Evidence for a Nonheme Fe(IV)=O Species in the Intramolecular Hydroxylation of a Phenyl Moiety

Steven J. Lange, Hiroyuki Miyake, and Lawrence Que, Jr.*

Department of Chemistry and
Center for Metals in Biocatalysis
University of Minnesota, 207 Pleasant Street SE
Minneapolis, Minnesota 55455

Iron(IV)−oxo species are proposed to be the key reactive species that effect substrate oxidation in many heme[1] and nonheme iron enzymes.[2−4] In cytochrome P450, the iron(IV)−oxo moiety is used in conjunction with a porphyrin radical to effect hydroxylations of alkanes and arenes. In methane monooxygenase (MMO), two iron(IV)−oxo species work in concert to achieve oxidation of methane to methanol.[5] In contrast, mononuclear nonheme iron enzymes such as isopenicillin N synthase, 2-oxo-glutarate-dependent enzymes, and the pterin-dependent phenylalanine, tyrosine, and tryptophan hydroxylases are proposed to carry out these 2-e$^-$ oxidations solely by an Fe(IV)−oxo moiety via an Fe(IV)=O/Fe(II) + [O] couple.[3,4] The Fe(IV)−oxo moiety is also frequently invoked in the mechanisms proposed for model systems, but there is no unequivocal evidence for the involvement of such a species in the literature.[6] Additionally the reactivity of the two well-characterized Fe(IV)=O systems is limited, and neither is capable of the 2-e$^-$ oxidations of relatively inert hydrocarbon substrates.[7] Thus, the important question remains as to whether an Fe(IV)=O alone is capable of such chemistry. In our efforts to learn more about the reactivity of an Fe(IV)=O species, we have taken advantage of the demonstration by Ingold and co-workers that alkylperoxoiron(III) complexes with tris(2-pyridylmethyl)amine (TPA)[8] decompose via O−O bond homolysis to generate an alkoxy radical and the desired iron(IV)−oxo species.[9] In this paper we demonstrate that the latter can then be trapped by introduction of a pendant phenyl group on one of the pyridines of TPA[10] to form a hydroxylated arene.

Mixing a 1:1 ratio of Fe(ClO$_4$)$_2$·6H$_2$O and 6-Ph-TPA[10] in CH$_3$-CN followed by addition of Et$_2$O induces crystallization of [Fe-(II)(6-Ph-TPA)(CH$_3$CN)$_2$](ClO$_4$)$_2$ (**1**). X-ray crystallography (Figure 1) confirmed the structure and further revealed the phenyl substituent to be well placed to react with a high valent Fe(IV)=O center.[11] Indeed, rotation of the phenyl substituent about the C$_{pyridyl}$–C$_{phenyl}$ bond brings the ortho hydrogen to within 1.9 Å of the iron center.[12]

Treatment of **1** with 3 equiv of ^{t}BuOOH under Ar in CH$_3$CN affords a product that exhibits a resonance Raman spectrum typical of ortho substituted Fe(III)-phenolates (see Supporting Information).[6e,13] Addition of triethylamine and NaBPh$_4$ to a methanol solution of this complex induces precipitation of [Fe$_2$-(μ-O)(6-C$_6$H$_4$O-TPA)$_2$](BPh$_4$)$_2$ (**2**) as an orange solid (70%).[14] Recrystallization from methanol/DMF affords crystals suitable for structure determination by X-ray crystallography.[15] As illustrated in Figure 2, the pendant phenyl groups of the 6-Ph-TPA ligands have been oxygenated at the ortho position and the resulting phenolates coordinate to the iron(III) centers. The geometry about the two iron(III) centers is octahedral, and they are related by an inversion center that also enforces a linear Fe−O−Fe bond.

