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A Role for Electron Transfer in Heme Catabolism? Structure and Redox Behavior of an Intermediate, (Pyridine)$_2$Fe(octaethyloxophlorin)

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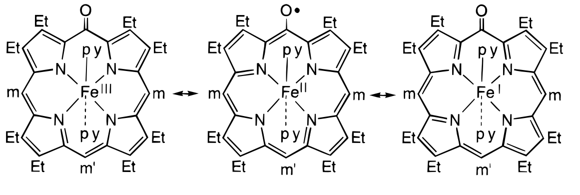
Oxidative heme catabolism, either by heme oxygenase[1] or by the coupled oxidation procedure,[2] results in the removal of a methine group from the iron porphyrin with the eventual release of carbon monoxide, iron, and biliverdin. Three molecules of dioxygen are involved in the overall reaction. It is widely believed that the process involves initial meso hydroxylation of the heme to form an iron oxophlorin (or iron meso-hydroxyporphyrin) as an intermediate. A number of hypothetical mechanistic schemes have been drawn to account for this process.[1,2] While many of these propose direct coordination of dioxygen to iron, few have been concerned with the effects of other axial ligands, and none have given any consideration to the significance of outer-sphere electron transfer reactions. Here we report the structural characterization of the iron complex, (pyridine)$_2$Fe(octaethyloxophlorin), (py)$_2$Fe(OEPO), **1**,[3] a likely intermediate in coupled oxidation,[2] and its redox behavior which includes the discovery of a facile, one-electron oxidation.



1a **1b** **1c**

Dark blue crystals of (py)$_2$Fe(OEPO) were obtained by dissolution of Fe$_2^{III}$(OEPO)$_2$[4] in dioxygen-free pyridine followed by precipitation through slow diffusion of dioxygen-free ethanol. The results of a single-crystal X-ray diffraction study are shown in Figure 1.[5] The iron is situated at a center of symmetry; hence, there is disorder in the position of the meso oxygen atom which has partial occupancies of 0.39 at O (and its symmetry-generated equivalent) and 0.11 at O′ (and its symmetry-generated equivalent).

The axial Fe−N(3) distance is 2.265(2) Å, and the in-plane Fe−N(1) and Fe−N(2) distances are 2.055(2) and 2.051(2) Å. These distances indicate that the $d_{x^2-y^2}$ and d_{z^2} orbitals are partially occupied and are consistent with high-spin Fe(III) or high-spin Fe(II).[6] The various magnetic, structural, and spectroscopic data that have been collected on this species[3] indicate that it has a complex electronic structure in which ligand and metal orbitals are mixed in a fashion that is not readily categorized by conventional oxidation and spin state assignments. While the overall oxidation level[3e] of (py)$_2$Fe(OEPO) is consistent with contributions from resonance structures **1a**, **1b**, and **1c**, the structural parameters, along with the observation that the known Fe(I) porphyrin is low spin,[6d] suggest that the contribution from **1c** is nearly negligible.

A cyclic voltammogram of (py)$_2$Fe(OEPO) in a dioxygen-free pyridine solution is shown in Figure 2. A reversible one-electron oxidation ($E_{1/2} = -192$ mV) to form [(py)$_2$Fe(OEPO)]$^+$ as well as a one-electron reduction ($E_{1/2} = -1023$ mV) to form [(py)$_2$Fe(OEPO)]$^-$ are observed. The oxidation process occurs at an unusually low potential. (The ferrocene/ferrocenium process occurs at +140 mV under these conditions.)

Initial spectroscopic characterization of the oxidation product, [(py)$_2$Fe(OEPO)]$^+$, has been obtained both through controlled oxidation of (py)$_2$Fe(OEPO) with diiodine or by reaction of pyridine with BrFe^{III}(OEPO•).[7] A common product is produced in either route. This even-electron species is characterized by an electronic absorption spectrum with $\lambda_{\max}$, nm (ϵ, L mol^{-1} cm^{-1}): 424 (9.9×10^4), 608 (1.0×10^4), and 648 (1.1×10^4) and by the ^{1}H NMR spectrum shown in part A of Figure 3. In contrast to (py)$_2$Fe(OEPO) whose ^{1}H NMR spectrum at 30 °C spans a 180 ppm range with meso resonances at −108 and −148 ppm, the spectrum of [(py)$_2$Fe(OEPO)]$^+$ nearly falls in the conventional diamagnetic region. However, the meso resonances occur at unusual upfield positions which are highly dependent on temperature. The temperature dependence is shown in part B of Figure 3 and suggests that this species has a diamagnetic ground state with a nearby, partially populated paramagnetic state.

