# Porphyrin Iron(III) Mixed Function Oxidases: An Evolutionary Endpoint for Transition Metal(III) Reactions with Oxygen Donors

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Peroxidases, catalases, and cytochrome P-450 enzymes have in common iron(III) protoporphyrin-IX as a cofactor. The reactions catalyzed by these enzymes can be, for the most part, duplicated by use of transition metal(III) porphyrins. Porphyrins serve admirably well as conjugated and rigidly planar ligands that prevent other than transformations of the ligated metal moiety at axial positions. The adjacent and distal axial positions serve to separate the enzyme-bound (distal) ligand from the reactive face (adjacent) of the iron(III). These features are not required, however, to mimic the chemical conversions that are catalyzed by peroxidases, catalases, and cytochrome P-450 enzymes. Indeed, other simple ligands may be used with a number of transition metals (cf. Samsel et al. 1985). Porphyrins were selected as components of living organisms at an early time, as attested to by the observation that petroleum is a rich source of porphyrins.

In the oxidation reactions catalyzed by peroxidases, catalases, and cytochrome P-450, an oxidant is initially formed that might be described as a "porphyrin-iron-oxene" compound. The formation of the porphyrin-iron-oxene compound occurs by reaction of the iron(III) porphyrin with a reagent that may be symbolically represented as Z-OH. With horseradish (HR) peroxidase (distal axial ligand a histidine imidazole) and catalase (distal axial ligand a tyrosine hydroxyl function), Z-OH represents HO-OH (for both enzymes) and, in addition, alkyl-O-OH (for the peroxidase). The porphyrin-iron-oxene compound formed in these reactions is known as compound I. Much evidence exists to support the structure of compound I as being an iron(IV)-oxo porphyrin $\pi$-cation radical (Eq. 1, where X is imidazole or tyrosine-$O^-$, cf. Moss et al. 1969; Dolphin et al. 1971; Schultz et al. 1979; Roberts et al. 1981).

$$(\text{Porph})\text{Fe}^{\text{III}}(\text{X}) + \text{Z-OH} \rightarrow ({}^{+}\cdot\text{Porph})(\text{X})\text{Fe}^{\text{IV}}(\text{O}) + \text{ZH} \quad (1)$$

With cytochrome P-450 enzymes (distal axial ligand a thiolate of cysteine) Z-OH may represent a host of oxidants (such as HO-OH, alkyl-O-OH, RC(O)O-OH, arene-IO, aniline N-oxides), but under physiological circumstances it represents $2e^- + O_2$ + one or two protons (building blocks for peroxide). Nothing is known about the structure of the porphyrin-iron-oxene intermediate formed with cytochrome P-450 because its formation (1) requires the presence of the substrate bound at the active site and (2) is rate-limiting in the oxidation of the substrate. When $R\text{-}S^-$ species are ligated to metal ions of higher oxidation states ($M^{n+1}$), they undergo $1e^-$ oxidation, and a covalent bond is formed between $RS\cdot$ and $M^n$ (Sawyer et al. 1986). It is safe to assume that the structure of the porphyrin-iron-oxene species of cytochrome P-450 reflects this feature so that the second $1e^-$ deficiency resides with the thiolate ligand rather than in the porphyrin ring system as a porphyrin $\pi$-cation radical (Eq. 2). Chemical systems that incorporate such features have not been prepared.

$$(\text{Porph})(\text{CysS}^-)\text{Fe}^{\text{III}} + \text{Z-OH} \rightarrow (\text{Porph})(\text{CysS}\cdot)\text{Fe}^{\text{IV}}(\text{O}) + \text{ZH} \quad (2)$$

