Verdoheme-like Oxaporphyrin Formation by Oxygenation of a Co(II) Porphyrinyl Naphthoic Acid. A New Model of Heme Degradation

C. K. Chang,* Gladys Avilés, and Nilkamal Bag

Department of Chemistry, Michigan State University
East Lansing, Michigan 48824

Received September 20, 1994

Biological heme degradation catalyzed by heme oxygenases (HO) is the source of bile pigments as well as endogenous carbon monoxide, a putative second neural messenger.[1,2] The major events occurring in HO-dependent transformations are O_2 binding and activation at the heme iron, followed by hydroxylation at the porphyrin meso position to form an α-hydroxyprotoheme, which then reacts with a second mole of O_2 to give verdoheme with concomitant elimination of the meso carbon as CO. Finally verdoheme reacts with a third molecule of O_2 to form biliverdin. While recent characterization of HO[3,4] and model studies based on coupled oxidation[5−8] have shed much light on the first and last stages of this process, mechanistic description for the conversion of *meso*-hydroxyheme to verdoheme remains poorly defined. Sano and coworkers[7] have demonstrated that this conversion can occur spontaneously in solution without enzyme. The driving force is suggested to arise from the facile formation of an oxophlorin radical that promotes oxygen attack at a porphyrin ring carbon. However, without additional evidence, little is certain about how the oxygenation and the elimination of CO actually happen. It would be of great value if a parallel of this reaction could be found. We here report such an example.

During the course of our study of H-bonding effects on heme−oxygen binding, we synthesized a naphthoic acid porphyrin **1**[9] and observed a curious reaction which bears much similarity to the verdoheme formation. When Co(II) complex **1a** was exposed to air in CH_2Cl_2, the color quickly changed from red to green in less than 1 min. The green product, after being isolated by chromatography, has a molecular ion of 538 (by FAB-MS) consistent with the oxaporphyrin cation **2**. The absorption spectral features of **2** are solvent dependent (Figure 1). Even though **2** may become Co(III) in air, the observation that an EPR-active material can be obtained initially without any reducing agent present during the entire process argues that

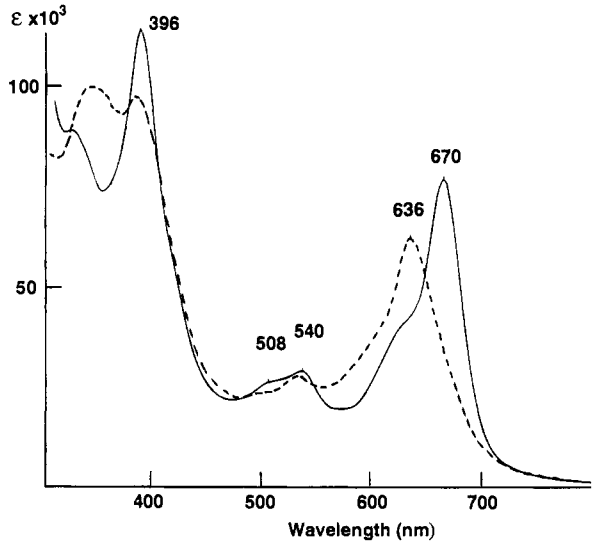
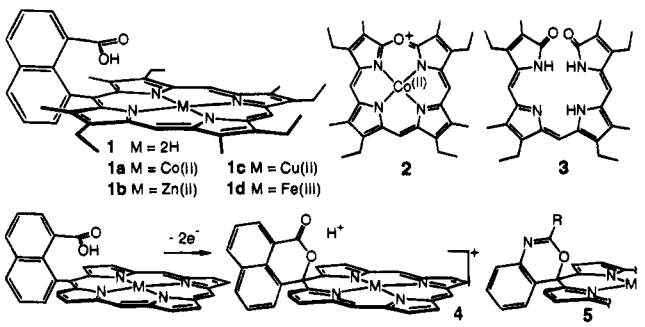


Figure 1. Absorption spectra of the paramagnetic oxaporphyrin **2** (- - -) in 5% MeOH/CH_2Cl_2 and (—) after being dried in air and dissolved in CH_2Cl_2. Both spectra share the distinctive features of verdoheme. The 670-nm spectrum, based on its similarity to a structurally characterized Co(III) oxaporphyrin[10] and its diminished spin concentration (10−15% by EPR), belongs to cobaltic species.



