Bioinorganic Chemistry

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A Biomimetic Ferric Hydroperoxo Porphyrin Intermediate**

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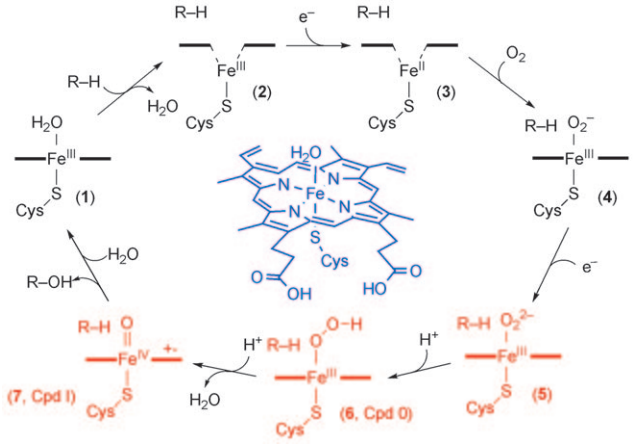
Heme enzymes, such as cytochrome P450 (CYP 450), peroxidase, catalase, heme oxygenase, and nitric oxide synthase, catalyze a diverse array of important metabolic transformations that require the binding and activation of dioxygen.[1] Our understanding of dioxygen activation chemistry by heme enzymes, especially the structures of reactive intermediates and the mechanistic details of dioxygen activation and oxygenation reactions, has improved greatly with the intensive mechanistic studies of the enzymes and their model compounds. For example, the catalytic cycle of dioxygen activation and oxygen-atom transfer reactions by CYP 450 has been well established (see Scheme 1).[2] The catalytic cycle starts at the resting state (**1**), at which a water molecule is bound to the sixth binding site of the iron center *trans* to the axial cysteinate ligand. When the substrate enters the binding pocket, this water molecule is released (**2**), which triggers a reduction from the reductase domain to yield a ferrous complex (**3**). Dioxygen then binds to the ferrous heme to form a ferric superoxo species (Fe^{III}–O_2^-, **4**). These steps are all relatively slow, and biochemical studies have revealed evidence and spectroscopic information on each of these species. The subsequent reduction of **4** to a ferric peroxo species (Fe^{III}–O_2^{2-}, **5**), however, is the rate-limiting step and brings the catalytic cycle into a gray zone, in which intermediates are short-lived and, as a consequence, are difficult to trap and characterize. A proton transfer then gives a ferric hydroperoxo complex (Fe^{III}–OOH, **6**), which is known as Compound 0 (Cpd 0), and a subsequent proton transfer generates a putative iron(IV) oxo porphyrin π-cation radical intermediate (**7**), which is equivalent to the Compound I (Cpd I) of horseradish peroxidase. Cpd I is believed to be the active oxidant that effects the oxygenation of organic substrates. Thus, the identification and spectroscopic and structural characterization of **7** has been the focus of research in enzymatic reactions over the past several decades. However, despite all efforts, the nature of the active oxidant remains elusive, and only indirect evidence of its existence has been found, namely through kinetic isotope effect studies and product distributions.[3] The latter observations were supported by computational modeling that has identified Cpd I as the active species of CYP 450.[4]



Scheme 1. Proposed catalytic cycle of CYP 450. The active site of the enzymes, where O_2 activation occurs, is shown in the center, and key intermediates, such as ferric peroxo (**5**), ferric hydroperoxo (Cpd 0, **6**), and iron(IV) oxo porphyrin π cation radical species (Cpd I, **7**), are highlighted in red.

In biomimetic reactions, a number of iron(IV) oxo porphyrin π-cation radicals have been synthesized and characterized with various spectroscopic techniques, and their reactivities have been extensively investigated in oxygenation reactions.[5] However, biomimetic studies have often bypassed several important steps in the catalytic cycle, such as the binding of dioxygen and the reduction and protonation steps (see Scheme 1), by reacting terminal oxidants, such as iodosylbenzene (PhIO) and *m*-chloroperbenzoic acid (*m*-CPBA), with iron(III) porphyrins (**2**) to form iron(IV) oxo