Aside from the structures of the porphyrin-iron-oxene species of HR peroxidase, catalase, and cytochrome P-450, a complete description of these systems requires a knowledge of the mechanisms of formation of the porphyrin-iron-oxene species and a description of their reactions with substrates. The electrochemical stepwise oxidation of iron(III)-hydroxy porphyrins to iron(IV)-oxo porphyrins (compound II oxidation level) and iron(IV)-oxo porphyrin $\pi$-cation radicals (compound I oxidation level) have been investigated (Calderwood et al. 1985; Lee et al. 1985; Calderwood and Bruice 1986). Also, the second-order rate constants have been determined for "oxene" equivalent transfer from a series of percarboxylic acids and alkyl hydroperoxides (YOOH) to meso-tetrakis(phenyl)porphinato transition metal(III) chlorides ([TPP]$Cr^{III}$[Cl], [TPP]$Fe^{III}$[Cl], [TPP]$Mn^{III}$[Cl], [TPP]$Co^{III}$[Cl]) (Lee and Bruice 1985, 1986; Yuan and Bruice 1985a,b, 1986; Balasubramanian and Bruice 1987). All reactions involving oxene equivalent transfer from percarboxylic acids were shown to involve heterolytic O–O bond scission (Eq. 3), and the logs of the second-order rate constants $k_{YOOH}$ were determined to be linearly related to the $pK_a$ of the leaving carboxylic acid (YOH).

$$\text{R-C(O)-O-O-H} + ([\text{TPP}]\text{M}^{\text{III}}[\text{Cl}]) \xrightarrow{k_{YOOH}} \text{R-C(O)-O-H} + ([\text{TPP}]\text{M}^{\text{V}}[\text{Cl}][\text{O}]) \quad (3)$$

The slopes of plots ($\beta_{lg}$) of log $k_{YOOH}$ versus $pK_a$ of YOH were found to be markedly negative (−0.35 to −1.25), which is expected for such a polar reaction. When $k_{YOOH}$ values for alkyl hydroperoxides were determined and included in the plots of log $k_{YOOH}$ versus $pK_a$ of YOH for percarboxylic acids, it was found that a single linear free-energy line was obtained with

(TPP)$Cr^{III}$(Cl). Thus, alkyl hydroperoxides most likely react with (TPP)$Cr^{III}$(Cl) to provide (TPP)$Cr^{V}$(O)(Cl) by heterolytic O–O bond scission. With imidazole-ligated meso-tetrakis(phenyl)porphinato manganese(III) chloride ([TPP][ImH]$Mn^{III}$[Cl]) as well as (TPP)$Fe^{III}$(Cl) and (TPP)$Co^{III}$(Cl), there was found to be a break in the slope of the linear free-energy plots with the most electron-rich alkyl peroxides residing on a line of very small negative slope. This has been interpreted as a change of mechanism from heterolytic to homolytic O–O bond scission (Eq. 4).

$$\begin{aligned}
&\text{Alkyl-O-OH} + ([\text{TPP}]\text{Fe}^{III}[\text{Cl}]) \xrightarrow{K_{YOOH}} ([\text{TPP}]\text{Fe}^{IV}[\text{Cl}][\text{OH}]\text{Alkyl-O}\cdot)\\
&([\text{TPP}]\text{Fe}^{IV}[\text{Cl}][\text{OH}]\text{Alkyl-O}\cdot) \xrightarrow{\text{fast}} ([^{+}\cdot\text{TPP}]\text{Fe}^{IV}[\text{O}][\text{Cl}]) + \text{Alkyl-OH}\\
&([\text{TPP}]\text{Fe}^{IV}[\text{Cl}][\text{OH}]\text{Alkyl-O}\cdot) \xrightarrow{\text{fast}} (\text{TPP})\text{Fe}^{III}(\text{Cl}) + \text{Alkyl-O}^{+} + \text{HO}^{-}\\
&([\text{TPP}]\text{Fe}^{IV}[\text{Cl}][\text{OH}]\text{Alkyl-O}\cdot) \xrightarrow{\text{fast}} ([\text{TPP}]\text{Fe}^{IV}[\text{Cl}][\text{OH}]) + \text{Alkyl-O}\cdot
\end{aligned} \tag{4}$$

Studies have been initiated to determine the role of general catalysis in the transfer of an oxene equivalent from Z-OH species to iron(III) and manganese(III) porphyrins (Bruice et al. 1986; Zipplies et al. 1986; Balasubramanian et al. 1987). These and the investigations that will follow are predicated on the proposed role of an imidazolyl group of a histidine residue as a general base at the active site of peroxidases. These investigations are being carried out in water using water-soluble metalloporphyrins that do not form $\mu$-oxo dimer. In the reaction of the manganese(III) porphyrin with $H_2O_2$ (Eq. 5, where X=$H_2O$, $HO^-$, or imidazole) the rate-controlling oxene equivalent transfer is not subject to general catalysis (by any of the oxygen-centered or nitrogen-centered bases and acids investigated) at any pH.