When the reaction is performed at low temperature (−60 °C), we observe a transient blue species formulated as [Fe(III)(OO-^{t}Bu)(6-Ph-TPA)]$^{2+}$ (intermediate **A** in Scheme 1), based on the strong resemblance of its UV−visible, resonance Raman, and EPR spectra to those of the related [Fe(III)(OO^{t}Bu)(6-Me-TPA)]$^{2+}$.[16,17] This blue alkylperoxo species decays over ca. 4 h at −60 °C to a yellow species that gives rise to the final Fe(III) complex of the hydroxylated ligand (see Supporting Information). The nature of the yellow species has not yet been determined but may be **B** or **C**. However its observation suggests that O−O bond homolysis occurs prior to hydroxylation of the phenyl moiety, as reaction of the phenyl substituent with the alkylperoxoiron(III) species (**A**) would form **2** directly.

The alkoxy radical formed in the homolysis of intermediate **A** is not likely to participate directly in the oxidation. For example, no phenol is formed in the reaction of [Fe(TPA)(CH$_3$CN)$_2$]$^{2+}$ and ^{t}BuOOH in the presence of 1000 equiv of benzene, conditions that result in cyclohexane hydroxylation. Furthermore, **2** is also obtained when MPPH[8] is used as the oxidant,[18] albeit in lower yield. When **1** is oxidized by 2 equiv of MPPH, 0.25 equiv of **2**, 0.7 equiv of benzyl alcohol, 0.35 equiv of bibenzyl, and 0.1 equiv of benzaldehyde are formed.[19] O−O bond homolysis of MPPH

(1) Sono, M.; Roach, M. P.; Coulter, E. D.; Dawson, J. H. *Chem. Rev.* **1996,** *96,* 2841−2887.

(2) Waller, B. J.; Lipscomb, J. D. *Chem. Rev.* **1996,** *96,* 2625−2657.

(3) Que, L., Jr.; Ho, R. Y. N. *Chem. Rev.* **1996,** *96,* 2607−2624.

(4) Kappock, T. J.; Caradonna, J. P. *Chem. Rev.* **1996,** *96,* 2659−2756.

(5) (a) Lee, S.-K.; Nesheim, J. C.; Lipscomb, J. D. *J. Biol. Chem.* **1993,** *268,* 21569−21577. (b) Lee, S. K.; Fox, B. G.; Froland, W. A.; Lipscomb, J. D.; Münck, E. *J. Am. Chem. Soc.* **1993,** *115,* 6450−6451. (c) Liu, K. E.; Wang, D.; Huynh, B. H.; Edmondson, D. E.; Salifoglou, A.; Lippard, S. J. *J. Am. Chem. Soc.* **1994,** *116,* 7465−7466. (d) Liu, K. E.; Valentine, A. M.; Wang, D.; Huynh, B. H.; Edmondson, D. E.; Salifoglou, A.; Lippard, S. J. *J. Am. Chem. Soc.* **1995,** *117,* 10174−10185. (e) Shu, L.; Nesheim, J. C.; Kauffmann, K.; Münck, E.; Lipscomb, J. D.; Que, L., Jr *Science* **1997,** *275,* 515−518.

(6) (a) Funabiki, T.; Yokomizo, T.; Suzuki, S.; Yoshida, S. *Chem. Commun.* **1997,** 151−152. (b) Hage, J. P.; Sawyer, D. T. *J. Am. Chem. Soc.* **1995,** *117,* 5617−5622. (c) Kitajima, N.; Ito, M.; Fukui, H.; Moro-oka, Y. *J. Am. Chem. Soc.* **1993,** *115,* 9335−9336. (d) Ménage, S.; Galey, J.-B.; Dumats, J.; Hussler, G.; Seité, M.; Luneau, I. G.; Chottard, G.; Fontecave, M. *J. Am. Chem. Soc.* **1998,** *120,* 13370−13382. (e) Hegg, E. L.; Ho, R. Y. N.; Que, L., Jr. *J. Am. Chem. Soc.* **1999,** *121,* 1972−1973.

