Coupled Oxidation of Heme without Pyridine. Formation of Cyano Complexes of Iron Oxophlorin and 5-Oxaporphyrin (Verdoheme) from Octaethylheme

Alan L. Balch,*,† Richard Koerner,† Lechosław Latos-Grażyński,‡ Jane E. Lewis,† Tamara N. St. Claire,† and Edward P. Zovinka†

Departments of Chemistry, University of California, Davis, California 95616, and University of Wrocław, Wrocław, Poland

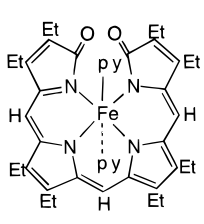
Received May 1, 1997[⊗]

Oxidative degradation of iron porphyrins under coupled oxidation conditions, which generally has involved pyridine as solvent and source of axial ligands, results in the production of verdoheme, the iron complex of 5-oxaporphyrin in which an oxygen atom replaces a methine group. Under pyridine-free conditions, $ClFe^{III}(OEP)$ (OEP is the dianion of octaethylporphyrin) and $\{Fe^{III}(OEPO)\}_2$ (OEPO is the trianion of octaethyloxophlorin) have been converted into the 5-oxaporphyrin complex (verdoheme) $\{(NC)_2Fe^{III}(OEOP)\}$ (OEOP is the anion of octaethyl-5-oxaporphyrin). Additionally, through control of the cyanide ion concentration, the oxidation of $ClFe^{III}(OEP)$ can be stopped to produce $\{Fe^{III}(OEPO)\}_2$ or allowed to proceed to give $\{(NC)_2Fe^{III}(OEOP)\}$. A convenient method for the preparation of $\{Fe^{III}(OEPO)\}_2$, and from it the free base H_2OEPOH, is reported. Anionic complexes, $[(NC)(py)Fe(OEPO)]^-$ and $[(NC)_2Fe(OEPO)]^{2-}$, related to these oxidative degradation processes have been obtained through the addition of bis(triphenylphosphine)iminium cyanide to $(py)_2Fe(OEPO)$ in pyridine solution or through cleavage of $\{Fe^{III}(OEPO)\}_2$ with potassium cyanide in methanol or with tetra(*n*-butyl)ammonium cyanide in chloroform solution. The products have been characterized principally through their characteristic ^1H NMR spectra, which show sizable hyperfine shifts with meso resonances upfield and methylene resonances with both up- and downfield shifts. In the presence of air, $[(NC)_2Fe(OEPO)]^{2-}$ undergoes oxidation to form the 5-oxaporphyrin complex (verdoheme) $\{(NC)_2Fe^{III}(OEOP)\}$. Treatment of $BrFe^{III}(OEPO^{\bullet})$ with CN^- produces $[(NC)_2Fe(OEPO)]^-$, which can also be obtained through oxidation of $[(NC)_2Fe(OEPO)]^{2-}$ with diiodine.

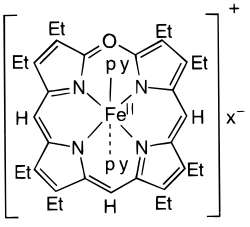
Introduction

Heme degradation is an important process for the disposal of unwanted heme,[1] for neurotransmission (through carbon monoxide production),[2] and for catalysis (where catalyst lifetime is dependent on its oxidative stability).[3] Until recently, however, the coordination chemistry (axial ligation, metal ion oxidation, and spin states) of the process of oxidative heme degradation has received scant attention.

The coupled oxidation process, in which heme degradation is brought about by dioxygen in the presence of a reducing agent (ascorbic acid or hydrazine),[4,5] has been widely used as a model for biological heme catabolism.[1] This process has generally involved pyridine as both solvent and source of axial ligands. In early work, Lemberg found that protoheme underwent negligible coupled oxidation in the absence of pyridine, but found that certain proteins when complexed with protoheme promoted coupled oxidation.[6,7] Since proteins, as well as pyridine, ligate to the iron atom of the heme molecule at one or both axial positions, binding of such axial ligands may activate the heme group and render the methine positions susceptible to oxidative cleavage. We have recently shown that coupled oxidation of $(py)_2Fe^{II}(OEP)$ (py, pyridine; OEP, dianion of octaethylporphyrin) produces **1**,[8] the iron(III) complex of



1



2

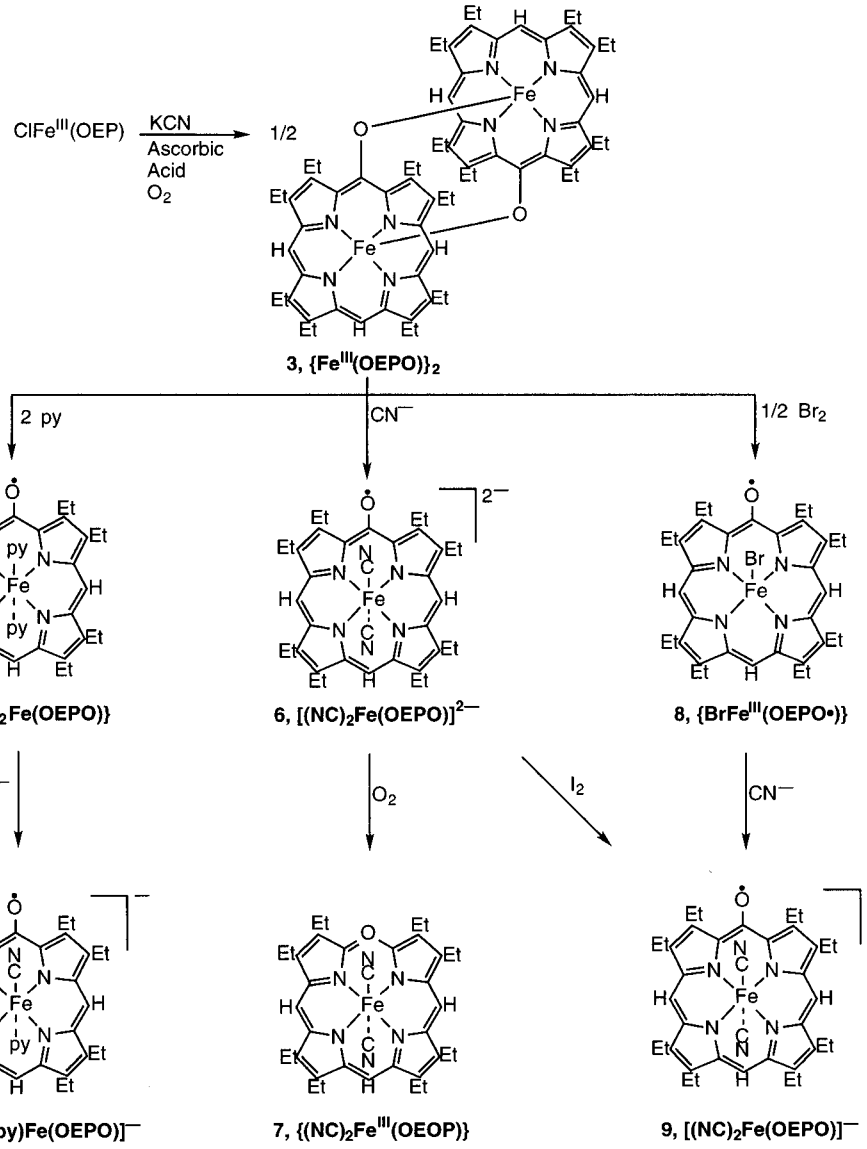
octaethylbilindione (a biliverdin analog) along with the long-known verdoheme, **2**, which is a diamagnetic iron(II) complex

