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$^{\bullet}NO_2$-Mediated *meso*-Hydroxylation of Iron(III) Porphyrin

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$^{\bullet}NO_2$ generated from $^{\bullet}NO$ and air add to iron(III) porphyrin upon nitration, which isomerized spontaneously to hydrolysis, yielding a *meso*-hydroxylated cation that seized by the end oxidation product, a nitrate ion, through extensive hydrogen bonding.

Nitric oxide ($^{\bullet}NO$) mediates a variety of physiological functions.[1] In endothelial cells, it is likely depleted from the tissue through oxidative interaction with oxyhemoglobin. The $^{\bullet}NO$-induced oxidation of oxyhemoglobin proceeds via several intermediates to form methemoglobin with a heme ($Fe^{III}OH_2$) moiety and nitrate.[2] The formation of methemoglobin triggers the onset of methemoglobinemia, which markedly up-regulates hemoxygenase. Nevertheless, in the catabolic degradation of heme by hemoxygenase, its oxidative degradation to biliverdin starts with the formation of the initial intermediate, α-hydroxyheme.[3] Phlorins, with a hydroxyl group in the meso position, are known, and their metal complexes have been synthesized.[4] Woodward suggested isoporphyrins as tautomers of porphyrins with a saturated *meso*-carbon.[5] Isoporphyrins with tetraaryl derivatives were studied with relevance to heme degradation processes.[6] In the tissue biochemistry, the individual roles of O_2 and $^{\bullet}NO$ are paramount, notwithstanding that both are mediators for oxidative/nitrative stress injury.[7,8] The possibility of synergistic/antagonistic involvement of $^{\bullet}NO$ with O_2 in heme proteins arouses our interest, and the present work is related to the chemistry of iron(III) porphyrin as a model of a heme protein with $^{\bullet}NO$ under exposure of air.

meso-Tetrakis(3,4,5-trimethoxyphenyl)porphyrinatoiron(III) chloride (**1**) was prepared by an alternative method[9] and, as expected, it did not react with O_2 in dichloromethane (DCM) even upon standing for days. However, **1** in the reduced state as iron(II) was readily oxygenated by O_2 to yield a μ-oxo-bridged dimeric iron(III) porphyrin unit.[10] $^{\bullet}NO$ with its low ionization potential is known to release its odd electron in reductive nitrosylation, but with the $\{Fe^{III}N_4\}$ group present in **1**, such a reaction did not occur. However, **1** in DCM containing $^{\bullet}NO$ and blanketed with air-saturated hexane upon standing for 1 week led to the crystallization of aquachloro-*meso*-hydroxytetrakis(3,4,5-trimethoxyphenyl)porphyrinatoiron(III) nitrate(**2**).[11] Native α-hydroxyheme exists as a mixture of iron(III) phenolate, iron(III) keto anion, and iron(II) keto π neutral radical resonance structures.[12] The stability of the cation of **2** in enol form lies in its involvement in hydrogen bonding. In the lattice (Figure 1b), two such units of **2** are held together using an intricate network of hydrogen bonding. This network involves two *meso*-hydroxyl groups (O1) from two porphyrins, two axially attached water molecules (O14) trans to a chloro group, and two nitrate anions (O15, O16, and O17) (Figure 1b). The molecular structure of the cation of **2** is presented in Figure 1a. The asymmetric unit contains one aquachloro-*meso*-hydroxytetrakis(3,4,5-trimethoxyphenyl)porphyrinato-