$$\begin{aligned}
&(\text{Porph})\text{Mn}^{III}(\text{X}) + \text{H}_2\text{O}_2 \rightarrow (\text{Porph})\text{Mn}^{V}(\text{O})(\text{X}) + \text{H}_2\text{O}\\
&(\text{Porph})\text{Mn}^{V}(\text{O})(\text{X}) + \text{H}_2\text{O}_2 \rightarrow (\text{Porph})\text{Mn}^{III}(\text{X}) + \text{O}_2 + \text{H}_2\text{O}
\end{aligned} \tag{5}$$

When an iron(III) porphyrin is used as catalyst, the single nitrogen base buffer employed (collidine/collidine$\cdot H^+$) is a catalyst, but a number of oxygen bases and acids are not. Much remains to be done to gain an understanding of the role of general catalysis in these systems.

A question that begs an answer concerns the rates of oxygen transfer from porphyrin-iron-oxene and porphyrin-manganese-oxene species to organic substrates. The two reactions of interest are oxygen insertion (Eq. 6) and epoxidation (Eq. 7). These are the most interesting reactions catalyzed by cytochrome P-450.

$$(^{+}\cdot\text{Porph})\text{Fe}^{IV}(\text{O})(\text{X}) + \text{RCH}_3 \rightarrow (\text{Porph})\text{Fe}^{III}(\text{X}) + \text{RCH}_2\text{OH} \tag{6}$$

$$(^{+}\cdot\text{Porph})\text{Fe}^{IV}(\text{O})(\text{X}) + \begin{array}{c}\diagdown\\ \text{C}{=}\text{C}\\ \diagup\end{array} \rightarrow (\text{Porph})\text{Fe}^{III}(\text{X}) + \begin{array}{c}\text{O}\\ \diagup\ \diagdown\\ -\text{C}-\text{C}-\\ |\quad |\end{array} \tag{7}$$

The dynamics of the reactions of Equations 6 and 7 cannot be explored with the enzyme, since the formation of the porphyrin-iron-oxene compound is rate-determining. Until the present, the same may be said of chemical systems. Meunier and co-workers (1984) introduced the use of $ClO^-$ as an oxene equivalent transfer agent and studied the epoxidation of alkenes by using a rapidly stirred biphasic system consisting of an organic phase containing manganese(III) porphyrin plus nitrogen base ligand, a phase transfer agent, and a basic aqueous phase containing LiOCl. Epoxides were obtained in high yield. Collman and co-workers (1983, 1984, 1985a,b) subsequently carried out a number of kinetic studies with the Meunier machine. From these studies they proposed that the rates of epoxidation, rather than oxene equivalent transfer to manganese(III) porphyrin, are rate-determining and that intermediate to epoxide formation there arises a metallaoxetane. Though the evidence for formation of a stable metallaoxetane intermediate has been questioned (Nakagaki et al. 1987), the facility of the oxene equivalent transfer to the metal center has not (Eq. 8a: where ImR represents a N-substituted imidazole).

$$([\text{P}]\text{Mn}^{III}[\text{ImR}])^{+} + \text{ClO}^{-} \rightarrow (\text{P})\text{Mn}^{V}(\text{O})(\text{ImR}) + \text{Cl}^{-} \tag{8a}$$

$$(\text{P})\text{Mn}^{III}(\text{OH})(\text{ImR}) + (\text{P})\text{Mn}^{V}(\text{O})(\text{ImR}) \rightarrow 2(\text{P})\text{Mn}^{IV}(\text{O})(\text{ImR}) + \text{H}^{+} \tag{8b}$$

$$(\text{P})\text{Mn}^{V}(\text{O})(\text{ImR}) + \begin{array}{c}\diagdown\\ \text{C}{=}\text{C}\\ \diagup\end{array} \rightarrow ([\text{P}]\text{Mn}^{III}[\text{ImR}])^{+} + \begin{array}{c}\text{O}\\ \diagup\ \diagdown\\ -\text{C}-\text{C}-\\ |\quad |\end{array} \tag{8c}$$

