Mary Glascock and Tatyana Spolitek, we have used rapid-scan stopped-flow to study the reaction of oxyferrous P450-CAM with reduced putidaredoxin (Pdx) and have observed a novel 'perturbed oxyferrous' species that may be a direct spectral signature of the Pdx effector role during P450-CAM catalysis. Further, we have optimized conditions for the single mix reaction of meta-chloroperbenzoic acid with substrate-free ferric P450-CAM to form P450 Compound I so as to allow for more complete spectral characterization of the latter.

Although Compound I is the ultimate O-atom transfer catalyst, the peroxo- and hydroperoxoferric forms have been proposed as secondary oxidants. With Shengxi Jin, Roshan Perera and Thomas A. Bryson, we have probed their reactivity using T252A P450-CAM, a mutant that does not hydroxylate camphor, and therefore does not efficiently form P450 Compound I. However, it still accepts electrons to form hydrogen peroxide, presumably via the same two intermediates. Thus, it is the ideal mutant to test whether either of these two states are capable of oxygenating substrates. We have synthesized a series of camphor analogues to examine the mechanism of olefin epoxidation, ether O-dealkylation, amine N-dealkylation, and thioether S-dealkylation. These studies probe the involvement of second oxidants in P450-catalyzed O-atom transfers.

(Support: NIH GM 26730. Thanks to Stephen G. Sligar for the T252A expression system.)