In pyridine solution (py)$_2$Fe(OEPO) is very air sensitive, and in the presence of dioxygen it is converted into diamagnetic verdoheme,[2c,e] the paramagnetic iron(III) complex of octaethylbilindione[2d] and other paramagnetic products. The observation that the one-electron process that interconverts (py)$_2$Fe(OEPO) and [(py)$_2$Fe(OEPO)]$^+$ occurs at an especially low potential indicates that such one-electron processes need consideration in formulating an overall mechanism for heme destruction. In the coupled oxidation process in pyridine, is it necessary for an oxidant (dioxygen) to convert (py)$_2$Fe(OEPO) into [(py)$_2$Fe(OEPO)]$^+$ before further oxidation at the meso

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(1) (a) O'Carra, P. In *Porphyrins and Metalloporphyrins*; Smith, K. M., Ed.; Elsevier: New York, 1975; p 123. (b) Schmid, R.; McDonagh, A. F. In *The Porphyrins*; Dolphin, D., Ed.; Academic Press: New York, 1979; Vol. 6, p 258. (c) Brown, S. B. In *Bilirubin*; Heirwegh, K. P. M., Brown, S. B., Eds.; CRC Press, Inc.: Boca Raton, FL, 1982; Vol. 2, p 1. (d) Bissell, D. M. In *Liver: Normal Function and Disease. Bile Pigments and Jaundice*; Ostrow, J. D., Ed.; Marcel Decker, Inc.: New York, 1986; Vol. 4, p 133. (e) Maines, M. D. *Heme Oxygenase: Clinical Applications and Functions*; CRC Press: Boca Raton, FL, 1992. (e) Chang, C. K.; Avilés, G.; Bag, N. *J. Am. Chem. Soc.* **1994**, *116*, 12127.

(2) (a) Warburg, O.; Negelein, E. *Chem. Ber.* **1930**, *63*, 1816. (b) Lagarias, J. C. *Biochim. Biophys. Acta* **1982**, *717*, 12. (c) Balch, A. L.; Latos-Grażyńsky, L.; Noll, B. C.; Olmstead, M. M.; Sżterenberg, L.; Safari, N. *J. Am. Chem. Soc.* **1993**, *115*, 1422. (d) Balch, A. L.; Latos-Grażyński, L.; Noll, B. C.; Olmstead, M. M.; Safari, N. *J. Am. Chem. Soc.* **1993**, *115*, 9056. (e) Balch, A. L.; Koerner, R.; Olmstead, M. M. *J. Chem. Soc., Chem. Commun.* **1995**, 873.

(3) For previous spectroscopic work on this species, see: (a) Bonnett, R.; Dimsdale, M. J. *J. Chem. Soc., Perkin Trans. 1* **1972**, 2540. (b) Sano, S.; Sugiura, Y.; Maeda, Y.; Ogawa, S.; Morishima, I. *J. Am. Chem. Soc.* **1981**, *103*, 2888. (c) Sano, S.; Sano, T.; Morishima, I.; Shiro, Y.; Maeda, Y. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 531. (d) Morishima, I.; Fujii, H.; Shiro, Y.; Sano, S. *J. Am. Chem. Soc.* **1986**, *108*, 3858. (e) Balch, A. L.; Noll, B. C.; Reid, S. M.; Zovinka, E. P. *Inorg. Chem.* **1993**, *32*, 2610. (f) Morishima, I.; Fujii, H.; Shiro, Y.; Sano, S. *Inorg. Chem.* **1995**, *34*, 1528.

(4) (a) Masuoka, N.; Itano, M. A. *Biochemistry* **1987**, *26*, 3672. (b) Balch, A. L.; Latos-Grażyński, L.; Noll, B. C.; Olmstead, M. M.; Zovinka, E. P. *Inorg. Chem.* **1992**, *31*, 2248.

(5) Blue plates of (C$_5$H$_5$N)$_2$Fe(C$_{36}$H$_{43}$N$_4$O) crystallize in the triclinic space group $P\bar{1}$ with $a = 9.838(3)$ Å, $b = 10.221(3)$ Å, $c = 10.493(2)$ Å, $\alpha = 99.57(2)°$, $\beta = 90.08(2)°$, $\gamma = 113.68(2)°$, $V = 947.8(6)$ Å^3, and $Z = 1$. Refinement of 3341 reflections with 255 parameters and one restraint yielded wR2 = 0.079 (based on F^2) and a conventional R1 = 0.036 (based on F) for 2985 reflections with $I > 2.0\ \sigma I$. The crystal used for data collection was transferred to a dioxygen-free environment and dissolved in dioxygen-free pyridine. The UV−vis spectrum of this sample was identical to those reported previously.[3]

(6) (a) Scheidt, W. R. In *The Porphyrins*; Dolphin, D., Ed.; Academic Press: New York, 1978; Vol. 3, p 463. (b) Scheidt, W. R.; Reed, C. A. *Chem. Rev.* **1981**, *81*, 543. (c) Scheidt, W. R.; Lee, J. Y. *Struct. Bonding* **1987**, *64*, 1. (d) Mashiko, T.; Reed, C. A.; Haller, K. J.; Scheidt, W. R. *Inorg. Chem.* **1984**, *23*, 3192.

