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Oxoferryl Porphyrin/Hydrogen Peroxide System Whose Behavior is Equivalent to Hydroperoxoferric Porphyrin

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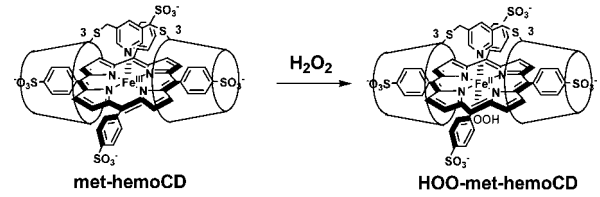
Abstract: The reaction between H_2O_2 and a pyridine-coordinated ferric porphyrin encapsulated by a cyclodextrin dimer yielded a hydroperoxoferric porphyrin intermediate, $PFe^{III}-OOH$, which rapidly decomposed to oxoferryl porphyrin ($PFe^{IV}=O$). Upon reaction with H_2O_2, $PFe^{IV}=O$ reverted to $PFe^{III}-OOH$, which was converted to carbon monoxide-coordinated ferrous porphyrin under a CO atmosphere. $PFe^{IV}=O$ in the presence of excess H_2O_2 behaves as $PFe^{III}-OOH$.

In the catalytic cycles of heme enzymes such as peroxidase, catalase, and cytochrome P450, it is assumed that hydroperoxoferric porphyrin complexes ($PFe^{III}-OOH$, known as Compound 0) serve as the precursors of oxoferryl porphyrin π-cation radicals [$(P^{\bullet+})Fe^{IV}=O$, known as Compound I], which are reactive species commonly associated with the oxidation reactions catalyzed by these enzymes.[1] It is presumed that the O−O bond of Compound 0 undergoes rapid heterolysis to yield Compound I. However, in spite of numerous attempts to detect Compound 0,[1−4] definitive evidence for the formation of $PFe^{III}-OOH$ under physiological conditions has not been reported. The present study shows that $PFe^{III}-OOH$ placed in a hydrophobic environment easily decomposes to yield oxoferryl porphyrin, $PFe^{IV}=O$, whose behavior is equivalent to $PFe^{III}-OOH$ in the presence of excess H_2O_2.

We previously found that 5,10,15,20-tetrakis(4-sulfonatophenyl)porphyrinatoiron(II) (Fe^{II}TPPS) forms a very stable 1:1 inclusion complex (hemoCD) with a per-*O*-methylated β-cyclodextrin (CD) dimer having a pyridine linker (Py3CD, CAS Registry no. 848412-43-1).[5] Ferrous hemoCD is quite stable and reversibly binds O_2 and CO in aqueous solution at room temperature.[5] Fe^{III}TPPS/CD systems exhibit unique behavior because of the isolation of the metal centers of the porphyrins from the aqueous bulk phase. Taking these findings into consideration, we employed ferric hemoCD [met-hemoCD; see the Supporting Information (SI)] as a heme enzyme model. In the present study, we sought to detect the formation of a hydroperoxoferric porphyrin complex, $PFe^{III}-OOH$, in aqueous solution at room temperature via reaction of H_2O_2 with met-hemoCD (Scheme 1).

The addition of H_2O_2 to a solution of met-hemoCD (a mixture of Fe^{III}TPPS and Py3CD in a 1:1.2 molar ratio) in aerobic phosphate buffer at pH 7.0 and 25 °C caused a gradual change in the absorption spectrum with isosbestic points (Figure 1b). The absorption maxima of met-hemoCD were observed at 418 and 571 nm and were shifted to 422 and 558 nm, respectively, upon addition of H_2O_2. The absorption spectrum of the product ($\mathbf{1^H}$) is very similar to that of an oxoferryl complex prepared by the reaction of a cationic ferric porphyrin with mCPBA.[6] After 20 min, the formation of $\mathbf{1^H}$

