Heme Cleavage with Remarkable Ease: Paramagnetic Intermediates Formed by Aerobic Oxidation of a Meso-Amino-Substituted Iron Porphyrin

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The oxidative stability of the porphyrin macrocycle is essential for the functioning of the many heme proteins that expose hemes to oxidants such as dioxygen and hydrogen peroxide and use highly oxidized iron porphyrins to carry out their functions.[1] Such highly oxidized forms include the ferryl ion ($Fe^{IV}=O$) found in compound II of horseradish peroxidase as well as the oxidized porphyrin radical found along with the ferryl ion in compound I.[1] Despite the high oxidation states involved, the heme molecule remains intact in the course of the oxygen atom-transfer processes. To control the level of heme, nature utilizes heme oxygenase, which oxidizes heme, as a substrate, and converts it into biliverdin, free iron ion, and carbon monoxide in a process that involves the activation of three molecules of dioxygen.[2,3] Coupled oxidation, treatment of heme with dioxygen in the presence of a reducing agent, has been used to mimic heme degradation in the presence of dioxygen.[4] Both processes involve introduction of meso hydroxy group as a fundamental step of the heme cleavage. Here we show that introduction of a single amino group at a meso position can render a heme extremely sensitive to ring opening by dioxygen.

Exposure of a red pyridine (py) solution of diamagnetic $(py)_2Fe^{II}(H_2N\text{-}OEP)$ (**1**) ($H_2N\text{-}OEP$ is the dianion of *meso*-amino-octaethylporphyrin) at 23 °C to air results in the immediate formation of a green intermediate **2**. Under identical conditions, $(py)_2Fe^{II}(OEP)$ is unchanged by exposure to air. Relevant UV/vis absorption spectra are shown in Figure 1. The changes seen in Figure 1, particularly the drop in the intensity of the Soret band at 420 nm, indicate that the porphyrin has undergone ring opening to produce an open-chain tetrapyrrole. Verdoheme, the green pigment with an oxygen atom in place of a meso CH unit, is not formed since its characteristic absorptions at 384 and 651 nm are absent in the spectrum in Figure 1.[6]

Concurrently the formation of the green intermediate **2** has been monitored by ¹H NMR spectroscopy. As seen in Figure 2, the paramagnetically shifted spectrum consists of three meso-H resonances at 99, 50, and −38 ppm and 16 resonances due to the methylene protons. A COSY experiment (Supporting Information) reveals that the methylene resonances are coupled pairwise as shown in Figure 2 and that they are also coupled to methyl resonances which lie in the crowded region from 0 to 10 ppm. The assignment of the meso resonances has been confirmed by the observation of the ²H NMR spectrum of (**2**-*meso-d₃*) (trace B of Figure 2) which was formed by oxygenation of $(py)_2Fe^{II}(H_2N\text{-}OEP\text{-}meso\text{-}d_3)$.

The overall pattern of resonances with 16 methylene resonances and three meso resonances indicates that oxygenation is accompanied by a significant lowering of the symmetry of the porphyrin and is consistent with the formation of either of the two hypothetical species shown as **2** in Scheme 1. The photooxidation
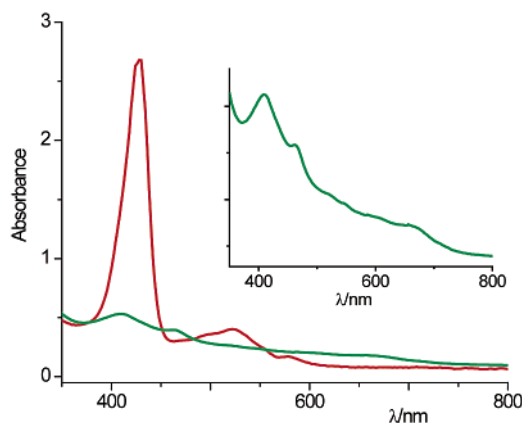
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Figure 1. The UV/vis absorption spectra of a pyridine solution of $((py)_2Fe^{II}(H_2N\text{-}OEP)$ before (red line) and after (green line) exposure to air. The inset shows an expanded version of the spectrum of the green intermediate **2**.
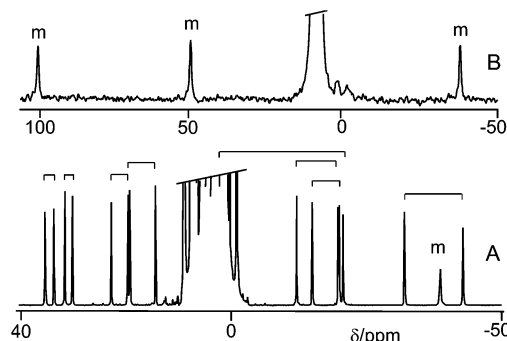

