

Formation of a Highly Oxidized Iron Biliverdin Complex upon Treatment of a Five-Coordinate Verdoheme with Dioxygen

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Verdoheme, a green iron porphyrin with an oxygen atom in place of one of the methine units as seen in ClFe^{II}(OEOP) **1** in Scheme 1, is an intermediate in the oxidation of heme by heme oxygenase.¹ To release biliverdin (a bilindione), the final product of heme oxidation, verdoheme must undergo ring opening. This process may occur either by hydrolysis or by oxidation² and is reported to produce an iron complex of biliverdin. It has been suggested that a five-coordinate Fe(II) form of verdoheme may be involved in the oxidative ring opening.^{2a,3} Consequently, we have investigated the oxidation of the five-coordinate verdoheme, XFe^{II}(OEOP) **1**,⁴ to determine the nature of the putative biliverdin complex produced. Previously, characterization of the structure and properties of iron biliverdin complexes has been hampered by their tendency to readily lose iron.⁵ Here, we report the clean conversion of a five-coordinate verdoheme to a highly oxidized iron biliverdin complex utilizing O₂ as the oxidant.

Treatment of a green solution of XFe^{II}(OEOP) **1** (X = Cl or Br)⁴ with dioxygen results in the formation of **2** within a matter of minutes. The reaction has been monitored by ¹H NMR spectroscopy. The results are shown in Figure 1. Trace A shows the previously reported spectrum of ClFe^{II}(OEOP) before addition of dioxygen.⁴ After addition of dioxygen, the spectrum shown in trace B is obtained. The resonances of ClFe^{II}(OEOP) have disappeared, and new resonances due to **2** (X = Cl) have developed. The spectrum of **2** (X = Cl) consists of three widely dispersed meso proton resonances at 35, -28, and -167 ppm, eight methylene resonances in the downfield region, and another eight methylene resonances in the upfield region. In addition, there are eight methyl resonances in the 6 to -3 ppm region that are not shown in Figure 1. This pattern of resonances is consistent with the structures shown in Scheme 1 where the five-coordinate nature of the iron and the helical nature of the ligand render the three meso protons and the 16 methylene protons inequivalent.

The reaction to form **2** (X = Cl or Br) is irreversible, and the product is stable to further exposure to dioxygen. Oxidation of solutions of ClFe^{II}(OEOP) or BrFe^{II}(OEOP) produces significantly different spectra so the halide ions remain coordinated in solution (see Supporting Information, Figure S1). Although a few iron(III) complexes of meso-substituted porphyrins are oxidized by dioxygen,⁶ the six-coordinate iron(III) verdoheme Cl₂Fe^{III}(OEOP)⁴ is not affected by exposure to dioxygen.

A crystalline sample of **2** (X = Cl) has been obtained as a chloroform solvate by slow diffusion of diethyl ether into a solution of the complex in chloroform. The structure of the complex has been determined by X-ray crystallography.⁷ Figure 2 shows two views of the molecule. The complex involves a five-coordinate iron atom bound to the ring-opened tetrapyrrole through its four nitrogen

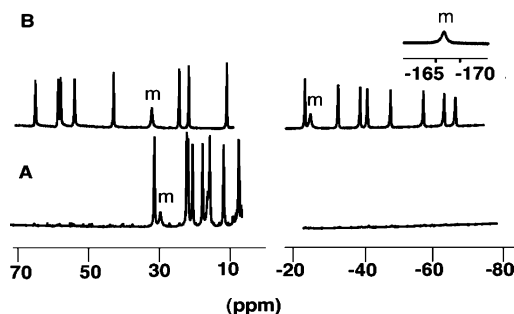
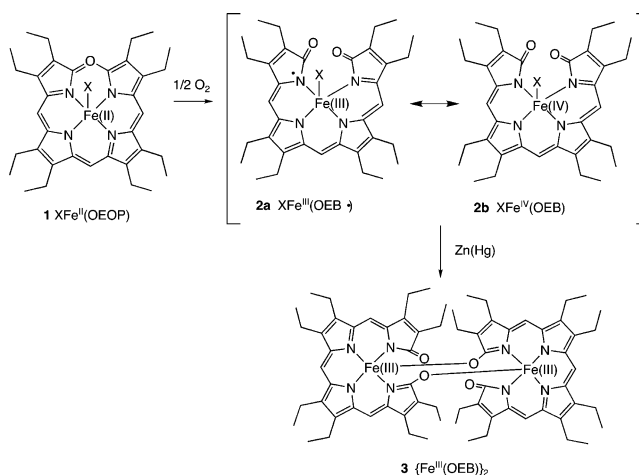


