Biomimetic Oxidation Studies. 9. Mechanistic Aspects of the Oxidation of Alcohols with Functional, Active Site Methane Monooxygenase Enzyme Models in Aqueous Solution[1a]

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The syntheses of biomimetic complexes that mimic the major structural features of the hydroxylase component of methane monooxygenase enzyme (MMO) and, more importantly, that provide similar alkane functionalization activity, in the presence of an oxidant, have been of great interest to the discipline of bioinorganic chemistry.[2] Previous studies with MMO mimics on several aspects of the alkane functionalization chemistry, including product formation and mechanisms, have been performed exclusively in organic solvents such as acetonitrile, since solubility of the substrates, the biomimetic catalysts, $[(Fe_2O(\mu\text{-OAc})(L)_2]^{3+}$/$[(Fe_2O(\eta^1\text{-}H_2O)_2(L)_2]^{4+}$, and the oxidants, H_2O_2/TBHP, were problematical.[3]

As far as we have been able to discern, no MMO biomimetic oxidation studies, as described above, have been conducted in H_2O, a solvent of both biological compatibility and significant environmental interest. In this communication, we will demonstrate the feasibility of conducting biomimetic oxidation studies in H_2O with soluble substrates, i.e., alcohols (cyclohexanol, benzyl alcohol), using H_2O-stable MMO mimics at pH 4.2, $[Fe_2O(\eta^1\text{-}H_2O)(\eta^1\text{-}OAc)(L)_2]^{3+}$ (L = TPA, **1**; BPIA, **2**), $[Fe_2O(\eta^1\text{-}H_2O)_2(BIPA)_2]^{4+}$ (**3**), and the oxidant, *tert*-butyl hydroperoxide (TBHP).[4]

These latter complexes, **1**−**3**, were generated *in situ* using the μ-OAc complexes, **4**−**6**, $[Fe_2O(\mu\text{-OAc})(L)_2]^{3+}$ (L = TPA, **4**; BPIA, **5**; BIPA, **6**), by following this conversion by UV−vis from pH 6 to 4 (supporting information). It was clear from the UV−vis data, and subsequent ^{1}H NMR spectral data, that for **1** and **2**, the OAc ligand was still bound to the Fe metal center, but we suggest that it is bonding as an η^1-OAc rather than a μ-OAc ligand, with an η^1-H_2O ligand on the other Fe metal center. We will demonstrate that the presence of an η^1-H_2O ligand at an Fe metal center, i.e., **1**, dramatically affects oxidation rates of alcohols in aqueous solution at pH 4.2.[3a]

More importantly, we will show that, in H_2O, the predominant role of the biomimetic catalysts **1**−**3** is to homolytically decompose TBHP and that the generated *t*-BuO$^\bullet$ and *t*-BuOO$^\bullet$ radicals initiate homolytic C−H bond abstraction from the alcohol substrate to predominantly provide the aldehyde/ketone product. It is interesting to note that a recent report[5] provided data concerning the decomposition of TBHP in the solvent acetonitrile, in the presence of a mononuclear complex, $[FeCl_2(TPA)]^+$, that produced *t*-BuO$^\bullet$ radicals, and not a Fe=O intermediate, as previously suggested.[3a,b]

Initial experiments on the fate of the oxidant, TBHP, in the presence of biomimetic catalysts **1**−**3** were modeled after the elegant kinetic and mechanistic studies of Bruice and coworkers[6] on similar reactions with H_2O-soluble cytochrome P-450 biomimics. The decomposition product data of TBHP with **1**−**3** at pH 4.2 (Table 1, supporting information) show that, when H_2O is used as a solvent, the following products are formed (average percentages of **1**−**3**): $(CH_3)_2CO$, 75−85%, CH_3OH, 10−25%, CH_2O, >10%, and *t*-BuOH, 15−24%. As well, only a small amount of the coupling product *t*-$BuOOCH_3$ (<3%) was detected, while $(t\text{-BuO})_2$, was not observed.

Significantly, in H_2O, the precursor to $(CH_3)_2CO$ and CH_3OH appears to be the *t*-BuO$^\bullet$ radicals, which strongly implies that the homolytic cleavage of the O−OH or OO−H bonds of TBHP by **1**−**3** is the predominant pathway of TBHP decomposition.[7] We also observed that added *t*-BuOH was not oxidized in the reaction of **1** with TBHP, indicating that *t*-BuO$^\bullet$ radicals are not produced by a 1e$^-$ oxidation of *t*-BuOH.

