# Modeling Nonheme Diiron Enzymes: Hydrocarbon Hydroxylation and Desaturation by a High-Valent $Fe_2O_2$ Diamond Core

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Methane monooxygenase (MMO),[1,2] stearoyl ACP $\Delta^9$-desaturase ($\Delta$9D),[3] and ribonucleotide reductase (RNR)[4,5] comprise a new class of metalloenzymes that activate dioxygen at a nonheme diiron active site[6] to carry out diverse functions such as the hydroxylation of methane, the desaturation of saturated fatty acids, and the generation of the catalytically essential Tyr radical for ribonucleotide reduction. The mechanisms for dioxygen activation appear to involve high-valent species as indicated by the spectroscopic properties of intermediates detected in rapid kinetic studies of $O_2$ and the diiron(II) forms of MMO (intermediate **Q**)[7−9] and RNR (intermediate **X**).[10−12] However, the absence of a porphyrin requires a mechanistic paradigm that differs from the high-valent iron−oxo porphyrin radical commonly implicated as the key oxidant for heme enzymes.[13] Instead, species with a high-valent $Fe_2(\mu\text{-}O)_2$ diamond core have been proposed to serve as the oxidizing intermediates for this class of enzymes.[14] This hypothesis derives from the recent synthesis of a series of metastable Fe(III)Fe(IV) complexes with the $Fe_2(\mu\text{-}O)_2$ diamond core using the tetradentate tripodal ligand TPA and its methylated derivatives, $[Fe_2(\mu\text{-}O)_2L_2]^{3+}$ (Figure 1) (**1**, L = TPA; **2**, L = 5-Me$_3$-TPA; **3**, L = 6-Me-TPA);[15−17] these complexes represent the first high-valent nonheme iron−oxo species to be synthesized. In this paper, we demonstrate that an $Fe_2(\mu\text{-}O)_2$ species can carry out oxidation reactions corresponding to those associated with MMO, $\Delta$9D, and RNR.

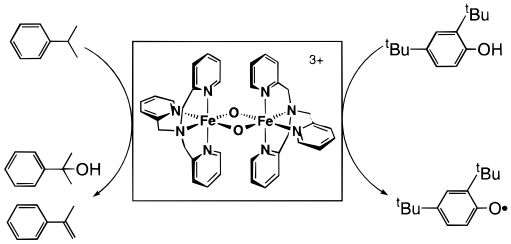


**Figure 1.** Reactions of $[Fe_2(\mu\text{-}O)_2(TPA)_2]^{3+}$ (**1**) mimicking oxidations carried out by the diiron centers of methane monooxygenase, fatty acid desaturases, and ribonucleotide reductase.

**Table 1.** Hydrocarbon Oxidations by the High-Valent Nonheme Diiron Complex **1**[a]

| substrate | products | yield[b] |
|---|---|---|
| $PhCH(CH_3)_2$ | $PhC(OH)(CH_3)_2$ | 0.17[c] |
| (500 mM) | $PhC(CH_3)=CH_2$ | 0.27[c] |
| $PhCH(CH_3)_2$ | $PhC(OH)(CH_3)_2$ | 0.82 |
| (500 mM in air) | $PhC(O)CH_3$ | 0.15 |
| $PhCH_2CH_3$ | $PhCH(OH)CH_3$ | 0.14[c] ($k_H/k_D = 22 \pm 3$)[d] |
| (500 mM) | $PhCH=CH_2$ | 0.14[c] ($k_H/k_D = 28 \pm 3$)[d] |
| | $PhC(O)CH_3$ | trace |
| cycloheptane | no product | |
| (120 mM) | | |

[a] A typical reaction mixture consisted of 2 mM of **1** and substrate at −40 °C under Ar (except where noted) in acetonitrile with 0.75% $H_2O$. The lower concentration used for cycloheptane was because of its low solubility. [b] Yield given in moles of product/moles of **1**. All products were identified and quantified by gas chromatography. The **1**/substrate reaction stoichiometry for alkane oxidation under Ar is 2:1 with a maximum product yield of 50% based on **1**, since **1** is a one-electron oxidant and the products are oxidized by two electrons. In the presence of $O_2$, the sole function of **1** is to generate the substrate alkyl radical, so the reaction stoichiometry is 1:1 and the maximum yield of products under these conditions would be 100%, assuming no radical chain process. [c] Ratio unchanged over the course of the reaction. [d] Product isotope effects. Reactions were carried out on 1:4 to 1:10 mixtures of ethylbenzene and ethylbenzene-$d_{10}$ to improve the accuracy for measuring the amounts of the deuterated products.