† University of California.
‡ University of Wrocław.
⊗ Abstract published in *Advance ACS Abstracts,* July 15, 1997.

(1) (a) Maines, M. D. *Heme Oxygenase: Clinical Applications and Functions*. CRC Press Boca Raton, FL, 1992. (b) 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. (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) Torpey, J.; Ortiz de Montellano, P. R. *J. Biol. Chem.* **1996**, *271*, 26067. (e) Hawkins, B. K.; Wilks, A.; Powers, L. S.; Ortiz de Montellano, P. R. *Biochim. Biophys. Acta, Protein Struct.* **1996**, *1295*, 165. (f) Takahashi, S.; Ishikawa, K.; Takeuchi, N.; Ikeda-Saito, M.; Yoshida, T.; Rousseau, D. L. *J. Am. Chem. Soc*. **1995**, *117*, 6002. (g) Sun, J.; Wilks, A.; Ortiz de Montellano, P. R.; Loehr, T. M. *Biochemistry* **1993**, *32*, 14151. (h) Takahashi, S.; Wang, J.; Rousseau, D. L.; Ishikawa, K.; Yoshida, T.; Host, J. R.; Ikeda-Saito, M. *J. Biol. Chem.* **1994**, *269*, 1010. (i) Takahashi, S.; Wang, J.; Rousseau, D. L.; Ishikawa, K.; Yoshida, Takeuchi, N.; Ikeda-Saito, M. *Biochemistry* **1994**, *33*, 5531. (j) Wilks, A.;Ortiz de Montellano, P. R. *J. Biol. Chem.* **1993**, *268*, 22357. (k) Wilks, A.; Sun, J.; Loehr, T. M.; Ortiz de Montellano, P. R. *J. Am. Chem. Soc*. **1995**, *117*, 2925. (l) Hernandez, G.; Wilks, A.; Paolesse, R.; Smith, K. M.; Ortiz de Montellano, P. R.; La Mar, G. N. *Biochemistry*, **1994**, *33*, 6631. (m) Schmid, R.; McDonagh, A. F. In *The Porphyrins*; Dolphin, D., Ed.; Academic Press: New York, 1979; Vol. 6, p 258. (n) O'Carra, P. In *Porphyrins and Metalloporphyrins*; Smith, K. M., Ed.; Elsevier: New York, 1975; p 123.
(2) Verma, A.; Hirsch, D. J.; Glatt, G. E.; Ronnett, G. V.; Snyder, S. H. *Science* **1993**, *259*, 381.
(3) Traylor, P. S.; Dolphin, D.; Traylor, T. G. *J. Chem. Soc., Chem. Commun.* **1984**, 279.
(4) Warburg, O.; Negelein, E. *Chem. Ber.* **1930**, *63*, 1816.
(5) Lagarias, J. C. *Biochim. Biophys. Acta* **1982**, *717*, 12.
(6) Lemberg, R. *Biochem. J.* **1935**, *29*, 1322.
(7) Lemberg, R. *Rev. Pure Appl. Chem*. **1956**, *6*, 1.
(8) Balch, A. L.; Latos-Grażyński, L.; Noll, B. C.; Olmstead, M. M.; Safari, N. *J. Am. Chem. Soc.* **1993**, *115*, 9056.

S0020-1669(97)00503-X CCC: $14.00

Chart 1



of octaethyl-5-oxaporphyrin.[9,10] Additionally, a number of iron complexes that involve the *meso*-hydroxyoctaethylporphyrin, H_2-OEPOH (or octaethyloxophlorin), and octaethyl-5-oxaporphyrin, HOEOP, ligand systems have been thoroughly characterized.[11−17]

Recent work on the coupled oxidation of cobalt porphyrins has revealed that successful conversion of Co^{II}(OEP) into [Co^{II}(OEOP)]$^+$ (OEOP, anion of octaethyl-5-oxaporphyrin) and Cl_2Co^{III}(OEOP) depends critically on the solvent and its ability to function as an axial ligand.[18,19] Thus, coupled oxidation of Co^{II}(OEP) does not proceed in pyridine or pyridine/dichloromethane solutions but does work in a mixture of dichloromethane and tetrahydrofuran.

Here we describe some iron porphyrin chemistry relevant to heme degradation that occurs under pyridine-free conditions. We have found that iron complexes of octaethylporphyrin and octaethyloxophlorin air oxidize in the presence of ascorbic acid and cyanide ion to form either the dimeric complex {Fe^{III}(OEPO)}$_2$[11] or the octaethyl-5-oxaporphyrin complex {(NC)$_2$Fe^{III}(OEOP)}[16] (verdoheme) under varying reaction conditions. Some intermediates in this process have also been detected through the use of paramagnetic NMR studies.