Scheme 1



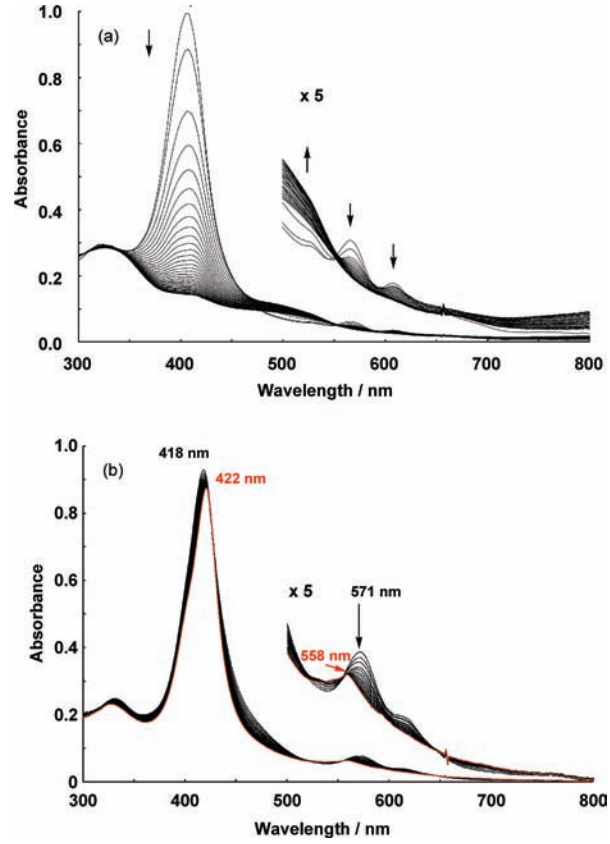


Figure 1. UV−vis spectral changes of Fe^{III}TPPS (1×10^{-5} M) during the reaction with H_2O_2 (2×10^{-4} M) in 5.0×10^{-2} M phosphate buffer in (a) the absence and (b) the presence of Py3CD (1.2×10^{-5} M) at pH 7.0 and 25 °C. The spectra were recorded at time intervals of 30 s. The spectrum shown in red was measured 20 min after the start of the reaction.

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ceased, and slow decomposition of $\mathbf{1^H}$ occurred. The half-life of $\mathbf{1^H}$ was 7 h at 25 °C. The decomposition product showed a noncharacteristic absorption spectrum that changed upon the addition of excess hydrochloric acid to exhibit the characteristic bands at 510 and 585 nm (see the SI). The final absorption spectrum thus obtained was in good agreement with that of a biliverdin-type compound derived from the reaction of Fe^{III}TPPS with H_2O_2.[7] These results suggest that $\mathbf{1^H}$ is the oxoferryl complex PFe^{IV}═O, which further decomposes to yield the biliverdin-type compound in the presence of excess H_2O_2. In the absence of Py3CD, Fe^{III}TPPS rapidly decomposed within 10 min without formation of $\mathbf{1^H}$ (Figure 1a). Encapsulation of Fe^{III}TPPS by Py3CD was essential for the formation of $\mathbf{1^H}$.

At 77 K, a frozen aqueous solution of $\mathbf{1^H}$ showed a resonance Raman (rR) band at 815 cm^{-1} that shifted to 779 cm^{-1} when $H_2{}^{18}O_2$ was used in place of $H_2{}^{16}O_2$ ($\Delta\nu = -36$ cm^{-1}; Figure 2). The observed isotope shift corresponded to the expected value for an Fe═O diatomic oscillator ($\Delta\nu = -37$ cm^{-1}).[8] The rR band was not affected by isotope substitution of H_2O_2 with D_2O_2. As a consequence, we concluded that the rR spectrum of $\mathbf{1^H}$ should be assigned to $\nu_{Fe(IV)=O}$ of oxoferryl porphyrin.