Complexes **1** and **2** have been formulated to have the $Fe_2(\mu\text{-}O)_2$ diamond core on the basis of electrospray ionization mass spectral, Raman, and EXAFS evidence.[16] Their characteristic intense green color (**1**, $\lambda_{max}$ 614 nm, $\epsilon$ 5500 $M^{-1}$ $cm^{-1}$; **2**, $\lambda_{max}$ 616 nm, $\epsilon$ 5200 $M^{-1}$ $cm^{-1}$) arising from the $Fe_2O_2$ core provides a convenient probe for monitoring oxidation reactions which are carried out at −40 °C in $CH_3CN$ to inhibit self decomposition. Thus, **1** and **2** can quantitatively oxidize 2,4-di-*tert*-butylphenol within seconds to its phenoxy radical (Figure 1), which dimerizes readily to form the 2,2′-biphenol as analyzed by NMR. Concomitantly the oxidant is converted to its ($\mu$-oxo)diiron(III) precursor complex as shown by NMR, demonstrating that **1** and **2** act as one-electron oxidants.[16] Thus, **1** and **2** mimic the role of RNR intermediate **X** in the assembly of the ($\mu$-oxo)diiron(III)-Tyr radical cofactor of RNR.[10−12]

Similarly, **1** oxidizes hydrocarbons and becomes reduced to its ($\mu$-oxo)diiron(III) precursor, while organic products derive from hydroxylation or desaturation of the substrate, similar to the action of MMO and $\Delta$9D, respectively (Figure 1). Cumene is converted to cumyl alcohol and α-methylstyrene, and ethylbenzene is converted to 1-phenylethanol and styrene; but cycloheptane is not oxidized (Table 1). The desaturation reaction in particular is a novel result for this nonheme oxidant

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(15) Abbreviations used: $R_3TACN$ = 1,4,7-trialkyl-1,4,7-triazacyclononane, TPA = tris(2-pyridylmethyl)amine, 5-Me$_3$-TPA = tris(5-methyl-2-pyridylmethyl)amine, 6-Me-TPA = *N*-(6-methyl-2-pyridylmethyl)-*N*,*N*-bis(2-pyridylmethyl)amine.
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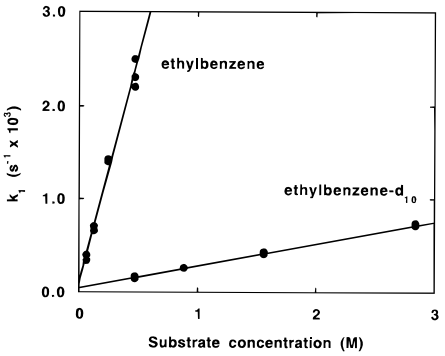


**Figure 2.** Concentration dependence of the decomposition of **1** (2 mM) in the presence of ethylbenzene or ethylbenzene-$d_{10}$ at −40 °C under Ar in $CH_3CN$.

and one that has not been associated with synthetic iron−oxo porphyrin complexes.[18−21]

Kinetic studies of **1** in the presence of ethylbenzene in $CH_3CN$ at −40 °C show that its decomposition is a process that is first order in **1** and first order in substrate, demonstrating that the rate-determining step entails a bimolecular collision between **1** and the substrate. The second-order rate constant for decomposition is $4.8 \times 10^{-3}$ $M^{-1}$ $s^{-1}$ for ethylbenzene and $2.4 \times 10^{-4}$ $M^{-1}$ $s^{-1}$ for ethylbenzene-$d_{10}$, corresponding to a $k_H/k_D$ of 20 (Figure 2); similarly, large isotope effects have been found for the cleavage of C−H bonds by the related $[Cu_2(\mu\text{-}O)_2(R_3TACN)_2]^{2+}$ ($k_H/k_D = 26 \pm 2$ for R = *i*-Pr and 40 for R = benzyl at −40 °C)[22] and by MMO intermediate **Q** ($k_H/k_D \geq 20$ at 4 °C).[23] Consistent with the kinetic results are $k_H/k_D$ values derived from product ratios in competition experiments using a mixture of ethylbenzene and ethylbenzene-$d_{10}$: $22 \pm 3$ for alcohol and 28±3 for styrene. These results demonstrate that cleavage of the substrate α-C−H bond is a major component of the rate-determining step for both hydroxylation and desaturation. However, cleavage of the $\beta$-C−H bond for the latter has a significantly smaller isotope effect (ca. 1.3), indicating that the desaturation involves asynchronous scission of the two C−H bonds, as has been observed for yeast Δ9D.[24]