As a further criterion of *t*-BuO$^\bullet$ radical formation, we used the diagnostic test of trapping the oxidized species (*t*-BuO$^\bullet$) with the water-soluble, pH-independent trapping agent 2,2′-azinobis-(3-ethylbenthiazoline-6-sulfonate) (ABTS), using GC to analyze for the formed $(CH_3)_2CO$ and *t*-BuOH.[6] The results (Figure 2, supporting information) show that as the ABTS concentration is increased, the $(CH_3)_2CO$ yield decreases from 85% to 3% and, concomitantly, that of *t*-BuOH increases from 15% to 97%. This result confirms that, indeed, the *t*-BuO$^\bullet$ radical is the major product from TBHP decomposition with **1**−**3** as catalysts.[6] Furthermore, the EPR spin radical trap 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO) has been employed to monitor the radical species formed in the reaction of TBHP with **1** at pH 4.2. Indeed, both radicals, *t*-BuO$^\bullet$ (predominant) and *t*-BuOO$^\bullet$, were detected in these DMPO EPR trapping experiments (Figure 3, supporting information).[8]

Since several mechanisms can be invoked in the formation of the *t*-BuO$^\bullet$ and *t*-BuOO$^\bullet$ radicals (for example, the Haber−Weiss process), we focused our efforts on attempting to define the role of **1**−**3** during the TBHP decomposition reaction using UV−vis and cyclic voltammetry (CV) techniques. Thus, the addition of TBHP to a solution of **1** was followed by UV−vis spectroscopy from 250 to 550 nm, which shows a loss of absorbance of all the major characteristic bands associated with the Fe−O−Fe core (isobestic point at 307 nm). Figure 1 demonstrates this phenomenon with **1** and TBHP concentrations of 10, 30, and 45 mM, with a dramatic loss in the absorbance at 458 nm (A_{458} values correspond to a 10%, 13%, and 17% loss of initial absorbance), including the anticipated color change from yellow-orange to colorless. It is important to note that the yellow-orange color returns after ~20 min of reaction time and is consistent with the presence of the original Fe^{3+} complex,

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(2) (a) Karlin, K. D. *Science* **1993**, *261*, 701. (b) Feig, A. L.; Lippard, S. J. *Chem. Rev.* **1994**, *94*, 759.

(3) (a) Buchanan, R. M.; Chen, S.; Richardson, J. F.; Bressan, M.; Forti, L.; Morvillo, A.; Fish, R. H. *Inorg. Chem.* **1994**, *33*, 3208 and references therein. (b) Leising, R. A.; Kim, J.; Perez, M. A.; Que, L., Jr. *J. Am. Chem. Soc.* **1993**, *115*, 9524 and references therein. (c) Menage, S.; Vincent, J. M.; Lambeaux, C.; Fontecave, M. *J. Chem. Soc., Dalton Trans.* **1994**, 2081. (d) Menage, S.; Vincent, J. M.; Lambeaux, C.; Chottard, G.; Grand, A.; Fontecave, M. *Inorg. Chem.* **1993**, *32*, 4766.

(4) (a) The tripodal ligands, L, are as follows: tris[(2-pyridyl)methyl]-amine (TPA), bis[(2-pyridyl)methyl][2-(1-methylimidazolyl)methyl]amine (BPIA), and bis[2-(1-methylimidazolyl)methyl][(2-pyridyl)methyl]amine (BIPA). (b) The precursor μ-OAc complexes, **4**−**6**, used to prepare the *in situ* formed complexes **1**−**3** were synthesized with procedures found in refs 3a,b (see the supporting information for the spectroscopic characterization of **1**−**3**).

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

(6) (a) Balasubramanian, P. N.; Lindsay Smith, J. R.; Davies, M. J.; Kaaret, T. W.; Bruice, T. C. *J. Am. Chem. Soc.* **1989**, *111*, 1477. (b) Arasasingham, R. D.; Jeon, S.; Bruice, T. C. *J. Am. Chem. Soc.* **1992**, *114*, 2536. (c) Almarsson, Ö.; Bruice, T. C. *J. Am. Chem. Soc.* **1995**, *117*, 4533 and references therein.

(7) The hypothetical formation of an intermediate $(CH_3)_3C-O^+$ species was ruled out, since cumyl hydroperoxide decomposition, catalyzed by **1** and **2**, produced neither phenol nor acetone; these latter products emanate from a $Ph(CH_3)_2C-O^+$ rearrangement (see ref 6).

(8) (a) O'Donnell, V.; Burkitt, M. J. *Biochem. J.* **1994**, *304*, 707. (b) Davis, M. J. *Biochem. Biophys. Acta* **1988**, *964*, 28.