In order to verify whether a hydroperoxoferric complex was formed as an intermediate, EPR spectra of a mixture of met-hemoCD and H_2O_2 were measured at 15 K (Figure 3; also see the SI). The mixture sample was prepared by freezing the met-hemoCD solution immediately after the addition of H_2O_2. Ferric met-hemoCD showed high-spin signals at $g = 6.03$ and 1.99, and the intensity of the signals diminished upon the addition of H_2O_2. Despite the EPR-silent character of the oxoferryl porphyrin, the freeze-quenched mixture of met-hemoCD and H_2O_2 showed the EPR signals of the iron porphyrin in the low-spin states. In the presence of H_2O_2, two sets of rhombic signals ($g_{1,2,3} = 2.24$, 2.14, 1.96; $g_{4,5,6} = 2.22$, 2.12, 1.97) were observed, together with a weak signal ($g = 2.00$) arising from organic radical(s). The two sets of anisotropic g values were observed in narrower regions of the magnetic field, which is characteristic of the EPR spectra of hydroperoxoferric porphyrins.[9] Tajima et al.[10] measured the EPR spectra of PFe^{III}−OOH(−OH) ($g = 2.257$, 2.156, 1.963) and PFe^{III}−OOH(−Im) ($g = 2.320$, 2.191, 1.943) (P = tetramesitylporphyrin, Im = imidazole) in a DMSO/methanol/H_2O mixed solvent at 77 K. On the basis of the results reported by Tajima et al., it is reasonable to assume that the species showing g_1, g_2, and g_3 is PFe^{III}−OOH(−Py), whose ferric center is axially coordinated by pyridine, and that the species showing g_4, g_5, and g_6 is PFe^{III}−OOH(−OH). The axial pyridine ligand of met-hemoCD might partially dissociate upon the coordination of HOO^-, affording a five-coordinate hydroperoxo complex in a high-spin state. The hydroxide anion might coordinate to the five-coordinate complex to form a six-coordinate PFe^{III}−OOH(−OH) complex that shows a set of rhombic signals at g_4, g_5, and g_6. Another possibility for the two sets of rhombic signals is the formation of PFe^{III}−OOH and PFe^{III}−OO^-. The formation of the dissociated form, PFe^{III}−OO^-, was ruled out on the basis of the fact that PFe^{III}−OOR (R = cumyl or *tert*-butyl) without a dissociable proton showed an EPR spectrum quite similar to that of PFe^{III}−OOH.[11] No rhombic signals were observed when the met-hemoCD solution was frozen at 2 min after the addition of H_2O_2. At higher concentrations of hemoCD (4×10^{-4} M) and H_2O_2 (8×10^{-3} M), the rapid formation of $\mathbf{1^H}$ (PFe^{IV}═O) occurred at room temperature. This might explain why the rhombic signals were not observed at 2 min after the start of the reaction. These results indicate that PFe^{III}−OOH is formed in the reaction of hemoCD with H_2O_2 and rapidly decomposes to PFe^{IV}═O and ·OH. Freeze-quenching enabled the detection of labile PFe^{III}−OOH.

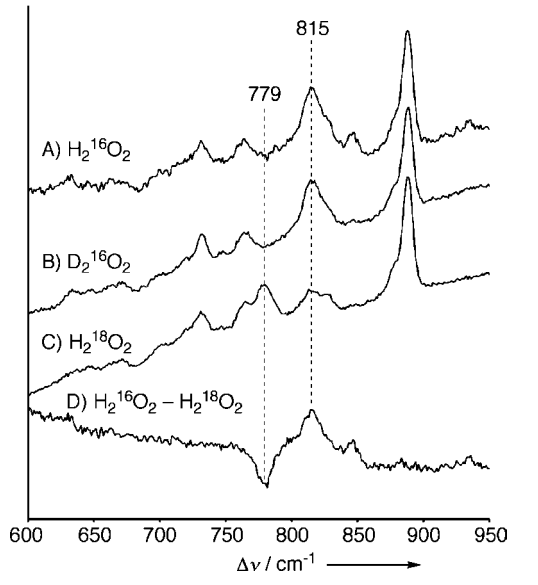


Figure 2. Resonance Raman spectra of met-hemoCD (4×10^{-4} M) with (A) $H_2{}^{16}O_2$ (2×10^{-3} M), (B) $D_2{}^{16}O_2$ (2×10^{-3} M), and (C) $H_2{}^{18}O_2$ (2×10^{-3} M) in 50 mM phosphate buffer at 77 K; the $H_2{}^{16}O - H_2{}^{18}O$ difference spectrum is shown in (D). The samples were irradiated with 407 nm laser light at a power of 1.0 mW.