Results

Synthetic Studies. The chemical transformations uncovered in this work are summarized in Chart 1. Four of the species—{Fe^{III}(OEPO)}$_2$,[11] {(py)$_2$Fe(OEPO)} (OEPO, trianion of octaethyloxophlorin),[12−14] {BrFe^{III}(OEPO•)},[15] and {(NC)$_2$Fe^{III}-(OEOP)}[16]—shown in the chart have been characterized previously by ^{1}H NMR and UV/visible spectroscopy, and three of

(9) Balch, A. L.; Koerner, R.; Olmstead, M. M. *J. Chem. Soc. Chem. Commun.* **1995**, 873.
(10) Balch, A. L.; Latos-Grażyński, L.; Noll, B. C.; Olmstead, M. M.; Szterenberg, L.; Safari, N. *J. Am. Chem. Soc.* **1993**, *115*, 1422.
(11) Balch, A. L.; Latos-Grażyński L.; Noll, B. C.; Olmstead, M. M.; Zovinka, E. P. *Inorg. Chem.* **1992**, *31*, 2248.
(12) Morishima, I.; Fujii, H.; Shiro, Y.; Sano, S. *J. Am. Chem. Soc.* **1986**, *108*, 3858.
(13) Balch, A. L.; Noll, B. C.; Reid, S. M.; Zovinka, E. P. *Inorg. Chem.* **1993**, *32*, 2610.
(14) Morishima, I.; Fujii, H.; Shiro, Y.; Sano, S. *Inorg Chem.* **1995**, *34*, 1528.
(15) Balch, A. L.; Latos-Grażyński, L.; Noll, B. C.; Szterenberg, L.; Zovinka, E. P. *J. Am. Chem. Soc.* **1993**, *115*, 11846.
(16) Balch, A. L.; Noll, B. C.; Safari, N. *Inorg. Chem.* **1993**, *32*, 2901.
(17) Balch, A. L.; Koerner, R.; Latos-Grażyński, L.; Noll, B. C. *J. Am. Chem. Soc.*, **1996**, *118*, 2760.
(18) Balch, A. L.; Mazzanti, M.; Olmstead, M. M. *J. Chem. Soc., Chem. Commun.* **1994**, 269.
(19) Balch, A. L.; Mazzanti, M.; St. Claire, T. N.; Olmstead, M. M. *Inorg. Chem.* **1995**, *34*, 2194.

these—$\{(py)_2Fe(OEPO)\}$,[17] $\{BrFe^{III}(OEPO^{\bullet})\}$,[15] and $\{(NC)_2Fe^{III}(OEOP)\}$[16]—were isolated and subjected to single-crystal X-ray diffraction studies. However, all of the chemical transformations shown in Chart 1 are new except for the conversions of dimeric $\{Fe^{III}(OEPO)\}_2$ into $\{(py)_2Fe(OEPO)\}$[13] and into $\{BrFe^{III}(OEPO^{\bullet})\}$,[15] which were described earlier.

Addition of $ClFe^{III}(OEP)$ to a methanolic solution of ascorbic acid and potassium cyanide leads to oxidative degradation of the porphyrin ring to form either the oxophlorin complex $\{Fe^{III}(OEPO)\}_2$ or the verdoheme $\{(NC)_2Fe^{III}(OEOP)\}$, depending upon reaction conditions. When $ClFe^{III}(OEP)$ is dissolved in a methanolic solution of ascorbic acid (46 equiv) and potassium cyanide (10 equiv) and is allowed to stand overnight under air, dimeric $\{Fe^{III}(OEPO)\}_2$ precipitates from the solution and may be collected by filtration. The identity of the precipitate was determined by comparison of the ^{1}H NMR and UV/visible spectra with those from an authentic sample.[11] This reaction represents a convenient method of preparation of not only $\{Fe^{III}(OEPO)\}_2$ but also of H_2OEPOH,[20] which may be obtained by treatment of $\{Fe^{III}(OEPO)\}_2$ with hydrogen chloride in 92% yield. We have found that the reaction solution used for the preparation of $\{Fe^{III}(OEPO)\}_2$ can be recycled. In a second use of the same reaction mixture, a 91% yield of $\{Fe^{III}(OEPO)\}_2$ was obtained. In a third use of the same reaction mixture, however, the yield dropped to 48%. Adding more ascorbic acid or more cyanide to the medium does not restore its ability to oxidize $ClFe^{III}(OEP)$ to $\{Fe^{III}(OEPO)\}_2$. It appears that a product is generated in the reaction mixture that retards the desired reaction. The nature of the inhibition has been briefly explored, and it is likely that cyanate formation is involved. Addition of an equivalent molar amount of potassium cyanate to a freshly prepared reaction mixture reduces the yield of $\{Fe^{III}(OEPO)\}_2$ to 80%, while addition of a 10-fold excess of potassium cyanate to the freshly prepared reaction mixture results in no precipitation of $\{Fe^{III}(OEPO)\}_2$.

If the coupled oxidation is run with a higher concentration of potassium cyanide, then the reaction produces the verdoheme $[(NC)_2Fe^{II}(OEOP)]^-$ rather than $\{Fe^{III}(OEPO)\}_2$. Thus, when the potassium cyanide concentration is increased to 127 equiv and the reaction solution is stirred vigorously under air for 3 h, a dark green solution is produced. The UV/visible spectrum of the reaction solution is similar to that of iron(II) verdoheme complexes.[16] Under these conditions, no precipitate of $\{Fe^{III}(OEPO)\}_2$ forms. Upon workup of the reaction solution in air (see Experimental Section), the oxaporphyrin complex $\{(NC)_2Fe^{III}(OEOP)\}$ is isolated in 48% yield. The ^{1}H NMR and UV/visible spectra of the product were identical to those from an authentic sample.