(7) (a) Kim, C.; Dong, Y.; Que, L., Jr. *J. Am. Chem. Soc.* **1997,** *119,* 3635−3636. (b) Groves, J. T.; Gross, Z.; Stern, M. K. *Inorg. Chem.* **1994,** *33,* 5065−5072.

(8) Abbreviations: TPA, tris(2-pyridylmethyl)amine; 6-Ph-TPA, bis(2-pyridylmethyl)-6-phenyl-2-pyridylmethylamine; 6-Me-TPA, bis(2-pyridyl-methyl)-6-methyl-2-pyridylmethylamine; MPPH, 2-methyl-1-phenyl-2-propyl hydroperoxide; DMF, *N,N*-dimethylformamide.

(9) MacFaul, P. A.; Ingold, K. U.; Wayner, D. D. M.; Que, L., Jr. *J. Am. Chem. Soc.* **1997,** *119,* 10594−10598.

(10) Chuang, C.-L.; Lim, K.; Canary, J. W. *Supramol. Chem.* **1995,** *5,* 39−43.

(11) Crystal data for **1** at 173 (2) K: monoclinic, *P2$_1$/n,* *a* = 10.0768 (4) Å, *b* = 11.0129 (5) Å, *c* = 28.3180 (12) Å, *β* = 91.302 (1)°, *V* = 3141.8 (2) Å^3, *Z* = 4. Crystal color: light yellow. On the basis of 5440 unique reflections (*R*$_{int}$ = 0.0452) and 448 variable parameters, *R* (*R*$_w$) = 0.065 (0.100), GOF (on *F*^{2}) = 1.080.

(12) The molecular modeling program Chem3D was used to manipulate the atomic coordinates obtained from the X-ray structure of **1**.

(13) Carrano, C. J.; Carrano, M. W.; Sharma, K.; Backes, G.; Sanders-Loehr, J. *Inorg. Chem.* **1990,** *29,* 1865−1870.

(14) Theoretically, 1.5 equiv of ^{t}BuOOH are required for complete conversion of **1** to **2**. However some of the ^{t}BuOOH are likely rendered ineffective by reaction with the alkoxy radical formed. Accordingly, we find that 2.5−3 equiv of ^{t}BuOOH is needed to achieve the highest yield of **2**.

(15) Crystal data for **2** at 173 (2) K: triclinic, *P1*, *a* = 9.8427 (7) Å, *b* = 13.9820 (9) Å, *c* = 15.5076 (10) Å, *α* = 64.763 (1)°, *β* = 77.158 (1)°, *γ* = 88.792 (1)°, *V* = 1875.6 (2) Å^3, *Z* = 2. Crystal color: red. On the basis of 6590 unique reflections (*R*$_{int}$ = 0.0279) and 502 variable parameters, *R* (*R*$_w$) = 0.0389 (0.0993), GOF (on *F*^{2}) = 1.015.

(16) Spectroscopic properties of **A**: λ$_{max}$ = 630 nm; EPR *g* values = 4.3 (high-spin component) 2.19, 2.12, 1.97 (low-spin component); Raman shifts (cm^{-1}) = 469, 502*, 644, 683*, 841, 873 (* = low-spin component).

(17) Zang, Y.; Kim, J.; Dong, Y.; Wilkinson, E. C.; Appelman, E. H.; Que, L., Jr. *J. Am. Chem. Soc.* **1997,** *119,* 4197−4205.

(18) Arends, I. W. C. E.; Ingold, K. U.; Wayner, D. D. M. *J. Am. Chem. Soc.* **1995,** *117,* 4710−4711.

(19) The product solution was analyzed by gas chromatography following removal of the iron(III) complexes by passage through a silica gel column.