Figure 2. (A) Portion of the 500-MHz ¹H NMR spectrum of the green intermediate **2** formed by addition of air to a pyridine-d_5 solution of $(py)_2Fe^{II}(H_2N\text{-}OEP)$ at 25 °C. The meso resonances at 100 and 50 ppm are omitted to allow expansion of the methylene resonances. (B) The 76.7 MHz ²H NMR spectrum of the green intermediate **2** formed by addition of air to a pyridine solution of $(py)_2Fe^{II}(H_2N\text{-}OEP\text{-}meso\text{-}d_3)$ at 25 °C. The scalar connectivity of the methylene resonances is marked in trace A.
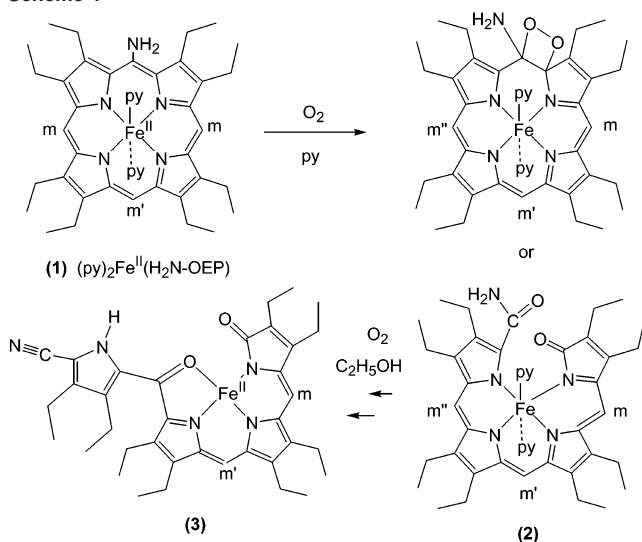
of magnesium porphyrins proceeds in an analogous fashion to produce formylbiliverdin.[5] The upfield and downfield shifts for methylene and meso protons is associated with the presence of a significant degree of ligand radical character. Iron−oxophlorin complexes[6−9] and a verdoheme hydrolysis product,[10] all with radical character, also show a pattern of methylene resonances with both upfield and downfield hyperfine shifts.

The conversion of **1** to **2** is clearly distinct from coupled oxidation, since no external reducing agent is needed to effect oxidative cleavage of $(py)_2Fe^{II}(H_2N\text{-}OEP)$ (**1**) and verdoheme is not formed.

Scheme 1

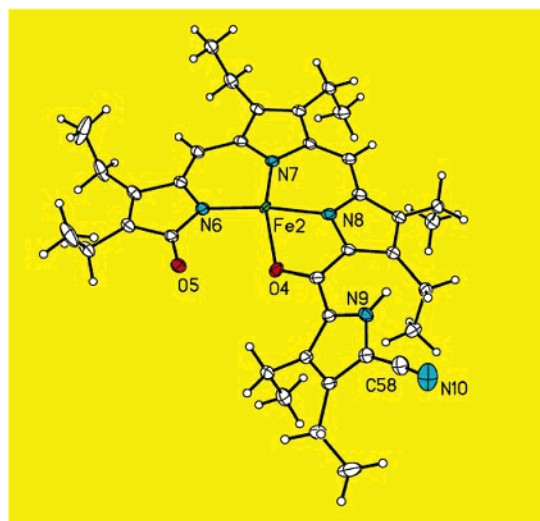(1) (py)$_2$Fe^{II}(H$_2$N-OEP)

(3)   (2)

Figure 3. A perspective view of **3** showing 50% thermal contours for all non-hydrogen atoms. Selected bond distances (Å): molecule 2: Fe2−N6 1.952(3), Fe2−N8 1.973(3), Fe2−N7 2.035(2), Fe2−O4 2.205(2), O5−C39 1.234(3), N10−C58 1.145(4); molecule 1 with coordinated ethanol: Fe1−N1 1.984(2), Fe1−N2 2.040(2), Fe1−N3 2.011(2), Fe1−O1 2.3155(19), Fe1−O3 2.111(2), O1−C15 1.250(3), O2−C1 1.249(3), O3−C37 1.451(4), N5−C20 1.151(4).