Treatment of a chloroform solution of $\{Fe^{III}(OEPO)\}_2$ with 10 molar equiv of bis(triphenylphosphine)iminium cyanide in air produces a deep blue solution from which the oxaporphyrin complex $\{(NC)_2Fe^{III}(OEOP)\}$ can be isolated in 95% yield. The identity of the product has been established by comparison of its UV/visible and ^{1}H NMR spectra with those of an authentic sample.[16] Previously $\{(NC)_2Fe^{III}(OEOP)\}$ had been prepared from the verdoheme $\{(py)_2Fe^{II}(OEOP)\}$ by oxidation with dioxygen in the presence of hydrogen chloride to form $\{Cl_2Fe^{III}(OEOP)\}$ and subsequent treatment of $\{Cl_2Fe^{III}(OEOP)\}$ with bis(triphenylphosphine)iminium cyanide.[16] The conversion of $ClFe^{III}(OEP)$ or $\{Fe^{III}(OEPO)\}_2$ into $\{(NC)_2Fe^{III}(OEOP)\}$ described here represents the first example of production of a 5-oxaporphyrin macrocycle via an oxidative process in which pyridine or another nitrogen donor from a protein is not involved.

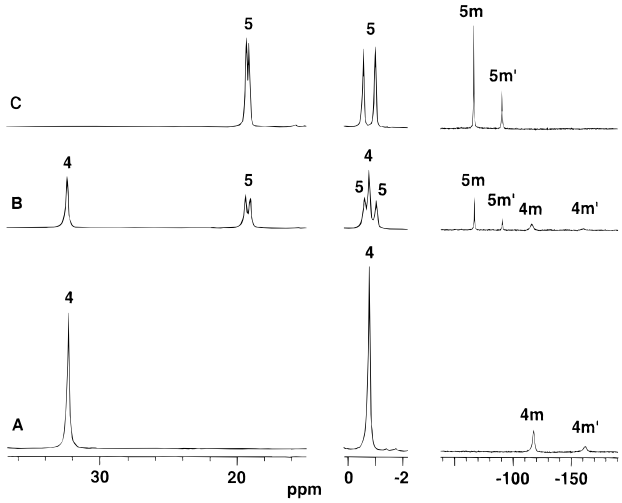


Figure 1. The 300 MHz ^{1}H NMR spectra of pyridine-d_5 solutions of (A) $\{(py)_2Fe(OEPO)\}$; (B) $\{(py)_2Fe(OEPO)\}$ and 0.5 molar equiv of bis(triphenylphosphine)iminium cyanide, and (C) $\{(py)_2Fe(OEPO)\}$ and 1.0 molar equiv of bis(triphenylphosphine)iminium cyanide at 25 °C. Resonances of $\{(py)_2Fe(OEPO)\}$ are labeled **4** while those of $[(NC)(py)Fe(OEPO)]^-$ are labeled **5** with subscripts m and m' denoting meso resonances.

Formation of $[(NC)(py)Fe(OEPO)]^-$. The reaction of $\{(py)_2Fe(OEPO)\}$ with bis(triphenylphosphine)iminium cyanide in dioxygen-free pyridine-d_5 solution has been monitored by ^{1}H NMR spectroscopy. Relevant data are shown in Figure 1. Trace A shows the familiar spectrum of $\{(py)_2Fe(OEPO)\}$[12-14] with two, upfield-shifted meso resonances and two of the four methylene resonances, one with an upfield shift and the other with a downfield shift. The other two methylene resonances along with the methyl resonances occur in the 1−10 ppm region. Trace B shows the spectrum of the solution after the addition of 0.5 equivalent of cyanide ion. Along with resonances from $\{(py)_2Fe(OEPO)\}$, new meso and methylene resonances are present which are assigned to the formation of $[(NC)(py)Fe(OEPO)]^-$. Trace C shows the spectrum of a solution to which 1 equiv of cyanide ion has been added. The resonances of the starting material, $\{(py)_2Fe(OEPO)\}$, are no longer present. The methylene resonances of the product, $[(NC)(py)Fe(OEPO)]^-$, that are visible in trace C, appear as diastereotopic pairs. Addition of up to 10 equiv of cyanide ion to the sample does not produce further changes in the spectrum. Hence, the equilibrium constant for the second replacement of pyridine by cyanide is substantially smaller than that of the first pyridine.

Figure 2 shows a magnitude COSY spectrum that was obtained from a pyridine solution of $[(NC)(py)Fe(OEPO)]^-$. Cross peaks are useful for identifying individual ethyl groups since they are expected between the methylene and methyl protons with ∼14 Hz coupling for geminal and 7 Hz coupling for vicinal protons.[21] Cross peaks from geminal coupling are seen in the four pairs of diastereotopic methylene resonances. Additionally these pairs of resonances have cross peaks that reveal coupling to the four methyl resonances that occur in the 1−3 ppm region. These spectral results indicate that the methylene protons in each ethyl group are inequivalent, a situation that is consistent with the presence of two different axial ligands, pyridine and cyanide, in the complex.

(20) For other methods of preparation of *meso*-hydroxyporphyrins (oxophlorins) see: (a) Barnett, G. H.; Hudson, M. F.; Smith, K. M. *J. Chem. Soc., Perkin Trans. 1* **1973**, 691. (b) Crossley, M. J.; King, L. G. Pyke, S. M. *Tetrahedron* **1987**, *43*, 4569.

(21) Keating, K. A.; de Ropp, J. S.; La Mar, G. N.; Balch, A. L.; Shiau, F.-Y.; Smith, K. M. *Inorg. Chem.* **1991**, *30*, 3258.