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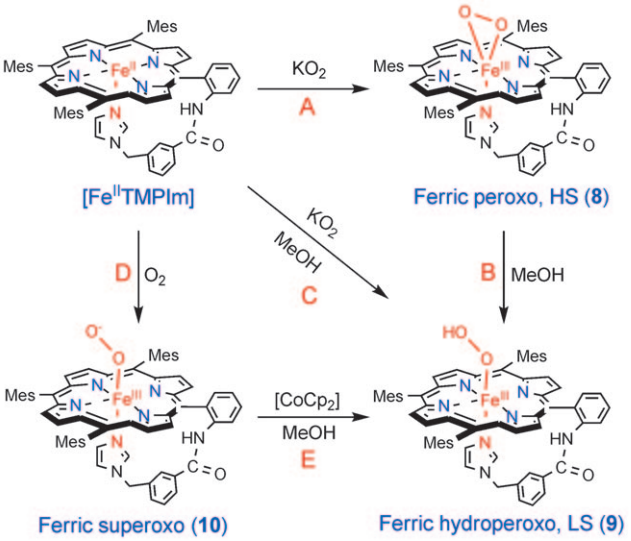
porphyrin π-cation radicals (**7**) directly. Such an approach does not mimic the natural pathway for generation of Cpd I via the important O_2-binding intermediates, such as **4**, **5**, and **6**. As a consequence, biomimetic studies of iron porphyrins to date have mainly focused on the chemistry of Cpd I.[5]

The ferric hydroperoxo intermediate (Cpd 0), which is a precursor of Cpd I, has been frequently proposed as a reactive species in heme enzymes. Indeed, several ferric hydroperoxo intermediates were generated by radiolytic reduction of the corresponding oxy ferrous heme proteins at cryogenic temperatures and were characterized spectroscopically and structurally by various spectroscopic methods, including EPR, ENDOR, Mössbauer, and resonance Raman (rR) spectroscopy.[6] In contrast, the synthesis and characterization of the ferric hydroperoxo intermediates in iron porphyrin model compounds were less successful. Only a couple of ferric hydroperoxo porphyrin compounds have been reported with absorption and EPR spectroscopic data.[7] Furthermore, to our knowledge, reactivities of ferric hydroperoxo porphyrin intermediates have never been investigated directly in nucleophilic and electrophilic reactions.

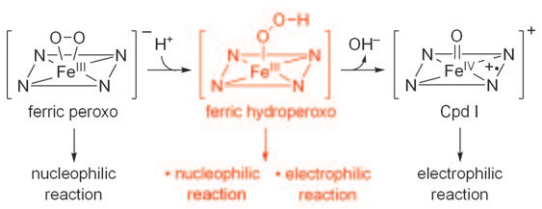
In this regard, a recent report by Naruta and co-workers is a significant advancement, as they prepared and thoroughly characterized a tractable ferric hydroperoxo porphyrin complex.[8] In this study, they showed that protonation of a side-on high-spin ferric peroxo species leads to the formation of the corresponding end-on low-spin ferric hydroperoxo intermediate. First, they used an iron imidazole-tailed porphyrin complex, [Fe^{II}(TMPIm)] (see structure in Scheme 2), to generate a high-spin ferric peroxo porphyrin complex, [(TMPIm)$Fe^{III}(O_2)]^-$ (**8**), by treating the starting iron(II) porphyrin with KO_2 in MeCN/THF at −30 °C (Scheme 2, reaction A). The intermediate **8** was characterized by electronic absorption, EPR, and resonance Raman (rR) spectroscopy. The authors provided the first reliable rR spectrum of a side-on iron(III) peroxo porphyrin intermediate (**8**; bands at 807 and 475 cm^{-1} assigned to ν(O–O) and ν(Fe–O) stretching vibrations, respectively).[9] Subsequently, addition of methanol to the solution of **8** at −65 °C afforded a low-spin ferric hydroperoxo porphyrin complex, [(TMPIm)Fe^{III}-$(O_2H)]^-$ (**9**; Scheme 2, reaction B). This intermediate, characterized by absorption, EPR, rR, and Mössbauer spectroscopy, exhibits spectroscopic data similar to those of ferric hydroperoxo intermediates identified in enzymatic reactions. Although the spectroscopic evidence supports an end-on ferric hydroperoxo structure, no direct structural information for **9**, such as X-ray crystallography or XAS/EXAFS (X-ray absorption spectroscopy/extended X-ray absorption fine structure) data, was provided in this study. Interestingly, the formation of a ferric hydroperoxo intermediate from a ferric peroxo intermediate does not occur in the absence of the axial imidazole ligand, suggesting that the axial ligand plays a crucial role in this reaction. It will, therefore, be of interest to understand the effect of the axial ligand on the conversion of ferric peroxo to ferric hydroperoxo species.[10]