(7) Balch, A. L.; Latos-Grażyński, L.; Noll, B. C.; Szterenberg, L.; Zovinka, E. P. *J. Am. Chem. Soc.* **1993**, *115*, 11846.

0002-7863/96/1518-2760$12.00/0

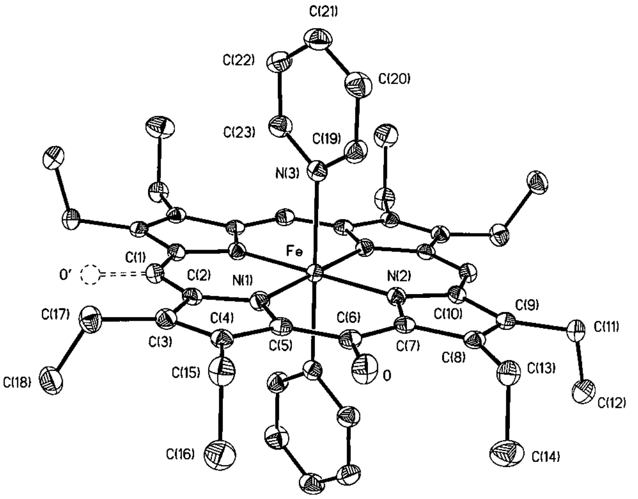

Figure 1. Perspective view of $(py)_2Fe(OEPO)$ with 50% thermal contours for all atoms. The site O′ which is shown with a dashed line is the site of minor fractional occupancy (0.11), while the site O has a fractional occupancy of 0.39. Bond distances (Å): Fe−N(1), 2.055(2); Fe−N(2), 2.051(2); Fe−N(3), 2.265(2); C(6)−O, 1.289(4); C(1)−O′, 1.340(13).

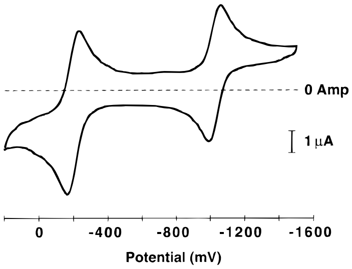

Figure 2. Cyclic voltammogram recorded from a dioxygen-free pyridine solution of $(py)_2Fe(OEPO)$ with 0.10 M tetrakis(*n*-butylammonium) perchlorate as supporting electrolyte. The reference electrode was silver/silver chloride, and the scan rate was 25 mV/s. The ferrocene/ferrocenium process occurs at +140 mV in this solvent/supporting electrolyte system.

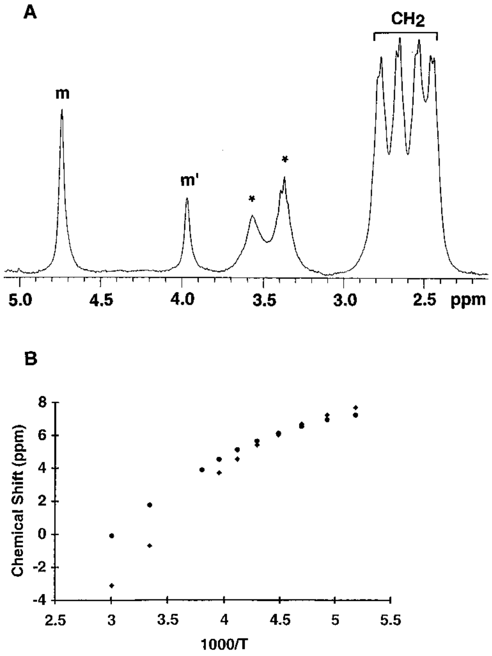

Figure 3. (A) Methylene (CH_2) and meso (m,m′) resonances in the ^1H NMR spectrum of $[(py)_2Fe(OEPO)]^+$ at −20 °C in dichloromethane-d_2. The sample was prepared by the addition of 0.8 μL of dioxygen-free pyridine-d_5 to a dioxygen-free dichloromethane-d_2 solution of $BrFe^{III}(OEPO^•)$.[7] The resonances denoted by * are due to the methylene protons of octaethylverdoheme, bis(pyridine)iron(II)(octaethyl-5-oxaporphyrin).[2e] (B) Plot of chemical shift versus 1/*T* for the two meso resonances.

carbon occurs, or is it necessary that a reductant protect $(py)_2Fe(OEPO)$ from this one-electron oxidation? The observation of the individual interconversions between well-defined iron complexes that are likely to be involved in heme degradation sets the stage for *in situ* monitoring of the coupled oxidation process and of heme oxidation catalyzed by heme oxygenase.

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Supporting Information Available: Tables of atomic coordinates, bond distances and angles, and anisotropic thermal parameters for $(py)_2Fe(OEPO)$ (9 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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