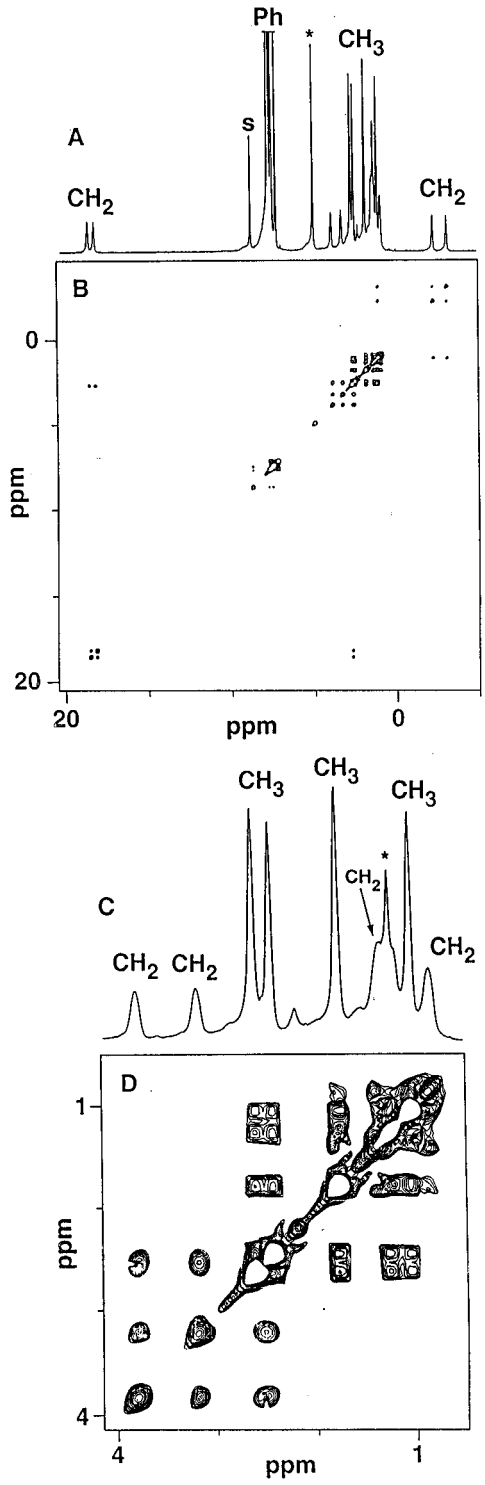


Figure 2. Magnitude COSY spectra for $[(NC)(py)Fe(OEPO)]^-$ in pyridine-d_5 at 23.6 °C. (A) and (B) show the full spectra, while (C) and (D) focus on the crowded 4−1 ppm region.

Cleavage of $\{Fe^{III}(OEPO)\}_2$ by Cyanide Ion. In the presence of a large excess of cyanide ion, $\{Fe^{III}(OEPO)\}_2$ undergoes cleavage slowly to form air-sensitive $[(NC)_2Fe(OEPO)]^{2-}$. Thus, treatment of a dioxygen-free methanol suspension of $\{Fe^{III}(OEPO)\}_2$ with a 100-fold molar excess of potassium cyanide results in the gradual dissolution of the iron complex and the eventual formation of a homogenous green solution. The ^{1}H NMR spectrum of the resulting solution is shown in Figure 3. The overall pattern of resonances, with two upfield-shifted meso resonances and methylene resonances in the 12 to −2 ppm region, is similar to that of $\{(py)_2Fe(OEPO)\}$.[12−14] Since only four methylene resonances

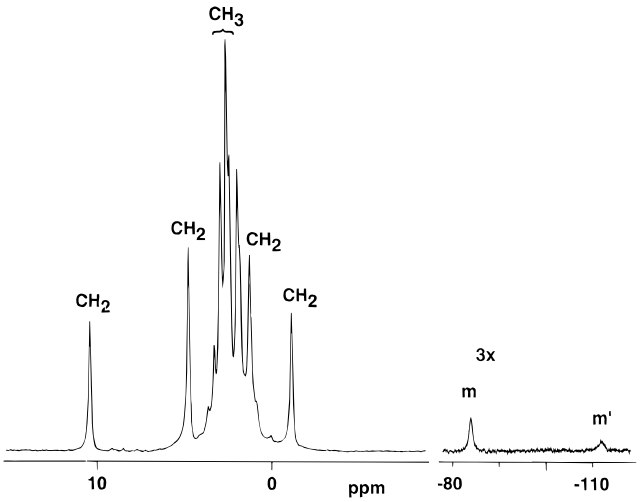


Figure 3. The 300 MHz ^{1}H NMR spectrum of a methanol-d_4 solution of $[(NC)_2Fe(OEPO)]^{2-}$ that was obtained from treating $\{Fe^{III}(OEPO)\}_2$ with potassium cyanide.

Table 1. Solvent Dependence of Chemical Shifts for $[(NC)_2Fe(OEPO)]^{2-}$

solvent	cyanide salt	methylene (ppm)				meso (ppm)	
dimethyl sulfoxide-d_6	KCN	12.2	2.6	0.76	−3.4	−65.8	−92.5
	BPCN[a]	12.1	*c*	*c*	−3.4	−65.0	−90.9
chloroform-*d*	BPCN	14.4	*c*	*c*	−0.015	−68.1	−90.9
methanol-d_4	KCN	10.4	2.5	1.80	−1.2	−86.2	−114.7
acetonitrile-d_3	TBA-CN[b]	14.6	*c*	*c*	−3.2	−59.6	−79.8

[a] Bis(triphenylphosphine)iminium cyanide. [b] tetra(*n*-butyl)ammonium cyanide. [c] Unable to determine chemical shift.

are observed, the axial ligation on both sides of the macrocycle is the same. Similarly, $\{Fe^{III}(OEPO)\}_2$ reacts with a 50-fold molar excess of bis(triphenylphosphine)iminium cyanide in dioxygen-free chloroform to form $[(NC)_2Fe(OEPO)]^{2-}$. The ^{1}H NMR spectrum of the complex anion shows a significant solvent dependence as summarized in Table 1, but the overall pattern is similar to that seen in Figure 3. The solvent dependance of the NMR spectra of related iron(III) porphyrin bis(cyano) adducts is well-known.[22,23] In particular, the large difference seen for the methanol solution when compared with the other solvents is readily explained by the presence of hydrogen bonding between the axial ligands and methanol.