0002-7863/95/1517-12356$09.00/0

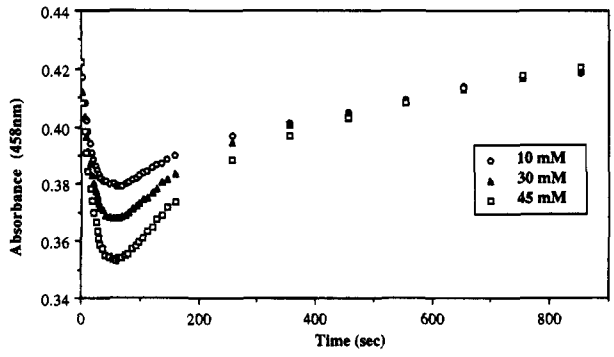

Figure 1. Changes in the absorbance at 458 nm as a function of time with different $[t\text{-BuOOH}]_i$. [catalyst **1**] = 0.5 mM. Reactions were initiated by addition of *t*-BuOOH.

1. As well, experiments in the presence of the substrates (butyl, cyclohexyl, and benzyl alcohol, cyclohexanone) at pH 4.5 show a *greater decrease* in the absorbance from 310 to 550 nm, which appears to depend on the concentration and the structure of the substrate (Figures 4 and 5, supporting information). These results suggest that the intermediate Fe complex does not have an absorbance in the visible spectrum. This phenomenon is consistent with the intermediacy of a stabilized $LFe^{3+}(\mu\text{-OH})Fe^{2+}L$ complex that would have no visible absorption[9] and, further, would imply a Haber–Weiss process for the formation of *t*-BuO• radicals.

Moreover, the redox properties of **1** have been evaluated in degassed aqueous solutions from pH 4 to 6, and two redox processes are displayed at +336 and +144 mV vs Ag/AgCl. The first quasi-reversible reduction wave corresponds to the mixed-valence $Fe^{2+}Fe^{3+}$ complex, while further reduction of this intermediate at +144 mV leads, presumably, to the formation of the $Fe^{2+}Fe^{2+}$ complex.[10] Previous CV results with the μ-OAc analog of **1** in CH_3CN displayed quasi-reversible redox behavior, with $Fe^{3+}(\mu\text{-O})Fe^{3+}/Fe^{3+}(\mu\text{-O})Fe^{2+}$ reduction potentials equal to −0.05 mV vs Ag/AgCl.[9a] *Thus, the reduction of* **1** *in* H_2O *appears to be favorable thermodynamically.*

We initially studied the rates of benzyl alcohol oxidation with **4** and TBHP as a function of pH (Figures 6 and 7, supporting information) and found a dramatic increase in the oxidation rate when the pH was decreased from 6 to 4. This result coincided with our UV–vis experiments, which showed the *in situ* formation of **1** as the pH was decreased from 6 to 4. We then studied the oxidation of cyclohexanol in H_2O at pH 4.2 with **1**–**3** as catalysts and TBHP as the oxidant and compared the reaction times, number of turnovers (NOT), and k_H/k_D values with those of similar reactions of **4**–**6** conducted in CH_3CN (Table 2, supporting information). In H_2O, cyclohexanone was detected as the predominant oxidation product (NOT 15–32). The pertinent results of these experiments are that all three complexes, **1**–**3**, provide the same k_H/k_D values, ~3.1,[11] for the cyclohexanol to cyclohexanone conversion, which is not the case in CH_3CN, where the structure of the $[Fe_2O(\mu\text{-OAc})L_2]^{3+}$ complexes appear to dictate k_H/k_D.[3a,b] Similar experiments with benzyl alcohol as the substrate gave benzaldehyde as the predominant product (NOT 16–28) and k_H/k_D values of ~4.0 for all three complexes, **1**–**3**, while in CH_3CN, the $[Fe_2O(\mu\text{-OAc})L_2]^{3+}$ complexes **4**–**6** provide k_H/k_D values with a range of 2.6–4.5. We interpret the alcohol conversion results (H_2O) in terms of a common radical initiator, which, from our preceding experiments in water on the decomposition of TBHP, clearly appears to be predominantly the *t*-BuO• radical.