While the green intermediate **2** is stable in solution for several days in the strict absence of air, it is transformed slowly by further air exposure over several hours into **3** in 80% yield (MALDI mass spectrum (negative ion mode), 632 (M − H), IR, ν(CN), 2211 cm^{-1}) which has been isolated by ethanol addition. The structure of **3** has been determined by X-ray crystallography.[11] The solid contains two independent molecules with no crystallographically imposed symmetry. A drawing of one is shown in Figure 3. The other molecule is similar but has an axial ethanol ligand. As seen in Figure 3, the (py)$_2$Fe^{II}(H$_2$N-OEP) (**1**) has been transformed into a linear tetrapyrrole which is opened at the meso carbon that once bore the amino group. The amino group is no longer present, but it and the adjacent meso carbon atom have been transformed into a cyano group, presumably by dehydration. Additional oxidation has occurred at a second meso carbon to introduce an oxygen atom, which is coordinated to iron. The terminal pyrrole ring is twisted away from the iron and is no longer coordinated.

The oxidative ring opening of (py)$_2$Fe^{II}(H$_2$N-OEP) is related to both the facile oxidation of iron oxophlorin and to the oxidative cleavage of a cobalt(II) porphyrin bearing another unique meso substituent (naphthoic acid) into cobalt verdoheme.[12] The mechanistic aspects of the process of cleavage of (py)$_2$Fe^{II}(H$_2$N-OEP), and in particular its relevance to the coupled oxidation mechanism, are currently under investigation.

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References

(1) Sono, M.; Roach, M. P.; Coulte, E. D.; Dawson, J. H. *Chem. Rev.* **1996**, *96*, 2841
(2) Maines, M. D. *Heme Oxygenase: Clinical Applications and Functions*; CRC Press: Boca Raton, FL, 1992.
(3) Ortiz de Montellano, P. R. *Acc. Chem. Res.* **1998**, *31*, 543.
(4) St. Claire, T. N.; Balch, A. L. *Inorg. Chem.* **1999**, *38*, 684.
(5) Wasser, P. K. W.; Fuhrhop, J.-H. *Ann. N. Y. Acad. Sci.* **1973**, *206*, 533.
(6) Balch, A. L.; Latos-Grażyński, L.; Noll, B. C.; Olmstead, M. M.; Szterenberg, L.; Safari, N. *J. Am. Chem. Soc.* **1993**, *115*, 1422.
(7) Morishima, I.; Shiro, Y.; Wakino, T. *J. Am. Chem. Soc.* **1985**, *107*, 1063.
(8) Balch, A. L.; Koerner, R.; Latos-Grażyński, L.; Noll, B. C. *J. Am. Chem. Soc.* **1996**, *118*, 2760.
(9) Kalish, H. R.; Latos-Grażyński, L.; Balch, A. L. *J. Am. Chem. Soc.* **1996**, *118*, 2760.
(10) Latos-Grażyński, L.; Wojaczyński, J.; Koerner, R.; Johnson, J. J.; Balch, A. L. *Inorg. Chem.* **2001**, *40*, 4971.
(11) Crystal data for C$_{38}$H$_{49}$Fe$_2$N$_5$O$_3$: dark green parallelepiped, 0.29 mm × 0.28 mm × 0.13 mm, triclinic, space group $P\bar{1}$, a = 13.2609(11) Å, b = 13.7486(10) Å, c = 22.1473(18) Å, α = 105.949(2)°, β = 94.927(3)°, γ = 108.663(4)°, V = 3610.7(5) Å^3, λ = 0.71069 Å, Z = 4, D_c = 1.250 Mg m^{-3}; μ = 0.460 mm^{-1}; ω scans, $2\theta_{max}$ = 61.0°; T = 91(2) K; 22060 refl. collected; 22048 independent (R_{int} = 0.043) included in the refinement; min/max transmission = 0.878/0.943; Patterson and difference Fourier methods solution (SHELXS-97, Sheldrick, 1990); full-matrix least squares based on F^2 (SHELXL-97; Sheldrick, 1998); R1 = 0.113, wR2 = 0.184 for all data; conventional R1 = 0.063 computed for 13860 observed data (>2 $\sigma(I)$) with 1 restraint and 898 parameters
(12) Chang, C. K.; Aviles, G.; Bag, N. *J. Am. Chem. Soc.* **1994**, *116*, 12127.

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