Figure 1. The 500 MHz ¹H NMR spectra at 22 °C of (A) a solution of ClFe^{II}(OEOP) in chloroform-*d* under dioxygen-free conditions and (B) after addition of O₂. Resonances in trace B are due to **2** (X = Cl). Resonances from the meso protons are labeled m, while the other resonances are due to methylene protons.

Scheme 1



atoms. The iron site is fully occupied in contrast to the situation with {Fe^{III}(OEB)}₂, which is the only other iron biliverdin complex crystallographically characterized and which underwent loss of iron during crystallization.⁵ The tetrapyrrole ligand assumes the helical shape that is characteristic of this type of ligand.⁸ A chloride ligand completes the iron coordination sphere. The Fe–N distances for the terminal pyrrole groups (Fe–N1, 2.018(2); Fe–N4, 2.016(2) Å) are somewhat shorter than those for the inner two pyrrole rings (Fe–N2, 2.049(2); Fe–N3, 2.070(2) Å). For comparison, the Fe–N distances in high-spin, five-coordinate iron(III) porphyrins fall in the range 2.06–2.09 Å.⁸ The Fe–Cl distance, 2.2694(8) Å, is similar to those found in a number of five-coordinate iron(III) porphyrins.⁶ The two C–O bond lengths (C1–O1, 1.219(4); C19–O2, 1.217(3) Å) are similar and are consistent with the presence of C=O units at the ends of the tetrapyrrole ligand.

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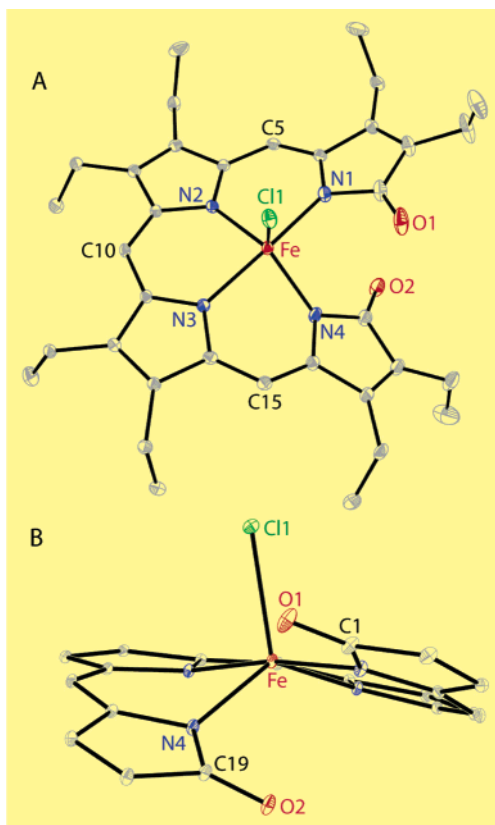


Figure 2. Two perspective views of **2** ($X = \text{Cl}$) showing 30% thermal contours for all non-hydrogen atoms. Selected bond distances (\AA): Fe–N1, 2.018(2); Fe–N2, 2.049(2); Fe–N3, 2.070(2); Fe–N4, 2.016(2); Fe–Cl1, 2.2694(8); C1–O1, 1.219(4); C19–O2, 1.217(3). Selected bond angles (deg): N1–Fe–N2, 87.51(9); N1–Fe–N3, 170.62(9); N1–Fe–N4, 94.43(9); N2–Fe–N3, 85.98(8); N2–Fe–N4, 133.83(9); N3–Fe–N4, 85.24(9); N1–Fe–Cl1, 95.74(7); N2–Fe–Cl1, 113.42(7); N3–Fe–Cl1, 93.05(6); N4–Fe–Cl1, 112.24(7).