Communications to the Editor

J. Am. Chem. Soc., Vol. 121, No. 26, 1999 6331
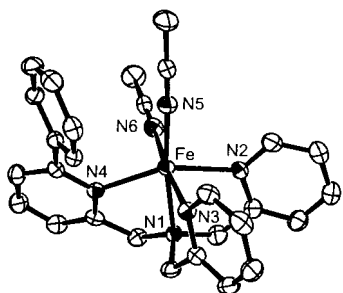
Figure 1. Thermal ellipsoid (50%) plot of **1**. Hydrogen atoms have been omitted for clarity. Selected interatomic distances (Å): Fe−N1 = 2.217 (3), Fe−N2 = 2.193 (3), Fe−N3 = 2.211 (3), Fe−N4 = 2.207 (3), Fe−N5 = 2.108 (4), Fe−N6 = 2.159 (4).
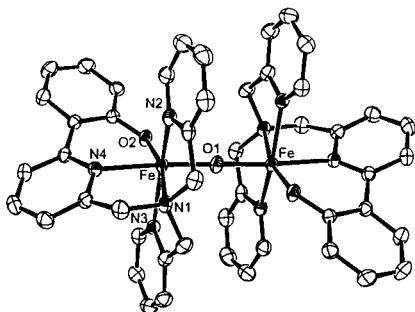

Figure 2. Thermal ellipsoid (50%) plot of **2**. Hydrogen atoms have been omitted for clarity. Selected interatomic distances (Å) and an angle (deg): Fe−N1 = 2.210 (2), Fe−N2 = 2.144 (2), Fe−N3 = 2.167 (2), Fe−N4 = 2.191 (2), Fe−O1 = 1.789 (1), Fe−O2 = 1.901 (1); Fe−O−Fe = 180.

Scheme 1. Proposed Mechanism
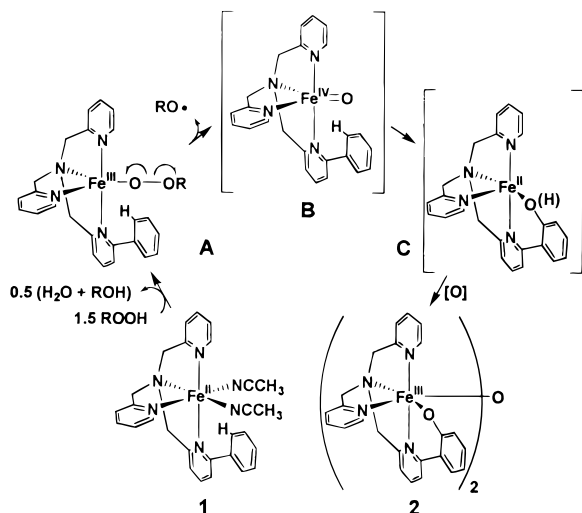
affords an alkoxy radical that very rapidly undergoes β-scission to give acetone and the relatively stable benzyl radical.[9] The observation of a significant amount of bibenzyl and only 0.1 equiv of benzaldehyde indicates that little or no dioxygen is generated in the reaction. We thus ascribe the benzyl alcohol formed in the reaction to the trapping of the transient Fe(IV)=O species (**B**) by the benzyl radical in competition with the pendant phenyl moiety.[9]

Isotope labeling experiments strongly support involvement of an Fe(IV)=O species. The resonance Raman spectrum of **2** shows a ν(Fe−OAr) mode at 619 cm^{-1} (Figure 3a)[20] that downshifts 9 cm^{-1} when ^{t}BuO^{18}OH[17] is used as the oxidant (Figure 3b). This shift confirms that the source of the oxygen atom in the product
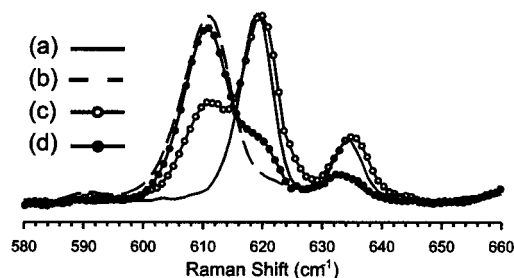
Figure 3. Resonance Raman spectra ($\nu_{\text{Fe−OAr}}$ region) of **2b** obtained with 514.5 nm excitation on frozen solutions: (a) from reaction of **1** with ^{t}BuOOH in CH$_3$CN, (b) from reaction of **1** with ^{t}BuO^{18}OH in CH$_3$CN, (c) from reaction of **1** with ^{t}BuOOH in CH$_3$CN/H$_2$^{18}O (20% H$_2$^{18}O by vol), (d) from reaction of **1** with ^{t}BuO^{18}OH in CH$_3$CN/H$_2$O (20% H$_2$O by vol).