We find (Nakagaki et al. 1987) that we can prepare homogeneous solutions of rather high $ClO^-$ concentration by simply shaking an organic solvent containing a high concentration of phase transfer agent with an aqueous solution of LiOCl. The organic solvent containing the $ClO^-$ may then be mixed on a stopped-flow bench with a like solution containing metalloporphyrin plus nitrogen base ligand and alkene substrate. Without substrate, the manganese(IV)-oxo species is formed due to the reactions of Equations 8a and 8b (Fig. 1), and at high substrate concentration there is scarcely any accumulation of manganese(IV)-oxo species due to the trapping of the manganese(V)-oxo porphyrin by alkene (Eq. 8c). By monitoring the rate of formation of manganese(IV)-oxo species (and its yield) as a function of alkene concentration, the second-order rate constants associated with Equations 8a and 8c may be determined. Thus, when employing meso-tetrakis-(2,4,6-trimethylphenyl)porphinato manganese(III) hydroxide with norbornene, the rate constant for Equation 8a is $\sim 6 \times 10^5\ \text{M}^{-1}\ \text{sec}^{-1}$ and the epoxidation rate of Equation 8c is $\sim 1.2 \times 10^4\ \text{M}^{-1}\ \text{sec}^{-1}$. This procedure is being extended to include other metalloporphyrins and other alkenes.

There is presently much interest in the mechanism of alkene epoxidation by hypervalent metallo-oxo porphyrin species. In the epoxidation of an alkene, a number of other products are generally obtained. The yields

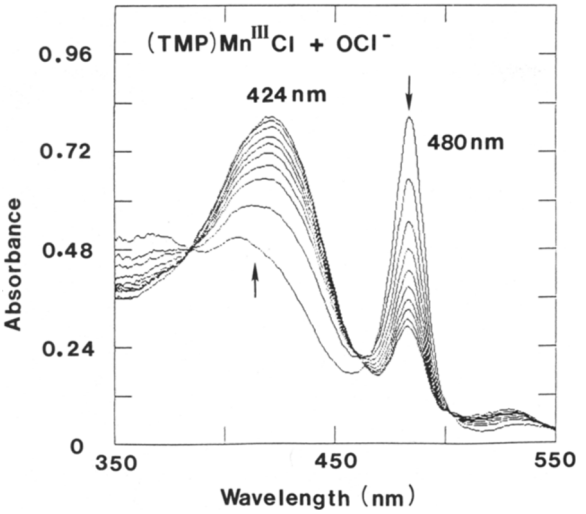
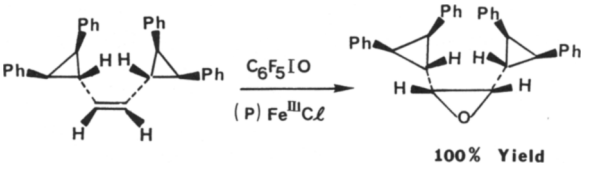



**Figure 1.** Repetitive spectral scans (10 msec between scans) using a homogeneous solution (wet $CH_2Cl_2$) showing the conversion of meso-tetrakis-(2,4,6-tetraphenyl)porphinato manganese(III) chloride (478 nm) to a manganese(IV)-oxo porphyrin species (426 nm) on reaction with $ClO^-$.

of these other products are dependent on conditions, the nature of the oxene equivalent transfer reagent, the alkene, and the metalloporphyrin. The question arises as to whether these additional products are derived from intermediates that are formed along the reaction path to epoxide. Does epoxide formation represent a multistep mechanism, or is it concerted? In Figure 2 are represented three pathways that can account for the products obtained in the epoxidation of *cis*-stilbene by $C_6F_5IO$ when catalyzed by a number of iron(III) tetraphenylporphyrins (A. Castellino and T. Bruice, in prep.). Pathway a involves the intermediacy of a porphinato iron(III)-oxo carbocation (2) that partitions between epoxide and the rearrangement products diphenylacetaldehyde (by phenyl migration), deoxybenzoin (by hydrogen migration), *trans*-stilbene oxide (by rotation about the C–C bond and ring closure), etc. In pathway b, the first step involves the formation of a caged pair consisting of a carbocation radical (1) and (Porph)$M^{IV}$(O). The reaction involves an outer sphere $1e^-$ transfer from the alkene to the hypervalent metallo-oxo porphyrin. Pathway c is consistent with the proposed radical character of the hypervalent metallo-oxo bond (Traylor et al. 1986). One could construct an alternate reaction scheme by having a concerted epoxidation mechanism and all other products arise as a result of competing reaction paths.

