Mechanistic study on *meso*-hydroxyoctaethylporphyrin formation from an $Fe^{III}(oep)–H_2O_2$ complex

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The benzoyl ester of an iron(III) *meso*-hydroxyoctaethylporphyrin complex, containing ^{18}O derived from molecular oxygen, is isolated from the reaction solution of the complex $[Fe^{III}(oep)(OOH)(OH)]^-$ which is prepared by reduction of $[Fe^{II}(oep)(py)(^{18}O_2)]$ with sodium ascorbate.

Haem enzyme–hydrogen peroxide complexes have been thought to be produced by the following two reactions: (*i*) coordination of hydrogen peroxide to the haem chromophore of enzymes, such as peroxides[1] and catalases,[2] (*ii*) one-electron reduction of the iron(II) oxygen complex of haem oxygenase[3] and P450.[4] We have reported the formation of the six coordinate $[Fe^{III}(oep)(OOH)(OH)]^-$ complex by reaction between hydrogen peroxide and $[Fe^{III}(oep)Cl]$.[5] In addition, the complex was similarly produced by reduction of the $Fe^{II}(oep)–O_2$ adduct with sodium ascorbate.[5] In the latter reaction system, the hydrogen peroxide complex was readily converted to octaethylverdohaem[6] at −30 °C. However, the possible transient intermediate species involved in the reaction pathway was still uncertain.[7] Here, the chemical reactivity of the hydrogen peroxide complex was studied by focusing upon formation of *meso*-hydroxy-oep derivatives, by means of EPR, optical absorption and product analysis using $^{18}O_2$.

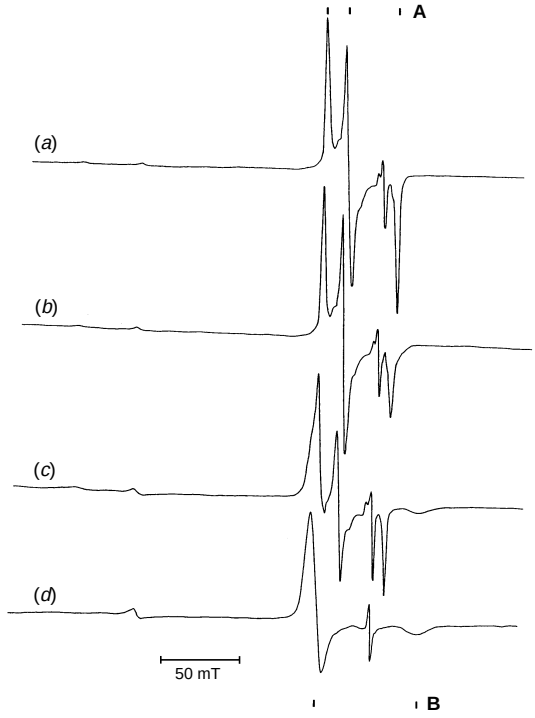


Fig. 1 (*a*) EPR spectrum observed at 77 K for the reaction solution containing $[Fe^{III}(oep)(OH)(OOH)]^-$; (*b*) with $^{17}O_2$ (39% enrichment); (*c*) after standing a solution of (*a*) for 30 min at −50 °C, and (*d*) after 60 min at −50 °C. The signal detected in the free-spin region is ascribed to the ascorbic acid radical.

A reaction solution containing $[Fe^{III}(oep)(OOH)(OH)]^-$ was prepared in a quartz EPR tube (5.0 mm diameter), by addition of aqueous sodium ascorbate (0.5 M, 0.016 ml) to a pre-cooled (−40 °C) dmf–pyridine (py) (9 : 1) solution of $[Fe^{III}(oep)Cl]$ (1.0 mM, 0.4 ml). After 3 min of continuous oxygen bubbling in the reaction mixture, the solution was frozen at −78 °C, and the atmosphere of the sample tube was changed to nitrogen. Then the sample was immediately frozen to 77 K. EPR signals [Fig. 1(*a*)] due to an iron(III) low-spin species (**A**; g_1 = 2.287, g_2 = 2.176, g_3 = 1.956) were clearly recorded, and the *g*-values agreed well with previous results[5] (Table 1). Fig. 1(*b*) show that a similar EPR spectrum was successfully detected using $^{17}O_2$ (39% enrichment), with the linewidth of the g_3 component broadened by unresolved splitting due to ^{17}O (I = 5/2).[8] This demonstrates the fact that the peroxide anion, ligated axially in **A**, was derived from atmospheric oxygen.