a cobaltous complex must be the immediate product. The oxaporphyrin macrocycle can be readily hydrolyzed by acid or base to give the metal-free biliverdin **3**.[11] It is remarkable that this conversion of **1a** to **2** is rapid, regiospecific, and quantitative.[12] The only other product is 1,8-naphthaldehydic acid, which can be isolated if due precaution is exercised to prevent its oxidation by air into 1,8-naphthalic anhydride.[13] Formation of the oxaporphyrin **2** requires a 1:1 ratio of O_2:**1a**, and the source of oxygen in **2** is molecular oxygen; quantitative incorporation of ^{18}O occurs if $^{18}O_2$ gas is used to react with **1a**. The reaction needs water to be present, but $H_2{}^{18}O$ is not incorporated into **2**. Under scrupulously anhydrous conditions, the reaction does not proceed to completion; it stops at a stage exhibiting a brownish color (λ_{max} 435, 569, 673, and 760 nm). At this point, the solution can be deaerated, and upon addition of O_2-free water, **2** is again obtained. Field desorption mass spectra of the intermediate indicate that its molecular ion is

(1) Maines, M. D. *Heme Oxygenase—Clinical Applications and Functions*; CRC Press: Boca Raton, FL, 1992.
(2) (a) Verma, A.; Hirsch, D. J.; Glatt, C. E.; Ronnett, G. V.; Snyder, S. H. *Science* **1993**, *259*, 381−384. (b) Zhuo, M.; Small, S. A.; Kandel, E. R. Hawkins, R. D. *Science* **1993**, *260*, 1946−1950.
(3) Wilks, A.; Ortiz de Montellano, P. R. *J. Biol. Chem.* **1993**, *268*, 22357−22362.
(4) (a) Sun, J.; Wilks, A.; Ortiz de Montellano, P. R.; Loehr, T. M. *Biochemistry* **1993**, *32*, 14151−14157. (b) Takahashi, S.; Wang, J.; Rousseau, D. L.; Ishikawa, K.; Yoshida, T.; Host, J. R.; Ikeda−Saito, M. *J. Biol. Chem.* **1994**, *269*, 1010−1014.
(5) (a) O'Carra, P. In *Porphyrins and Metalloporphyrins*; Smith, K. M., Ed.; Elsevier: New York, 1975; p 122. (b) Schmid, R.; McDonagh, A. F. In *The* Porphyrins; Dolphin, D., Ed.; Academic Press: New York, 1979; Vol. VI, p 258.
(6) Saito, S.; Itano, H. A. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 1393.
(7) (a) Sano, S.; Sugiura, Y.; Maeda, Y.; Ogawa, S.; Morishima, I. *J. Am. Chem. Soc.* **1981**, *103*, 2888−2890. (b) Sano, S.; Sano, T.; Morishima, I.; Shiro, Y.; Maeda, Y. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 531−535.
(8) (a) Balch, A. L.; Latos-Grazyński, L.; Noll, B. C.; Olmstead, M. M.; Szterenberg, L.; Safari, N. *J. Am. Chem. Soc.* **1993**, *115*, 1422−1429. (b) Balch, A. L.; Latos-Grazyński, L.; Noll, B. C.; Olmstead, M. M.; Safari, N. *J. Am. Chem. Soc.* **1993**, *115*, 9056−9061.
(9) Chang, C. K.; Kondylis, M. P. *J. Chem. Soc., Chem. Commun.* **1986**, 316−918.
(10) (a) Balch, A. L.; Mazzanti, M.; Olmstead, M. M. *J. Chem. Soc., Chem. Commun.* **1994**, 269−270. (b) A Co(II) oxaporphyrin has also been characterized: Balch, A. L.; Mazzanti, M.; St. Claire, T. N.; Olmstead, M. M. *Inorg. Chem.*, submitted by publication.

(11) **3** was verified by NMR, MS, etc., and was independently synthesized according to the literature: Smith, K. M. *J. Chem. Soc., Perkin Trans 1* **1972**, 1471−1475.
(12) Three individual researchers who have run this reaction more than 20 times invariably achieved 90−94% isolated yields.
(13) 1,8−Naphthaldehydic acid is notoriously susceptible to air oxidation. It was analyzed by GC using a 25-m ethyl silicone capillary column. Several commercial and freshly prepared samples (from acenaphthenequinone: Bader, H.; Chiang, Y. H. *Synthesis* **1976**, 249) were used as reference, and all were shown to be contaminated with variable amounts (20−60%) of 1,8−naphthalic anhydride. A CH_2Cl_2 solution (1 mL) of **1a** (3.5 mg, 5 μmol) was stirred with 0.2 mL (8 μmol) of O_2 for 10 min and without purification, this mixture was injected into the GC, which showed 8:2 ratio of 1,8-naphthaldehydic acid *vs* the anhydride in the product.