The UV/visible absorption spectrum of a chloroform solution of $[(NC)_2Fe(OEPO)]^{2-}$ is shown in Figure 4. The spectrum displays a Soret peak at 428 nm and broad absorptions at 634 and 678 nm which resemble corresponding features seen in the UV/visible spectrum of $\{(py)_2Fe(OEPO)\}$.[12,24]

Solutions of $[(NC)_2Fe(OEPO)]^{2-}$ are air sensitive. Upon exposure to air, the UV/visible and ^{1}H NMR spectra of solutions of $[(NC)_2Fe(OEPO)]^{2-}$ show changes that indicate that $\{(NC)_2Fe^{III}(OEOP)\}$ is eventually formed.

Formation of $[(NC)_2Fe(OEPO)]^-$. Air-sensitive solutions of $[(NC)_2Fe(OEPO)]^-$ can be obtained in two ways: through oxidation of $[(NC)_2Fe(OEPO)]^{2-}$ with diiodine or through treatment of $\{BrFe^{III}(OEPO^{\bullet})\}$[15] with excess tetra(*n*-butyl)-ammonium cyanide. Figure 5 shows the ^{1}H NMR spectrum of a sample of $[(NC)_2Fe(OEPO)]^-$ that was obtained through the

(22) La Mar, G. N.; Del Gaudio, J.; Frye, J. S. *Biochim. Biophys. Acta* **1977**, *498*, 422.
(23) Walker, F. A.; Simonis, U. *Biological Magnetic Resonance. NMR of Paramagnetic Molecules;* Berliner, L. J., Reuben, J., Eds.; Plenum Press: New York, 1993; Vol. 2, p 133.
(24) Bonnett, R.; Dimsdale, M. J. *J. Chem. Soc., Perkin Trans 1* **1972**, 2540.

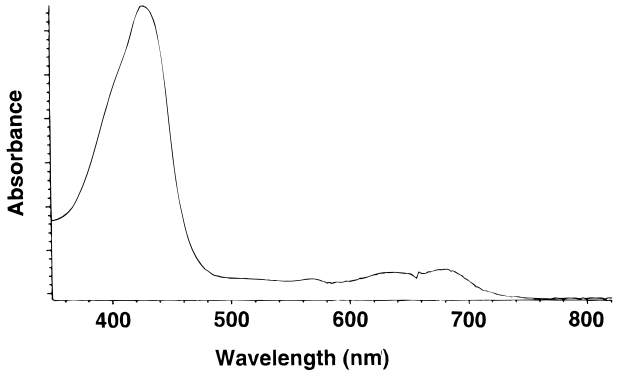


Figure 4. UV/visible spectrum of $[(NC)_2Fe(OEPO)]^{2-}$ in dichloromethane solution: λ_{max}, nm (ϵ, M^{-1} cm^{-1}; 408 (4.4×10^4), 428 (5.6×10^4), 634 (4.1×10^3), 678 (4.7×10^3).

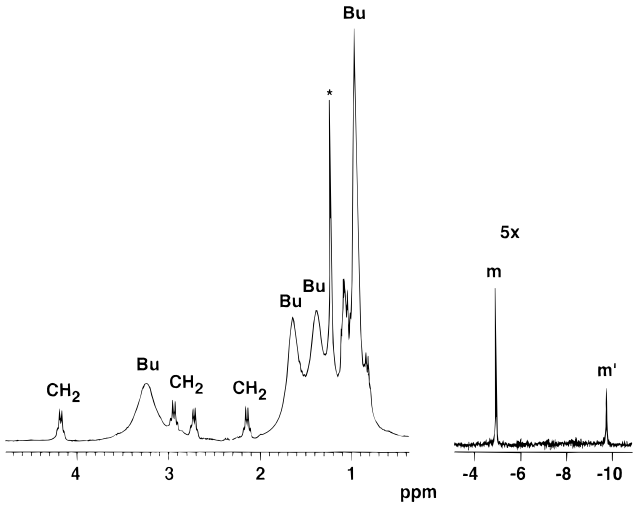


Figure 5. The 300 MHz ^1H NMR spectrum of $[(NC)_2Fe(OEPO)]^-$ obtained by treating $\{BrFe^{III}(OEPO^{\bullet})\}$ with 3 equiv of tetra(*n*-butyl)ammonium cyanide in chloroform-*d* at 25 °C. Resonances of the tetra(*n*-butyl)ammonium ion are identified by the label Bu, while the methylene and meso resonances of $[(NC)_2Fe(OEPO)]^-$ are identified by the labels CH_2 and m and m'. The resonance labeled * is from an impurity.

addition of 3 molar equiv of tetra(*n*-butyl)ammonium cyanide to a chloroform solution of $\{BrFe^{III}(OEPO^{\bullet})\}$. The spectrum shows two resonances at −9.8 and −5 ppm from the meso protons, four quartets in the 4.5−2.0 ppm region that are due to the methylene protons, and four resonances in the 2−0 ppm region that are due to the methyl protons. The overall pattern, with the slight upfield shift of the meso resonances, is similar to that observed for $[(py)_2Fe(OEPO)]^+$, the one-electron oxidation product of $\{(py)_2Fe(OEPO)\}$.[17] These meso resonances show a marked, non-Curie dependence on temperature which is shown in Figure 6. Upon cooling, these meso resonances shift downfield. The unusual temperature dependence of the ^1H NMR spectrum is readily understood if the even-electron complex, $[(NC)_2Fe(OEPO)]^-$, has an $S = 0$ ground state with a nearby $S = 1$ state that is significantly populated at 25 °C. Similar non-Curie behavior has been previously observed in the ^1H NMR spectra of the related complex, $[(py)_2Fe(OEPO)]^+$.[17]

Discussion

The results described here show that cyanide ion and pyridine behave similarly as axial ligands in oxidative heme degradation. Thus, an axial amine is not the only ligand that can promote methine bridge oxidation of iron porphyrins.