is the terminal oxygen atom of ^{t}BuOOH. The incorporation of the label into the phenolate appears nearly quantitative, as evidenced by the absence of a peak corresponding to the unlabeled species in both the resonance Raman and ESI mass spectra.[21] When **1** is oxidized with unlabeled ^{t}BuOOH in CH$_3$CN containing H$_2$^{18}O (∼20% by vol), peaks corresponding to both ν(Fe^{16}OAr) and ν(Fe^{18}OAr) are observed in the resonance Raman spectrum of **2** (Figure 3c). The complementary experiment with ^{t}BuO-^{18}OH in CH$_3$CN containing H$_2$^{16}O (∼20% by vol) (Figure 3d) corroborates this result, implicating an oxidant capable of exchange with water. Similar experiments with MPPH and H$_2$^{18}O also resulted in approximately 20% incorporation of ^{18}O into **2** and benzyl alcohol, consistent with the competitive trapping of the Fe(IV)=O moiety as discussed above. In these systems there are no plausible mechanisms for the ^{t}BuOOH or MPPH oxidants to exchange oxygen atoms with water. As high-valent metal−oxo species are known to be capable of oxygen atom exchange with water,[22] the observation of ^{18}O incorporation from H$_2$^{18}O is indirect but compelling evidence for the intermediacy of such species.

In summary, we have demonstrated arene hydroxylation by a nonheme Fe(IV)=O species. This reaction mimics that performed by pterin-dependent hydroxylases[23,24] and lends additional support for the hypothesis that Fe(IV)=O species can be the active species responsible for substrate oxidation in this class of oxygen-activating nonheme iron enzymes. We are currently examining the ability of other oxidants to achieve this oxidation, as well as ligand modifications that may allow for the buildup of the reactive iron−oxo intermediate **B**.

Supporting Information Available: X-ray crystallographic details for **1** and **2**, experimental procedures, UV−visible time trace of **A**, and resonance Raman and ESI mass spectra of **2** (PDF). X-ray crystallographic file in CIF format. This material is available free of charge via the Internet at http://pubs.acs.org.

JA990233U

(20) Pyrz, J. W.; Roe, A. L.; Stern, L. J.; Que, L., Jr. *J. Am. Chem. Soc.* **1985**, *107*, 614−620.

(21) The electrospray ionization mass spectrum of **2** shows a peak at *m/z* 1213.0 corresponding to {[Fe(III)$_2$(μ-O)(C$_{24}$H$_{21}$N$_8$^{18}O)$_2$](C$_{24}$H$_{20}$B)}$^+$ when ^{t}BuO^{18}OH is used as the oxidant. No peak at *m/z* 1209, corresponding to the ^{16}O derivative is observed.

(22) (a) Bernadou, J.; Fabiano, A.-S.; Robert, A.; Meunier, B. *J. Am. Chem. Soc.* **1994**, *116*, 9375−9376. (b) Lee, K. A.; Nam, W. *J. Am. Chem. Soc.* **1997**, *119*, 1916−1922.

(23) Hillas, P. J.; Fitzpatrick, P. F. *Biochemistry* **1996**, *35*, 6969−6975.

(24) Goodwill, K. E.; Sabatier, C.; Marks, C.; Raag, R.; Fitzpatrick, P. F.; Stevens, R. C. *Nat. Struct. Biol.* **1997**, *4*, 578−585.