A Novel *Meso*-Oxygenation of an Iron Porphyrin Complex Related to *Meso*-Hydroxylation Catalyzed by Heme Oxygenase

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A novel iron porphyrin complex, Fe^{III}(BMB*p*CPP) (**1a**), in which two *meso*-positions are less hindered, is converted to an iron(III) isoporphyrin complex (**3a**) via an intermediate (**2a**) by the reaction with 1 equiv of *p*NPBA in CH_2Cl_2 at -70 °C.

Heme degradation to biliverdin is catalyzed by heme oxygenase (HO) under O_2-NADPH-cytochrome P-450 reductase system.[1] It is widely believed that the initial O_2 binding and activation at the heme iron are responsible for the formation of α-*meso*-hydroxyheme and the following two sequential utilization of O_2 affords biliverdin IXα through verdoheme with simultaneous elimination of CO derived from the α-*meso*-carbon.[2] While recent model studies support hypothetical mechanistic schemes for the second (α-*meso*-hydroxyheme → verdoheme) and third step (verdoheme → biliverdin IXα) of HO-catalyzed reactions,[3] little is known about the mechanism for the first step of heme degradation, i.e., the conversion of heme to α-*meso*-hydroxyheme.[4]

Through mechanistic studies on the formation of oxo-iron(IV) porphyrin π-cation radicals by employing Fe(TPP) derivatives and peracids, we have shown that the formation of acylperoxo-iron(III) complexes and the following heterolytic O-O bond cleavage afford oxo-iron(IV) porphyrin π-cation radicals.[5] The Fe(TPP) derivatives (**1**) used for these studies require bulky substituents at the *meso*-positions such as mesityl and 2,6-dichlorophenyl groups to prevent heme degradation under the conditions. These results may imply that the introduction of one or two less hindered phenyl group(s) at *meso*-position(s) could allow us to examine details of heme degradation related to HO-catalyzed formation of α-*meso*-hydroxyheme. For this purpose, we have synthesized Fe^{III}[5,15-bis(mesityl)-10,20-bis(*p*-chlorophenyl)porphyrin] (**1a**)[6] and found new reactions related to the α-*meso*-hydroxyheme formation.

In a typical run, a dichloromethane solution of **1a**-trifluoroacetate (1.0×10^{-5} M) containing 4 equiv of TFA in a UV-cuvette was cooled to -70 °C. To the resulting solution was added 1.5 equiv of *p*-nitroperbenzoic acid (*p*NPBA) to give a complex (**3a**) *via* an intermediate (**2a**) as shown in Fig. 1. On the basis of two intense band in the near-IR region in the spectrum of **3a**, the formation of an isoporphyrin complex is evident.[7] As illustrated in Fig. 1 (inset), gentle absorption around 600 nm of **2a** is indicative of a porphyrin radical species,[8] though spectroscopic feature of **2a** is different from O=Fe^{IV}(BMB*p*CPP) π-cation radical prepared by the reaction of **1a** and pentafluoroiodosylbenzene. Further characterization of **2a** is under investigation. EPR spectrum (g = 6.18, 5.68, and 1.98) demonstrates **3a** being a typical rhombic iron(III) high spin complex. NMR measurements were employed for further characterization of **3a**. ^{1}H-NMR spectrum of **3a** gives four paramagnetically-shifted signals for β-pyrrole protons around

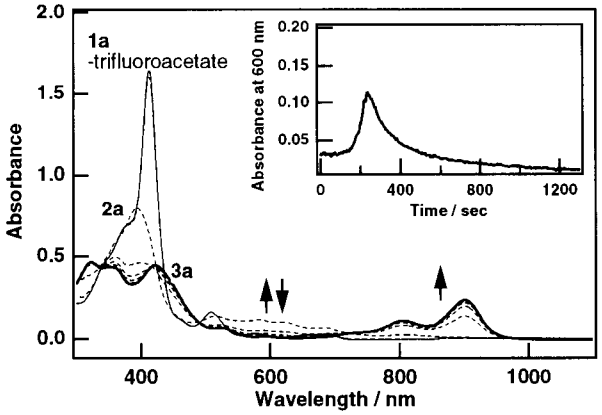

Figure 1. UV-vis spectral changes of **1a**-trifluoroacetate (—— ; [1.0×10^{-5} M]) by the addition of 1.5 equiv of *p*NPBA in dichloromethane containing 4 equiv of TFA at -70°C to yield **3a** (▬▬). The transitional spectra were shown with a broken line (- - - - -). The first spectrum was recorded immediately after the addition followed by 2-minute intervals. *inset*: time-dependent change of absorbance at 600 nm upon the addition of 1.5 equiv of *p*NPBA to the solution of **1a**-trifluoroacetate.