To assess the likelihood of formation of intermediate radical species, we have synthesized *Z*-1,2-bis(2',3'-diphenylcyclopropyl)ethene and examined its epoxidation (Eq. 9) by $C_6F_5IO$ with meso-tetrakis(pentafluorophenyl)porphinato iron(III) chloride catalyst (A.J. Castellino and T.C. Bruice, work in progress).




(9)

The *cis*-epoxide was formed in 80% yield based on the concentration of $C_6F_5IO$ oxene equivalent transfer reagent employed. The rate constant for the cyclopropylcarbinyl radical → allyl carbinyl radical rearrangement of Equation 10 has been clocked as $10^9$ $sec^{-1}$ (Mathew and Warkentin 1986).

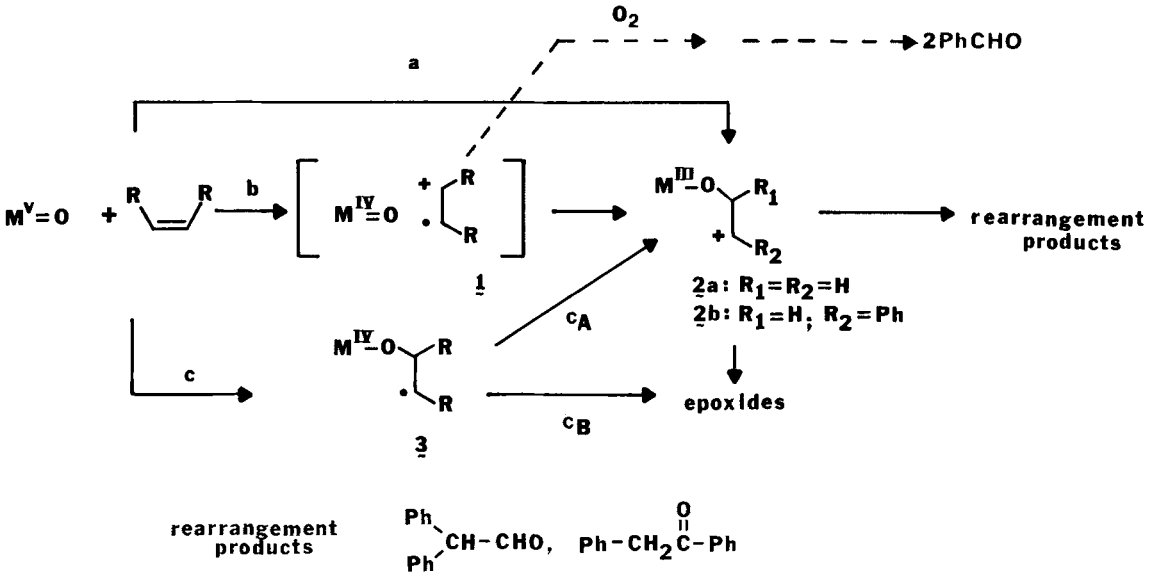
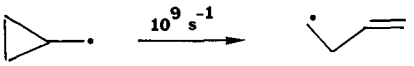
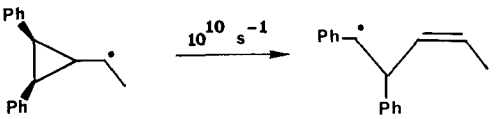



**Figure 2.** Plausible stepwise mechanisms for oxygen insertion into alkenes. $M^V$=O symbolically represents the formal state of the metalloporphyrin catalyst after oxene equivalent transfer. No structural inference is to be made from this representation.




(10)

Though not verified experimentally, it is our anticipation that the rearrangement of Equation 11 should have a clocked time of about $10^{10}$ $sec^{-1}$.




(11)

If this proves to be so, then the theoretical yield of *cis*-epoxide obtained for the reaction of Equation 9 would require that any radical intermediate present would have a half-life of about $10^{11}$ $sec^{-1}$. Such a result would make questionable the radical cation (1) and iron-oxo carbinyl (3) intermediates of Figure 2.

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