0002-7863/94/1516-12127$04.50/0

Scheme 1

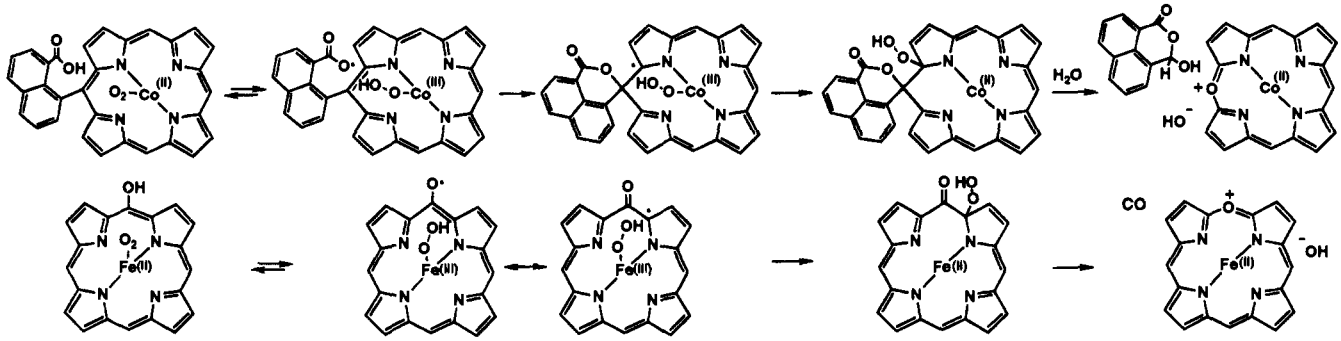


Table 1. Cyclic Voltammetric Data of **1** and Corresponding Methyl Ester Derivatives[a,b]

compound	first oxidation, E_{pa}, V	second oxidation, $E°$, V
Co (**1a**)	0.96 ($Co^{3+/2+}$ at 0.61 V)	
Zn (**1b**)	0.80	
Cu (**1c**)	0.86	
Co (**1a** Me ester)	0.81 ($Co^{3+/2+}$ at 0.60 V)	1.12
Zn (**1b** Me ester)	0.63	0.94
Cu (**1c** Me ester)	0.65	1.08

[a] Conditions: solvent, dichloromethane; supporting electrolyte, tetrabutylammonium perchlorate; working electrode, platinum; reference electrode, SCE; solute concentration, $\sim 10^{-3}$ M; temperature, 298 K. [b] $E° = 0.5(E_{pa} + E_{pc})$, where E_{pa} and E_{pc} are anodic and cathodic peak potentials, respectively; scan rate at 100 mV s^{-1}.

equivalent to an dioxygen adduct of the parent compound, with a structure much less symmetrical than **1a**, as shown by NMR.[14]

Porphyrin **1** is sterically congested, and the carboxylic group is found to lie parallel to the porphyrin plane in the X-ray structure.[15] There is a tendency for the carboxylate to attack at the porphyrin meso carbon to give a naphthalein-type lactone **4**. The lactonization is accompanied and facilitated by oxidation. For example, a CH_2Cl_2 solution of **1b** exposed to air quickly develops an intense EPR $g = 2$ signal, quite similar to that found with oxophlorin.[16] Furthermore, electrochemical studies of various metal complexes (**1a**–**d**) indicate that they undergo a reversible two-electron oxidation at potentials differing from the onset of the typical porphyrin cation radical formation (Table 1). Blocking the carboxylic acid by esterification restores the normal two one-electron oxidation reactions. The observed behavior is analogous to that of the *meso*-(*o*-anilido)porphyrins reported by Savéant and co-workers, who demonstrated a two-electron oxidation product to be an oxazine ring **5** formed upon condensation of a meso carbon of the porphyrin dication with the oxygen of the amide group.[17] The two-electron oxidation products of our naphthoic acid porphyrin complexes also display an isoporphyrin-like spectrum.