The metal plays a crucial role in directing the site of oxidation in such complexes. While oxygenation of both iron[8−10] and

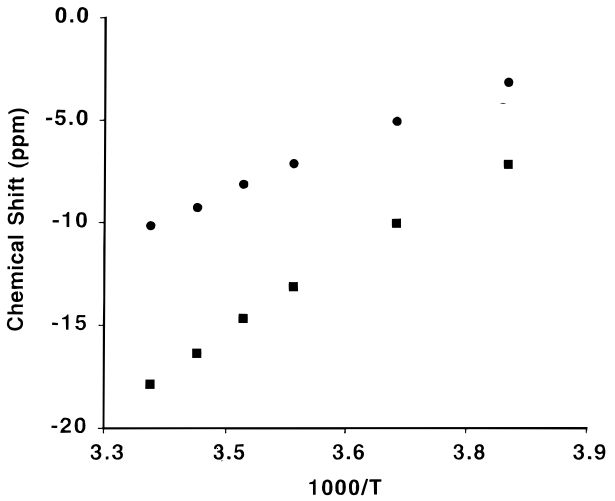
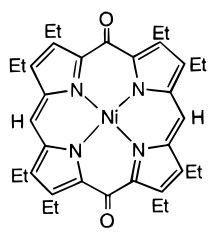
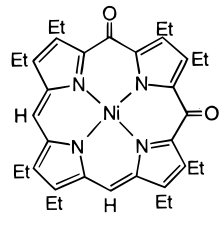


Figure 6. Chemical shift vs $1/T$ for the meso resonances of $[(NC)_2Fe(OEPO)]^-$ in chloroform-*d* solution.

cobalt[18,19] porphyrins yields a similar set of products, nickel porphyrin behaves differently. Recently this laboratory reported that in pyridine solution the nickel(II) complex $Ni^{II}(OEPOH)$ (OEPOH, dianion of *meso*-hydroxyoctaethylporphyrin), undergoes aerial oxidation to form the stable radical $(py)_2Ni^{II}(OEPO^{\bullet})$, which subsequently undergoes a slower oxidation at the other meso positions to yield the dioxoporphyrin complexes **10** and **11**.[25,26] Further work is clearly needed to elucidate how iron



10



11

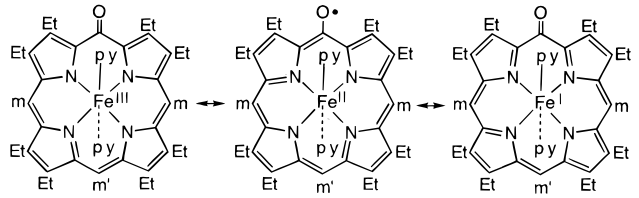
directs the oxidative reactivity away from the unoxygenated meso bridges and toward the oxygenated site so that C−C bond cleavage occurs.

The formation of $\{Fe^{III}(OEPO)\}_2$ during the modified coupled oxidation process developed here offers evidence that the initial step in heme oxidation involves oxygenation of a meso carbon. Although it has been widely assumed that oxidative heme degradation involves what is effectively hydroxylation at a meso position, direct evidence in the form of isolation of an intermediate from the reaction has been lacking. The ability to stop aerobic conversion of $ClFe^{III}(OEP)$ to $\{Fe^{III}(OEPO)\}_2$ (i.e., without further oxidation to from $\{(NC)_2Fe^{III}(OEOP)\}$) through control of the cyanide concentration, is largely due to the poor solubility of $\{Fe^{III}(OEPO)\}_2$ in methanol. Further oxidation of $\{Fe^{III}(OEPO)\}_2$ only occurs when a sufficient concentration of cyanide ion cleaves $\{Fe^{III}(OEPO)\}_2$ to form $[(NC)_2Fe(OEPO)]^{2-}$. Additionally, cleavage of $\{Fe^{III}(OEPO)\}_2$ by cyanide is a slow process, so that once precipitated, this dimeric complex is effectively protected from further reaction. However, it is possible to convert $\{Fe^{III}(OEPO)\}_2$ into the free ligand, H_2OEPOH, by treatment with acid, and this route from $ClFe^{III}(OEP)$ to H_2OEPOH is a convenient method for the preparation of H_2OEPOH.

(25) Balch, A. L.; Noll, B. C.; Phillips, S. L.; Reid, S. M.; Zovinka, E. P. *Inorg. Chem.* **1993**, *32*, 4730.
(26) Balch, A. L.; Olmstead, M. M.; Phillips, S. L. *Inorg. Chem.* **1993**, *32*, 3931.

The fact that the conversion of $ClFe^{III}(OEP)$ or $\{Fe^{III}(OEPO)\}_2$ to $\{(NC)_2Fe^{III}(OEOP)\}$ occurs in the presence of an excess of such a strongly bound ligand as cyanide suggests that the process does not require coordination of dioxygen by iron. Rather it is likely that dioxygen or a dioxygen-derived species (peroxide, superoxide, or hydroxyl radical) reacts directly at the porphyrin periphery to induce C−C bond cleavage in the coupled oxidation processes outlined here.

The new cyano complexes $[(NC)(py)Fe(OEPO)]^-$ and $[(NC)_2Fe(OEPO)]^{2-}$ have spectral characteristics that are similar to those of $\{(py)_2Fe(OEPO)\}$. The electronic structure of this compound has received considerable discussion.[12−14,17] The overall oxidation level is consistent with resonance structures **4a**, **4b**, and **4c** for $\{(py)_2Fe(OEPO)\}$. However, the properties



of $\{(py)_2Fe(OEPO)\}$ suggest a complex electronic structure with strong mixing of ligand and metal orbitals that is not readily explained by any one conventional oxidation and spin state assignment. Similar considerations also pertain to the electronic structures of $[(NC)(py)Fe(OEPO)]^-$ and $[(NC)_2Fe(OEPO)]^{2-}$. Additionally $[(NC)_2Fe(OEPO)]^{2-}$ and $\{(py)_2Fe(OEPO)\}$ are air sensitive, and both undergo conversion to oxaporphyrin (verdoheme) complexes in the presence of dioxygen.

The one-electron oxidation of $[(NC)_2Fe(OEPO)]^{2-}$ parallels the behavior of $\{(py)_2Fe(OEPO)\}$, whose redox properties have been recently described.[17] The two oxidation products, $[(NC)_2Fe(OEPO)]^-$ and $[(py)_2Fe(OEPO)]^+$, share common physical properties. Both have similar ^1H NMR spectral patterns in which the meso protons have an upfield bias at room temperature. The temperature dependence of these meso resonances for both species is non-Curie and indicative of a thermal equilibrium between a diamagnetic ground state and a paramagnetic excited state for these even-electron species.