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(11) Detailed synthesis: see the Supporting Information. Crystallographic data for **2**: $C_{57}H_{57}Cl_3FeN_5O_{17}$, M_r = 1246.28, 0.20 × 0.10 × 0.08 mm^3, triclinic, a = 14.899(5) Å, b = 15.181(5) Å, c = 16.891(5) Å, α = 67.672(5)°, β = 67.322(5)°, γ = 81.697(5)°, V = 3260.8(18) Å^3, space group $P\bar{1}$, Z = 2, ρ_{calcd} = 1.269, λ(Mo Kα) = 0.710 73 Å, T = 100(2) K, 22 021 reflections, 16 305 unique (15 581 observed, R_{int} = 0.0626), R1 = 0.0901, wR2 = 0.2453, for 739 parameters.
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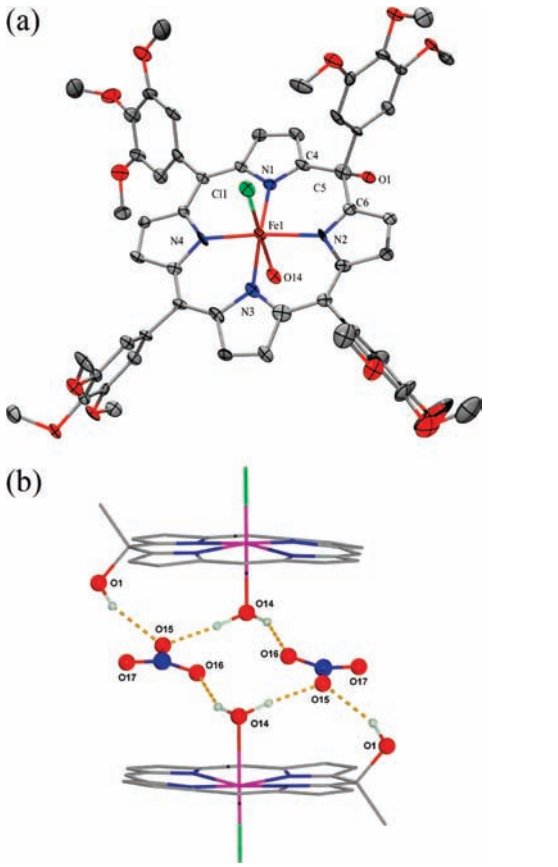

Figure 1. (a) Molecular structure (ORTEP view) of the cation of **2** with a partial atom labeling scheme (50% thermal probability ellipsoid; hydrogen atoms are omitted for clarity). (b) Intricate hydrogen-bonding network between two molecules involving a nitrate anion (other atoms are removed for clarity).

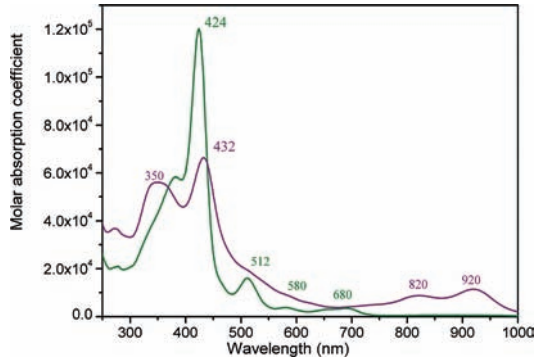

Figure 2. Electronic spectra of **1** (green) and **2** (violet) in DCM.

iron(III) cation and one nitrate anion. Bond distances between C5−C6 and C5−C4 in **2** (Figure 1a and Figure 8 in the Supporting Information) showed elongation compared to those in **1**,[13] and the planarity of the ring associated with FeN1N2C4C6C5 in **1** is also lost in **2**, showing C5 as sp^3-hybridized, disrupting the delocalization affecting the intensity of the Soret band. This is clearly observed in the electronic spectra of **1** and **2** (Figure 2), where the Soret band at 424 nm of **1** reduced its intensity and shifted its position in **2**.