100 ppm, consistent with the EPR results. In addition, observation of four signals for 8 β-pyrrole protons of **3a** clearly demonstrates that the ring symmetry of **3a** is C_s expected for the isoporphyrin structure. In order to determine whether new substituent is introduced at the less-hindered or hindered *meso*-position, a selectively deuterated iron complex, Fe^{III}[5,15-bis(mesityl)-10,20-bis(phenyl-d_5)porphyrin] (**1b**), was used to examine the oxidized complex (**3b**) by ^{2}H-NMR spectroscopy. While the ^{2}H-NMR spectrum of **1b**-trifluoroacetate shows one set of signals for *o*-, *m*-, and *p*-deuteriums due to the equivalence of two deuterated phenyl groups, that of **3b** gives signals which correspond to two different phenyl groups. Nonidentical deuterated phenyl groups are expected for the addition of the substituent at the less hindered *meso*-position. These results allow us to identify **3b** as an iron(III) isoporphyrin high spin complex bearing a substituent at the less hindered *meso*-position.

The involvement of *p*NPBA as the corresponding *p*-nitrobenzoyloxy group in **3a** was confirmed by electrospray ionization (ESI) mass measurement, i.e., the appearance of the parent ion peak (M^+) at 1099 is the indication of **3a** being consisted of **1a**, and trifluoroacetoxy and *p*-nitrobenzoyloxy groups. As reported by Gold et al.,[7b] treatment of **3a** with Cl^- could replace the axial ligand in **3a** with Cl^-. The ESI mass measurement of **3a** treated with tetra-*n*-butylammonium chloride gave the signal corresponding to the complex bearing the *p*-nitrobenzoyloxy group and chloride. For further confirmation

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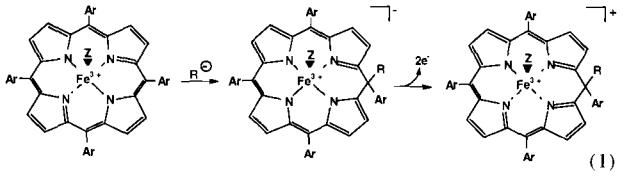
of the benzoyloxy group from the perbenzoic acid employed being on the *meso*-position, an IR spectrum of **3a** obtained by the reaction with *m*-chloroperbenzoic acid was measured at -70 °C. The C=O stretching of alkyl *m*-chlorobenzoate used to appear around 1710-1740 cm^{-1}, while if the trifluoroacetoxy group is on the *meso*-position as an ester, $\nu_{C=O}$ must appear around 1770-1790 cm^{-1}.[9] The IR spectrum of **3a** shows only the broad C=O stretching band at 1701cm^{-1} in the region between 1700 and 1800 cm^{-1}. Apparently, the trifluoroacetoxy group can not be the substituent at the *meso*-position. These results clearly demonstrate trifluoroacetate being exchangeable and the axial ligand in **3a**.

The conversion of the ferric porphyrin to the corresponding ferric isoporphyrin requires two electron oxidation of the porphyrin ring as depicted in Eq.1. Therefore,

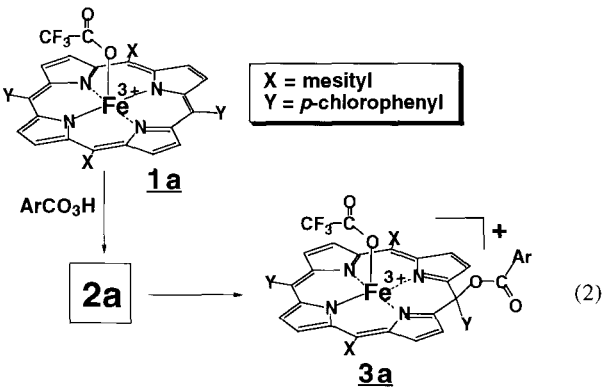


(1)

Iron(III) Porphyrin **Iron(III) Isoporphyrin**

it is reasonable to obtain a benzoyloxy group at the *meso*-position of **3a** instead of a perbenzoyloxy group due to the stoichiometry of the reaction.

In this report, we have shown the formation of isoporphyrin in the reaction of peracid and less sterically hindered iron porphyrin *via* an intermediate (Eq. 2). If the same reaction proceeded in the HO-catalyzed reaction *via* hydroperoxy-iron(III) intermediate, deprotonation from isoporphyrin can form α-*meso*-hydroxyheme. As reported by



(2)

Wilks et al, peracids such as *m*CPBA and peracetic acid produced compound II of HO instead of heme degradation, while hydrogen peroxide and ethyl hydroperoxide can be used to complete heme degradation in the presence of O_2.[4b] Oxidant dependence of HO-catalyzed reactions could be caused by either specific hydrogen bond with iron bound hydroperoxide (and ethyl peroxide) or steric hindrance of the distal site of HO,[10] since such effects are expected to alter the structure of the oxidant-iron(III) heme adduct. In fact, recent resonance Raman studies indicates that the molecular oxygen bound to a ferrous iron of HO is highly bent toward α-*meso*-carbon.[11] Thus, such structure dependency has been mimicked by changing substituents at the *meso*-position of Fe(TPP) complexes. Mechanistic studies including the characterization of **2a** for catabolic pathway giving **3a** is under investigation.

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