The ferric hydroperoxo porphyrin intermediate **9** could also be prepared by treating [Fe^{II}(TMPIm)] with KO_2 in the presence of methanol in EtCN/THF at −75 °C (Scheme 2, reaction C). Another method for the preparation of **9** is the generation of a ferric superoxo porphyrin complex, [(TMPIm)$Fe^{III}(O_2)$] (**10**), by treating [Fe^{II}(TMPIm)] with O_2 in the presence of methanol in EtCN/THF at −75 °C (Scheme 2, reaction D) and subsequent one-electron reduction of **10** by cobaltocene ([$CoCp_2$]; Scheme 2, reaction E). Thus, the authors provided several synthetic methods for the generation of this elusive ferric hydroperoxo species.

Naruta and co-workers have reported facile methods for the biologically important ferric O_2 adducts, including the title compound (i.e., ferric hydroperoxo intermediate), along with their spectroscopic characterization. This advance allows use of these intermediates in important mechanistic studies, such as the reactivity of ferric hydroperoxo species in oxygenation reactions[11] and the mechanism of the formation of Cpd I from the ferric hydroperoxo precursor by O–O bond cleavage (see Scheme 3).[12] In the former case, with the difficulties in trapping and characterizing key active species for CYP 450 enzymes, there are still controversies on the nature of the active oxidant (or oxidants) in the catalytic oxygenation of organic substrates by CYP 450 (i.e., debate on multiple oxidants vs. two-state reactivity).[4,11] For example, derivatives of $P450_{cam}$ with site-directed mutations that supposedly blocked the second proton-transfer step in the catalytic cycle retained significant activity in substrate oxygenation reac-



Scheme 2. Intermediates, such as ferric superoxo, peroxo, and hydroperoxo species, discussed herein. HS and LS stand for high spin and low spin, respectively. Mes = 2,4,6-$Me_3C_6H_2$, Cp = C_5H_5.



Scheme 3. Reactivities of ferric peroxo and hydroperoxo species and Cpd I in nucleophilic and electrophilic reactions, and the formation of Cpd I from ferric hydroperoxo species.

tions, which led to the suggestion of the involvement of a "second electrophilic oxidant" in the oxygenation reactions (i.e., two-oxidant scenario).[13] A series of computational and biomimetic studies on analogous complexes, however, disagree with this conclusion and identify Cpd I as the active species, whereas Cpd 0 is a sluggish oxidant.[14] However, no direct reactivity studies have been conducted with ferric hydroperoxo porphyrins generated in situ; therefore, direct use of the well-characterized ferric hydroperoxo porphyrins in reactivity studies will provide clues that help us resolve the long-standing controversy on the multiple oxidants versus two-state-reactivity hypothesis.

The synthetic ferric hydroperoxo porphyrins can also be used in investigating the formation mechanism of Cpd I from the ferric hydroperoxo precursor by O–O bond cleavage. Previously, mechanistic studies for O–O bond cleavage and Cpd I formation, which is the last step of the catalytic cycle (see Scheme 1 and Scheme 3), were mainly performed with synthetic ferric acylperoxo porphyrins [(Porp)Fe^{III}–OO-C(O)Ar], as the intermediates were easily prepared and well-characterized.[15] Furthermore, mechanisms of O–O bond cleavage of biologically important oxidants, such as hydrogen peroxide and alkyl hydroperoxides, by iron(III) porphyrins were mainly investigated under catalytic conditions.[16] Therefore, Naruta and co-workers' compound **9** (Scheme 2)[8] or new analogues, being much closer in their chemical nature to the biological intermediate **6** (Scheme 1), unquestionably will lead to new results concerning O–O bond cleavage of ferric hydroperoxo porphyrins. Such studies will contribute significantly to the understanding of dioxygen activation by heme enzymes and their models.

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