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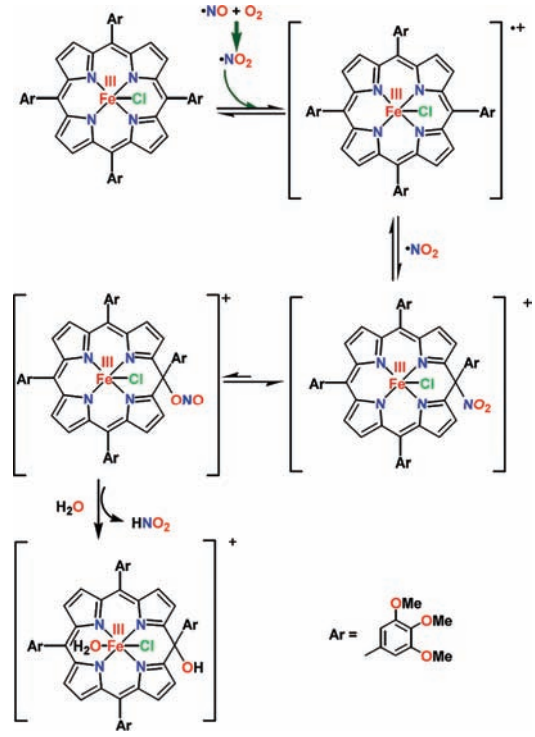
Scheme 1. Proposed Mechanism of *meso*-Hydroxylation by Nitration Followed by Hydrolysis[a]



[a] The counteranion NO_2^- is not shown.

The ready hydroxylation of **1** to **2** in the presence of both •NO and O_2 implied that the preferred reactive species may be •NO_2 with a σ-centered odd electron. The controlled reaction of **1** with •NO_2 in DCM showed a loss in the Soret band intensity of **1**, and the spectral feature of the reaction mixture changes to that of **2** (Figure 1 in the Supporting Information). This nitration at the meso position removes the delocalization in the porphyrin ring. The proposed mechanism of such a reaction is presented in Scheme 1.

The reaction may thus be initiated with the formation of a π-cation radical by the formal oxidation of **1**.[14] This is followed by the nucleophilic attack of a second •NO_2 directed to the meso position of the porphyrin ring forming C−NO_2 concomitant with the destruction of the delocalization of the porphyrin ring. C−NO_2 may be in equilibrium with its nitrito form, C−ONO, which responds to ready hydrolytic reaction (Scheme 1).[15] Such an interaction of NO_2 with *meso*-tetraphenyporphyrinatozinc, leading to the formation of a bound nitro group at the *meso*-carbon atom via a π-cation radical, has been shown by IR and UV−visible spectroscopy.[16] Subsequent hydrolysis of the zinc complex led to the formation of a *meso*-hydroxylated product.[17] The cationic iron complex ion is crystallized out by replacing the nitrite

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(17) The ZnTPP complex was subjected to a similar treatment (as described in ref 16), and the final nitro derivative was hydrolyzed to the corresponding *meso*-hydroxylated product, which has been characterized by X-ray crystallography (to be published separately).

by a nitrate anion for effective hydrogen bonding. The presence of a free nitrate ion in the aqueous NO_2 environment is important to stabilize the cationic derivative in a solid crystalline form as **2**. In solution, the final product is not stable and gradually degraded. It is difficult to predict the nature of the oxygen atom in the hydroxyl group arising from either the $^{\bullet}NO$ or O_2 molecule. With the resonance structure of $^{\bullet}NO_2$ followed by its isomerization to a nitrito group, the incorporation of the oxygen atom in the hydroxyl group would be of equal probability. Therefore, whether this hydroxylation falls under an oxygenase similar to the hemoxygenase type of reaction cannot be stated with certainty. It is interesting to note that this reaction does not occur in a dry solvent or even using HNO_2 as the possible nitrating agent. The presence of excess NO or O_2 over NO_2 does not appreciably influence the course of the reaction.

2 is stabilized by an extensive hydrogen-bonding network in the solid state (Figure 1b). However, in solution, such stability due to hydrogen bonding is lost, and so upon standing in solution, it changed to a green species resembling verdoheme from which ethylenediaminetetraacetic acid readily scavenges iron to produce yellow-orange biliverdin-type species (Figure 3 in the Supporting Information).

Thus, we show the formation of **2** as a model intermediate product possibly at the fate of methemoglobin in the tissue upon overexposure with $^{\bullet}NO$ under an oxygen flux, which is the first stage of heme degradation. This upon standing in solution changed to the established decomposition product from verdoheme to biliverdin.

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Supporting Information Available: Details of the synthesis of **1** and **2**, electronic spectra of other derivatives, EPR and IR spectra of **1** and **2**, a MS spectrum of **2**, and X-ray crystallographic data in CIF format for **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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