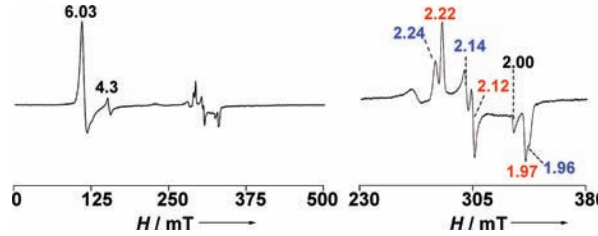


Figure 3. EPR spectrum of $\mathbf{1^H}$ generated in the reaction of met-hemoCD (4×10^{-4} M) with H_2O_2 (8×10^{-3} M) in 50 mM phosphate buffer at pH 7.0. The sample was frozen immediately after mixing met-hemoCD with H_2O_2.

The reaction of met-hemoCD (1×10^{-5} M) with H_2O_2 (2×10^{-4} M) yielded CO-coordinated ferrous hemoCD (CO-hemoCD, final yield 70%) under a CO atmosphere.[12] Because the previously prepared PFe^{IV}═O was slowly converted to CO-hemoCD (yield 65%) when the atmosphere was changed from air to CO (see the SI), it can be concluded that CO-hemoCD is formed via PFe^{IV}═O. PFe^{IV}═O itself does not yield CO-hemoCD in the absence of H_2O_2. Therefore, the oxoferryl complex must be converted to some sort of compound that reacts with CO. The most plausible reactions explaining the experimental results are shown below:

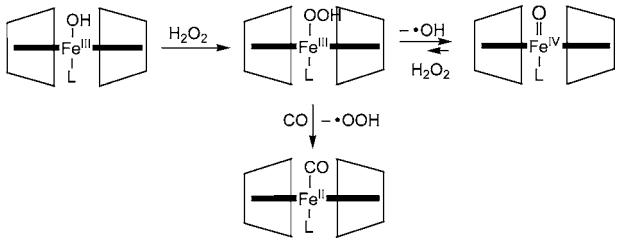
$$PFe^{IV}{=}O + H_2O_2 \rightarrow PFe^{III}{-}OOH + {\cdot}OH \qquad (1)$$

$$PFe^{III}{-}OOH + CO \rightarrow PFe^{II}{-}CO + {\cdot}OOH \qquad (2)$$

The charge of the HOO^- is transferred to Fe^{III}, providing the ferrous character of the iron center in PFe^{III}−OOH. As the tri-*O*-methylglucopyranose unit of Py3CD is a good radical scavenger (see the SI), the ·OH and ·OOH radicals could not be trapped by 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO). In addition, the ·OH radical might be consumed by the attack at the meso position of the porphyrin within the capsule formed by Py3CD.

It is concluded that hydroperoxoferric porphyrin is formed as an intermediate in the reaction of met-hemoCD with H_2O_2 and rapidly converted to the oxoferryl complex PFe^{IV}═O. PFe^{IV}═O,

Scheme 2



which is stabilized by Py3CD, slowly reacts with coexisting H_2O_2 to revert to $PFe^{III}-OOH$ (Scheme 2). In the absence of Py3CD, rapid decomposition of Fe^{III}TPPS occurred, and no reaction intermediate was observed spectroscopically. The effect of Py3CD is somewhat similar to that of a globin protein of metmyoglobin (metMb), which has been known to yield ferryl Mb in its reaction with H_2O_2.[13] Although Compound II-type ferryl Mb had been believed to be the only intermediate in the reaction of metMb with H_2O_2, formation of the Compound I-type oxoferryl Mb π-cation radical was demonstrated on the basis of UV−vis spectroscopy and distribution of the oxidation products.[14] In the present study, we could not observe the formation of an oxoferryl porphyrin π-cation radical using the stopped-flow technique. Given that oxoferryl porphyrin is greatly stabilized in the supramolecular system, we were able to find the unique reaction of $PFe^{IV}=O$ with H_2O_2 to yield hydroperoxoferric porphyrin, $PFe^{III}-OOH$, which reacts with CO to yield CO-coordinated ferrous porphyrin. The slow degradation of FeTPPS to yield the biliverdin-type compound can also be interpreted in terms of a slow reaction of $PFe^{IV}=O$ with H_2O_2 to afford $PFe^{III}-OOH$, which is the intermediate of the degradation reaction.

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Supporting Information Available: Additional data and explanation. This material is available free of charge via the Internet at http://pubs.acs.org.

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