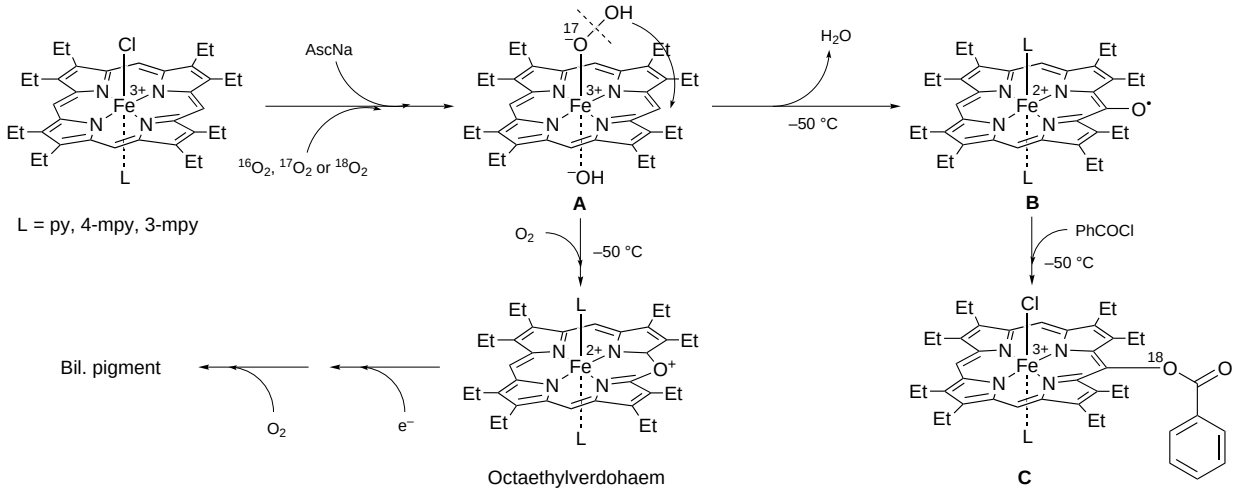
Quantitative EPR analysis was performed by means of graphic integration, using an ESPRIT ESR data analyser (JEOL). In the present case, the EPR spectrum recorded for a dmf solution of $[Cu^{II}(tpp)]$ (S = 1/2) was employed as a standard to calibrate the integrated EPR signal intensity of **A**. The concentration of **A** was evaluated to be *ca*. 0.8 mM. By using the dmf solution of $[Fe^{III}(oep)Cl]$ as the standard, the total concentration of observed iron(III) high-spin species (g = 6, 4.3) arising from remaining $[Fe^{III}(oep)Cl]$ and a decomposed tetrapyrrole iron complex,[8] was also estimated to be *ca*. 0.1–0.15 mM. Therefore the maximum percentage of **A** in the fresh frozen mixture was confirmed to be *ca*. 80% of the initial concentration of $[Fe^{III}(oep)Cl]$.

On standing the reaction solution at −50 °C for 30 min, new EPR signals ($g_\perp$ = 2.3, $g_\parallel$ = 1.8) began to merge with that of complex **A**, as shown in Fig. 1(*c*). After 60 min, signals due to

Table 1 EPR parameters at 77 K of $Fe^{III}(oep)–H_2O_2$ and *meso*-hydroxy complexes[a]

Complex	Ligand	EPR parameters		
		g_1	g_2	g_3
A	py	2.287	2.176	1.956
	py[b]	2.269	2.162	1.961
	py[c]	2.269	2.162	1.961
	OH[c,d,e]	2.286	2.171	1.953
	4-mpy	2.291	2.174	1.957
	3-mpy	2.29	2.17	1.95
	2-mpy	Not detected		
	2,6-dmpy	Not detected		
B	py	2.290	1.805[f]	
	py[g]	2.31	1.87[f]	
	4-mpy	2.310	1.768[f]	
	3-mpy	2.310	1.778[f]	
	2-mpy	Not detected		
	2,6-dmpy	Not detected		

[a] Reductant is sodium ascorbate unless indicated otherwise. [b] Prepared under $^{17}O_2$ (39% enrichment). [c] Ref. 5. [d] No reductant. [e] H_2O_2 added. [f] Axial symmetry $g_1 = g_\perp$, $g_2 = g_\parallel$. [g] Ref. 6.