The overall results suggest the intermediacy of a reduced Fe complex, such as $LFe^{3+}(\mu\text{-OH})Fe^{2+}L$, that occurs upon reaction with TBHP at pH 4.2, with concomitant formation of the *t*-BuO• radical.[6] Equations 1–6 depict the plausible decomposition pathway of TBHP with **1**–**3**, somewhat reminiscent of the Bruice et al. studies of TBHP with water-soluble Fe^{3+} porphyrin complexes.[6]

$$LFe^{3+}(\mu\text{-O})Fe^{3+}L + t\text{-BuOO–H} \rightarrow LFe^{2+}(\mu\text{-OH})Fe^{3+}L + t\text{-BuOO}^{\bullet} \quad (1)$$

$$t\text{-BuOO}^{\bullet} \rightarrow t\text{-BuO}^{\bullet} + {}^1/_2O_2 \quad (2)$$

$$t\text{-BuO}^{\bullet} \rightarrow (CH_3)_2C{=}O + CH_3^{\bullet} \xrightarrow{O_2,\ \text{fast}} CH_3OO^{\bullet} \quad (3)$$

$$t\text{-BuO}^{\bullet} + t\text{-BuOO–H} \rightarrow t\text{-BuOH} + t\text{-BuOO}^{\bullet} \quad (4)$$

$$t\text{-BuOO}^{\bullet} + CH_3^{\bullet} \rightarrow t\text{-BuOOCH}_3 \quad (5)$$

$$LFe^{2+}(\mu\text{-OH})Fe^{3+}L + t\text{-BuO–OH} \rightarrow LFe^{3+}(\mu\text{-O})Fe^{3+}L + t\text{-BuO}^{\bullet} + H_2O \quad (6)$$

The critical ramification of our studies is that MMO biomimetic oxidation reactions in H_2O, with TPHP as the oxidant, provide a pathway somewhat similar to those found in studies recently conducted in anhydrous organic solvents, as represented by CH_3CN;[5] we, however, are not fully convinced that Fe=O intermediates are not involved in the C–H functionalization of alkanes in CH_3CN, due to the variation of k_H/k_D values we observe with **4**–**6**. Therefore, we propose that, in water, the very positive $Fe^{3+}Fe^{3+}/Fe^{3+}Fe^{2+}$ reduction potentials for **1**–**3** favor the initial step in the Haber–Weiss process (eq 1), and in this sequence of events, the μ-oxo bridge would be protonated. Similar reasoning was suggested for semimetHr, where the protonation of the μ-oxo bridge occurs upon reduction of the diferric center to neutralize the added charge, thereby stabilizing the intermediate $Fe^{3+}Fe^{2+}$ complex.[9] Moreover, we also suggest that **1**–**3** catalyze the substrate alcohol oxidations, in the presence of TBHP, by a mechanism involving Fe^{3+} oxidation and Fe^{2+} reduction of TBHP to generate the *t*-BuO• radical. This radical subsequently initiates homolytic C–H bond abstraction from the alcohol substrate to provide the corresponding ketone/aldehyde product. Future reports of aqueous oxidation reactions with **1**–**3**, TBHP, and cyclohexane as the substrate will provide further evidence for *t*-BuO• radical involvement in micelles.

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Supporting Information Available: Figures 2–7, Tables 1–2, and the synthetic procedure for complexes **1**–**3** (9 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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(9) Enzymes containing $Fe^{3+}(\mu\text{-OH})Fe^{3+}$, $Fe^{3+}(\mu\text{-OH})Fe^{2+}$, and $Fe^{2+}(\mu\text{-OH})Fe^{2+}$ sites, or their synthetic models, do not provide any intense absorptions in the visible range. (a) Holz, R. C.; Elgren, T. E.; Pearce, L. L.; Zhang, J. H.; O'Connor, C. J.; Que, L., Jr. *Inorg. Chem.* **1993**, *32*, 5844. (b) Kurtz, D. M., Jr. *Chem. Rev.* **1990**, *90*, 585.

(10) (a) Armstrong, W. H.; Spool, A.; Papaefthymiou, G. C.; Frankel, R. B.; Lippard, S. J. *J. Am. Chem. Soc.* **1984**, *106*, 3667. (b) Hartman, J. A. R.; Radin, R. L.; Chaudhuri, P.; Pohl, K.; Wieghardt, K.; Nuber, B.; Weiss, J.; Papaefthymiou, G. C.; Frankel, R. B.; Lippard, S. J. *J. Am. Chem. Soc.* **1987**, *109*, 7387.

(11) For a comprehensive study of k_H/k_D with a variety of substrates, including secondary alcohols, with *t*-BuO• radicals, see: Paul, H.; Small, R. D.; Scaiano, J. C. *J. Am. Chem. Soc.* **1978**, *100*, 4520. The k_H/k_D values in the present study were determined by the procedure reported in the following: Fish, R. H.; et al. *New J. Chem.* **1992**, *16*, 727.