A proposed mechanism for hydrocarbon oxidation with cumene as an example is shown in Figure 3. In the first and rate-determining step, **1** abstracts a hydrogen atom from the substrate generating an intermediate alkyl radical which then reacts in a fast step with a second equivalent of **1** to yield alcohol or olefin (pathway a). The alcohol would result either from the capture of the alkyl radical by the $Fe_2O_2$ diamond core, analogous to the proposed "oxygen rebound" step in heme-catalyzed hydroxylations,[25] or from electron transfer between the alkyl radical and **1** and nucleophilic trapping of the resulting

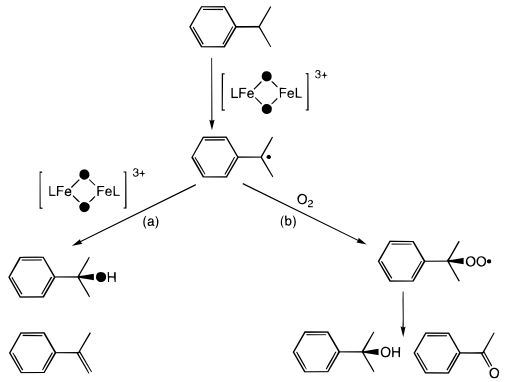


**Figure 3.** Proposed mechanism for alkane oxidation by **1**.

carbocation. In support, when cumene oxidation is carried out in the presence of $H_2{}^{18}O$ (250 equiv), the cumyl alcohol obtained is 77% labeled with $^{18}O$, consistent with the amount of $^{18}O$ incorporated into the bis($\mu$-oxo)diiron core by $H_2{}^{18}O$ exchange.[26] The olefin, on the other hand, would derive from the abstraction of a $\beta$ hydrogen by the $Fe_2O_2$ diamond core or its equivalent (e.g., electron transfer followed by loss of a $\beta$ proton). Thus, the oxidation of substrate requires 2 equiv of **1**; for cumene oxidation, the transformation efficiency is 88%. What governs the partitioning between hydroxylation and desaturation at this nonheme diiron center is not currently understood and presumably reflects a competition between C−O bond formation to form alcohol and loss of the $\beta$ hydrogen to form olefin, as manifested by the differing product isotope effects noted above.

Further support for the two-step mechanism derives from results obtained for the cumene oxidation under $O_2$. Under these conditions, cumyl alcohol and acetophenone are the reaction products, and neither product shows any $^{18}O$ incorporation when the reaction is carried out in the presence of $H_2{}^{18}O$. These products derive from the breakdown of cumylperoxy radical formed by the trapping of the intermediate alkyl radical by $O_2$ (pathway b, Figure 3).

In this study, we have demonstrated that **1**, the first high-valent nonheme iron−oxo complex to be synthesized,[16] can carry out a range of oxidations analogous to those associated with the diiron sites of RNR, MMO, and Δ9D. Because it is an Fe(III)Fe(IV) complex, **1** cannot be as powerful an oxidant[27] as MMO intermediate **Q** or its putative Δ9D analogue, both of which are formally Fe(IV)Fe(IV). Oxidation of hydrocarbons with stronger C−H bonds may be expected if **1** could be further oxidized to the Fe(IV)Fe(IV) state, by analogy to the reactivities of the oxoiron(IV) porphyrin and its one-electron oxidized counterpart.[18,20,28] The diverse reactivity exhibited by **1** thus supports a new paradigm in which an $Fe_2(\mu\text{-}O)_2$ diamond core serves as the common structural unit for the high-valent intermediates in the oxygen activation mechanisms of these nonheme diiron enzymes.[14]

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(26) It has not been possible to determine the extent of $H_2{}^{18}O$ exchange into **1** by mass spectrometry, due to its instability and the presence of interfering features from other components in the reaction mixture. However, the mass spectral analysis of the more stable **2** (0.8 mM in $CH_3CN$) treated with 250 equiv of $H_2{}^{18}O$ showed that 68% of the molecules were doubly $^{18}O$-labeled and 27% of the molecules were singly $^{18}O$-labeled. Transfer of one of the oxo groups to cumene would then afford about 80% yield of labeled cumyl alcohol.
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