Scheme 1 Possible reaction mechanisms for the formation of *meso*-hydroxy-oep *via* $[Fe^{III}(oep)(OH)(OOH)]^-$

the new species (denoted **B**; $g_\perp$ = 2.290, $g_\parallel$ = 1.805) was exclusively observed [Fig. 1(*d*)], while those of **A** completely disappeared. **A** was unstable under oxygen, and immediately changed to a green diamagnetic species (388, 493, 527 and 650 nm), after oxygen gas bubbling into the solution at −50 °C. The resulting species was isolated and identified as bis(pyridine)(octaethylverdohaem) iron(II),[9] from its NMR, and optical absorption parameters. This observation indicates that **B** is one of the intermediate species in the process of verdohaem derivative formation from **A**.

Similar EPR measurements were performed using methylated pyridine derivatives, 2-, 3-, 4-mpy, and 2,6-dmpy, in order to clarify the role of **A** in the process of verdohaem formation. Similar EPR spectra to **A** were recorded for the reaction systems composed of 3- and 4-mpy, and the observed EPR parameters agreed well to those of **A** (Table 1). Furthermore, the EPR signal of **B** was clearly detected with 3- and 4-mpy instead of py, and the observed *g*-values agreed with each other, although formation of **A** and **B** was not detected using 2-mpy or 2,6-dmpy. EPR signals of $[Fe^{III}(oep)Cl]$ completely disappeared, suggesting that the complex is reduced to the iron(II) form Fe(oep) and the formation of the oxygen complex $[Fe^{II}(oep)(O_2)]$ could be markedly suppressed. Accordingly, this provided experimental evidence that the $Fe^{II}(oep)$–O_2 complex is converted to **B** *via* **A** (Scheme 1).

In terms of the observed *g*-parameters, **B** is assigned as the porphyrinoxyl radical form of iron(II) *meso*-hydroxyoctaethylporphyrin (Table 1);[10] however, the origin of the oxygen atom of the hydroxy group was still debatable. MS measurements were thus performed for **B** isolated from $^{18}O_2$ (96% enrichment) as its benzoyl ester by addition of a dmf solution of benzoyl chloride (0.1 M, 0.4 ml) to a solution of **B** at −50 °C. The brownish yellow species obtained, **C** [λ_{max} = 379, 508, 535, 636 nm ($CHCl_3$) g = 6, 2 at 77 K] gave an IR band at 1740 cm^{-1} due to ν(CO) of the benzoyl ester moiety. NMR was consistent for a *meso*-benzoyloxyoctaethylporphyrin iron(II) bispyridine complex.[11] MS for **C**, isolated from $^{16}O_2$ and $^{18}O_2$ atmosphere, revealed weak parent ion peaks (*m/z* 743, 745), and major fragment peaks ($M^+ - Cl^-$ at *m/z* 708, 710), respectively. In addition, a common fragment peak was detected for both samples at *m/z* 588, which corresponds to Fe(oep) after fragmentation of the chlorine and benzoyl moieties from **C**. Based on the observed *m/z* value **C** was formulated as $C_{43}H_{48}N_4{}^-(^{16}O)_2Cl$ and $C_{43}H_{48}N_4{}^-(^{16}O)(^{18}O)Cl$, respectively, indicative that the oxygen atom attached at the *meso* carbon was derived from molecular oxygen. Detectable fragment peaks due to the ^{16}O structure were scarcely detected in the mass spectrum of the ^{18}O analogue, indicating that the oxygen atoms of H_2O and OH^-, derived from aqueous sodium ascorbate, are not the oxygen source in **C**.

Noting that **B** was produced *via* **A**, the axially ligating OOH^- is regarded as the most probable oxygen source. These results are reasonably interpreted by assuming an intramolecular electrophilic addition of OOH^- to the porphyrin ring resulting in the formation of the cationic form of *meso*-hydroxyporphyrin,[12] which was readily converted to **B** by loss of two protons,[10] under alkaline conditions (Scheme 1). In the process of O–O bond cleavage in **A** the formation of high-valent iron species are also postulated, however, these species are expected to be shortlived in the presence of ascorbate, and readily reduced to **B** with loss of water. Consequently, the haem H_2O_2 complex is the most important intermediate in the process of *meso*-oxygenation. The results described here demonstrate the role of the haem–OOH^- complex in the process of haem metabolism catalysed not only by the coupled oxidation of haem,[7] but also by naturally occurring haem-oxygenases.[12]

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