In conclusion, this work demonstrates considerable parallels in the species present in the coupled oxidation process when either cyanide ion or pyridine is present as axial ligand. Since cyanide ion is frequently added to heme proteins to produce the low spin state of the iron and thus to facilitate their study by ^1H NMR techniques,[27] the results reported here have added relevance to the reactivity that may be seen with such cyanide-treated proteins.

Experimental Section

Preparation of Compounds. Authentic samples of $\{Fe^{III}(OEPO)\}_2$,[11] $\{(py)_2Fe(OEPO)\}$,[13,17] and $\{BrFe^{III}(OEPO^{\bullet})\}$[15] were prepared as described previously except that $\{Fe^{III}(OEPO)\}_2$ was recrystallized from dichloromethane/methanol before use.

Preparation of $\{Fe^{III}(OEPO)\}_2$. Potassium cyanide (65.5 mg, 1.01 mmol) and ascorbic acid (0.7973 g, 4.53 mmol) were dissolved in 50 mL of methanol. $ClFe^{III}(OEP)$ (61.4 mg, 0.0984 mmol) was added to the methanol solution, and the reaction was stirred in air for 12−18 h. During this time, the product precipitated as a dark black powder. The product was collected by filtration, washed with methanol, and air-dried. Yield: 52.2 mg (0.0432 mmol), 87.7% . The product was identified by comparison of its ^1H NMR spectrum with that of an authentic sample.

Preparation of $\{(NC)_2Fe^{III}(OEOP)\}$ Directly from $ClFe^{III}(OEP)$. $ClFe^{III}(OEP)$ (113 mg, 0.181 mmol) was added to a solution of ascorbic acid (1 g, 5.68 mmol) and potassium cyanide (1.5 g, 23.0 mmol) in 200 mL of methanol and stirred vigorously in air for 3 h. The dark green solution of $[Fe^{II}(OEOP)(CN)_2]^-$ was evaporated to dryness under reduced pressure, redissolved in 100 mL of dichloromethane, and washed with water (4 × 200 mL) in the presence of air. The resulting blue dichloromethane solution was dried over anhydrous sodium sulfate, filtered, and evaporated to dryness under reduced pressure. The solid was redissolved in a minimum volume of dichloromethane, and diethyl ether was slowly added. The recrystallized material was collected by filtration, washed with diethyl ether (20 mL), water (5 mL), methanol (10 mL), and diethyl ether (50 mL), and placed under a vacuum. Yield: 56.4 mg, 48.3%. The identity of the product was confirmed by comparison of its UV/visible and ^1H NMR spectra with those of an authentic sample of $\{(NC)_2Fe^{III}(OEOP)\}$.

Preparation of $\{(NC)_2Fe^{III}(OEOP)\}$ from $\{Fe^{III}(OEPO)\}_2$. Bis(triphenylphosphine)iminium cyanide (42 mg, 0.075 mmol) was added to a solution of 9 mg (0.0075 mmol) of $\{Fe^{III}(OEPO)\}_2$ in 15 mL of chloroform. The solution was stirred with exposure to air for 5 min. The resulting green solution was evaporated to dryness under vacuum. The colored solid was dissolved in a minimum volume of dichloromethane. This solution was subjected to chromatography on a 2.5 × 5 cm silica gel column with elution with chloroform. The blue band that eluted was collected and evaporated to dryness. The solid was purified by recrystallization from dichloromethane/diethyl ether. Yield 9.1 mg, 95%.

Preparation of H_2OEPOH from $\{Fe^{III}(OEPO)\}_2$. A 12.7 mg (0.0105 mmol) sample of $\{Fe^{III}(OEPO)\}_2$ was dissolved in 50 mL of chloroform. Hydrogen chloride was bubbled through the solution for 1 min. The solution was then stirred for 12−18 h. The color of the reaction solution changed from brown to purple. The solution was washed with aqueous sodium bicarbonate, and then the organic layer was separated and dried over sodium sulfate. After decanting the organic solution and removing the solvent on a rotary evaporator, the remaining residue was dissolved in a minimum of chloroform and subjected to chromatography on a silica gel column with chloroform as the eluant. The blue fraction was collected to yield 10.4 mg (92.4%) of H_2OEPOH. The product was characterized by comparison of the absorption and ^1H NMR spectra to those of an authentic sample.

Instrumentation ^1H NMR spectra were recorded on a General Electric QE-300 FT spectrometer operating in the quadrature mode (^1H frequency is 300 MHz). The spectra of paramagnetic complexes were collected over a 50−100 kHz bandwidth with 8K complex data points. For a typical spectrum, between 500 and 2000 transients were accumulated with a 50 ms delay time. The signal-to-noise ratio was improved by apodization of the free induction decay. The residual ^1H spectra of chloroform-*d* or dichloromethane-d_2 were used as secondary references. To obtain unambiguous methyl and methylene assignments in the diamagnetic region, an inversion recovery sequence was used with τ values of ∼100 ms.

The MCOSY spectrum[21] was obtained after collecting a standard 1D reference spectrum. The 2D spectrum was collected by use of 512 t_1 increments that consisted of 400 scans each with a block size of 1024 complex points. A spectral bandwidth of 10 kHz in both dimensions was utilized as the minimum necessary to observe the resonances of interest. A preacquisition delay of 150 ms was used. Prior to the collection of the first block, eight dummy scans were performed. The data were apodized in both dimensions by an unshifted squared sine bell function, and zero filled to form a 1K × 1K matrix prior to Fourier transformation. A magnitude calculation was performed on the resulting spectrum; this was followed by symmetrization.

Acknowledgment. We thank the National Institutes of Health (Grant GM26226) for support.

IC970503E

(27) For examples, see: La Mar, G. N.; Chen, Z.; Vyas, K.; McPherson, A. D. *J. Am. Chem. Soc.* **1995**, *117*, 411. Sette, M.; de Ropp, J. S.; Hernández, G.; La Mar, G. N. *J. Am. Chem. Soc.* **1993**, *115*, 5237.