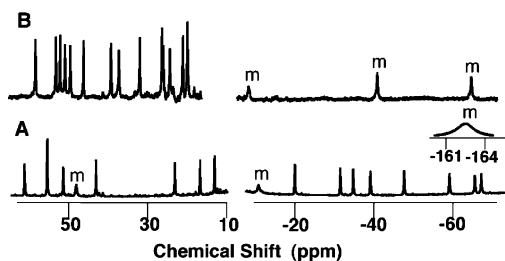


Figure 3. The 500 MHz ^1H NMR spectra at 22 $^\circ\text{C}$ of a solution of **2** ($X = \text{Br}$) in benzene- d_6 (A) before and (B) after treatment with zinc amalgam. The spectrum in (B) is that of $\{\text{Fe}^{\text{III}}(\text{OEB})\}_2$ (see ref 5). Resonances from the meso protons are labeled m, and all other resonances come from methylene protons.

Reduction of **2** ($X = \text{Cl}$ or Br) with zinc amalgam produces the previously characterized dimer, **3** $\{\text{Fe}^{\text{III}}(\text{OEB})\}_2$.⁵ Trace A of Figure 3 shows the ^1H NMR spectrum of **2** ($X = \text{Br}$) before reduction, and trace B shows the spectrum after reduction. In trace B, the resonances of **2** have vanished and have been replaced with those of $\{\text{Fe}^{\text{III}}(\text{OEB})\}_2$, which has three meso resonances in the upfield

region and 16 methylene resonances in the downfield portion. These results indicate that **2** is an oxidized complex that can be formulated by the resonance structures shown in Scheme 1. These involve either an $\text{Fe}(\text{IV})$ ion bound to a bilindione trianion or an $\text{Fe}(\text{III})$ ion bound to an oxidized, dianionic form of the ligand. The unusual observation of meso and methylene resonances that have both upfield and downfield hyperfine shifts suggests that significant radical character is present in **2**.^{2c,4,10} Examples of bilindione dianion radical coordination with metals other than iron have been observed.⁹ Density functional theory has been able to reproduce the sign alteration and shifts detected in the meso and methylene hyperfine shifts for such coordinated ligand radicals.¹⁰

The results reported here demonstrate that five-coordinate $\text{Fe}(\text{II})$ verdoheme is unusually reactive toward O_2 and that a highly oxidized iron biliverdin complex is cleanly formed in the process. The characteristic ^1H NMR spectrum of **2** should be useful in identifying this species if it forms during the functioning of heme oxygenase. Studies of the mechanism of the oxidation of this five-coordinate verdoheme, which is likely to involve attack of the dioxygen on the exposed face of the macrocycle, are in progress. While the oxidation state of the iron biliverdin complex formed in heme oxygenase has not been determined, this complex also involves a five-coordinate structure similar to **2** but with an axial histidine ligand in place of the chloride ligand.¹¹

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Supporting Information Available: Figure S1 and X-ray crystallographic data for **2** ($X = \text{Cl}$) in CIF format. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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- (7) Crystal data for of **2** ($X = \text{Cl}$) $\cdot\text{CHCl}_3$; $\text{C}_{36}\text{H}_{44}\text{Cl}_4\text{FeN}_4\text{O}_2$: reddish-black needle, triclinic, space group $P1$, $a = 10.201(2)$, $b = 14.015(3)$, $c = 14.074(3)$ \AA , $\alpha = 86.764(4)^\circ$, $\beta = 76.115(4)^\circ$, $\gamma = 69.759(4)^\circ$, $V = 1831.9(7)$ \AA^3 , $Z = 2$, $D_c = 1.382$ Mg/m^3 , $T = 90(2)$ K; $R1 = 0.069$, $wR2 = 0.126$ for all data; conventional $R1 = 0.045$ computed for 7177 observed data ($>2\sigma(I)$) and 454 parameters.
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