Given these observations, we interpret the spontaneous degradation of **1a** in Scheme 1. The acid proton dramatically enhances O_2 binding to the Co(II) porphyrin by H-bonding to or protonation of the cobaltic superoxide adduct. This is quickly followed by the lactonization occurring at the meso carbon imparting a radical nature to the macrocycle. This species with unpaired spin density at the porphyrin ring skeleton could then react with O_2 or, more likely, with the cobalt-bound hydroperoxy radical (*vide infra*). Eventually, in the presence of H_2O, the meso carbon is eliminated as part of the leaving naphthaldehydic acid. The mechanism for the final steps is unknown, but the overall sequence is electron-neutral, and there is no need for oxidizing or reducing equivalents.

In comparison with the *meso*-hydroxyheme, the close similarities between the two systems become immediately obvious. In both cases it is the propensity of forming a phlorin radical[18] that brings about an intramolecular, net two-electron reduction of dioxygen. Both sequences are electronically self-sufficient to account for their spontaneity and high yields. Furthermore, the same regiochemistry observed here is important. MO calculations of an oxophlorin radical have predicted the highest spin density on the γ-meso position opposite to the oxy group[19] and therefore would render a direct reaction between molecular oxygen and the phlorin radical unlikely. The fact that specific cleavage of the substituted meso carbon occurs in both Fe and Co systems would argue for a specific role of the substituent (hydroxy or naphthoic acid) in directing the incoming attack—possibly by a 1,3-sigmatropic shift of the metal-bound hydroperoxy group already tilting toward the substituent via H-bonding. In the hydroxyheme system, internal electron transfer to the coordinated O_2 and the irreversible formation of a carbon-bound peroxy adduct occur relatively fast. In the naphthoic acid system, multiple energy barriers have been added to the sequence, and the internal redox reaction is now coupled to the lactonization, which could be rate-limiting. If the Co(II) in **1a** is replaced by Fe(II), other autoxidation pathways (e.g., μ-peroxo dimer formation between two molecules) for the heme iron become so competitive that only the Fe(III) complex **1d** is obtained with *no* degradation of the macrocycle. Likewise, any condition that normally catalyzes Co(II) porphyrin autoxidation would prevent the ring degradation even for **1a**. Thus, noncoordinating solvents (e.g., CH_2Cl_2 and benzene) provide the best environment for generating **2**, while in DMF or solvents containing nitrogen donors, reversible O_2 binding can still be observed at subzero temperatures,[9] as low temperature retards both cobaltous ion oxidation and the lactonization.

The discovery of the cobalt model reaction for heme degradation firmly establishes the importance of the macrocycle as donor for activating both O_2 and the ring during verdoheme formation, and the additional requirement of H_2O in the cobalt system may provide a much needed window to reveal how the meso carbon is eliminated from the porphyrin ring.

Acknowledgment. This work was supported in part by a grant from NIH (GM34468). We thank Michael Kondylis for first discovering the anomaly of **1a** and Professor Alan Balch for comments and providing results prior to publication.

(14) The paramagnetic **2**, as well as the initial porphyrin **1a**, exhibits broadened but discernible NNR peaks of two methyl singlets and ethyl peaks expected for the type IV symmetry. The intermediate, however, has multiple sets of methyl and ethyl peaks.

(15) Chang, C. K.; Peng, S.-M., manuscript in preparation.

(16) (a) Fuhrhop, J.-H.; Besecke, S.; Subramanian, J. *J. Chem. Soc., Chem. Commun.* **1973**, 1–2. (b) Bonnett, R.; Dimsdale, M. J.; Sales, K. D. *J. Chem. Soc., Chem. Commun.* **1970**, 962–963.

(17) El–Kasmi, A.; Lexa, D.; Maillard, P.; Momenteau, M.; Savéant, J.-M. *J. Am. Chem. Soc.* **1991**, *113*, 1586–1595.

(18) Redox relationship:

$$\text{phlorin} \xrightarrow[-H^+]{-e^-} \text{phlorin radical} \xrightarrow[-H^+]{-e^-} \text{isoporphyrin}$$

(19) (a) Morishima, I.; Fujii, H.; Shiro, Y.; Sano, S. *J. Am. Chem. Soc.* **1986**, *108*, 3858–3860. (b) Balch, A. L.; Noll, B. C.; Reid, S. M.; Zovinka, E. P. *J. Am. Chem. Soc.* **1993**, *115*, 2531–2532.