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The Reaction of a High-Valent Nonheme Oxoiron(IV) Intermediate with Hydrogen Peroxide**

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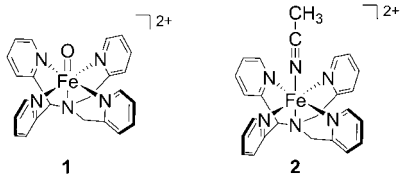
Reactive oxygen species (ROS) are versatile small molecules that under normal homeostatic control are essential for physiological signaling, whereas an improper balance can lead to aging and age-related diseases.[1–4] A main regulatory mechanism for the ROS hydrogen peroxide (H_2O_2) is associated with heme enzymes called catalases.[5,6] These metalloenzymes dismutate two molecules of H_2O_2 via the proposed reaction pathways shown in Equations (1) and (2).[5–8] Initial oxidation of the Fe^{III} resting state with H_2O_2 generates a high-valent oxoiron(IV) porphyrin π-cation radical, also known as compound I, $[(P^{\bullet+})Fe^{IV}{=}O]^+$, where P = porphyrinate dianion [Eq. (1)]. The reaction of compound I with a second molecule of H_2O_2 results in the return to the resting state of the enzyme with the release of water and dioxygen [Eq. (2)]. Considering the reactivity of compound I toward H_2O_2 in heme enzymes, a similar interaction with H_2O_2 has not been reported for oxoiron(IV) intermediates involved in the catalytic cycles of mononuclear nonheme iron enzymes.[9–11]

$$[(P)Fe^{III}]^+ + H_2O_2 \rightarrow [(P^{\bullet+})Fe^{IV}{=}O]^+ + H_2O \quad (1)$$

$$[(P^{\bullet+})Fe^{IV}{=}O]^+ + H_2O_2 \rightarrow [(P)Fe^{III}]^+ + O_2 + H_2O \quad (2)$$

Although the different iron coordination spheres, distal pocket environments, and spin states of the oxoiron(IV) intermediates found in heme and nonheme iron enzymes afford specific oxidative reactivities, similar reactions can occur in both enzyme families, for example C–H bond activation in the nonheme iron enzyme taurine α-ketoglutarate dioxygenase (TauD) and heme-based cytochrome P450 enzymes.[9–18] These comparable reactions lead to the question of whether and possibly how nonheme oxoiron(IV) species play a role similar to that of catalases in modulating the fate of H_2O_2. The feasibility of such reactions may first be validated through the use of biomimetic complexes, which also have provided vast insights into the mechanisms of other chemical reactions underlying biological functions of oxoiron(IV) and other iron–oxygen intermediates (for example, organic transformations, electron transfer, and interaction with reactive nitrogen species (RNS)).[13,16,19–22] Examples of interactions of oxometal complexes with H_2O_2 have been reported, and these generally involve coordination of peroxide to the metal center or exchange of the oxo ligand with peroxide.[23–27] Alternatively, the direct reaction of H_2O_2 with terminal or bridging oxo ligands through hydrogen atom transfer, generating O_2, has been described for only a few complexes of V, Cr, Mn, and Ru.[23,27–31] In iron chemistry, reaction of oxoiron(IV) complexes with H_2O_2 has been suggested to occur at intermediate steps upon mixing of Fe^{II} or Fe^{III} complexes with H_2O_2 or O_3, although direct reactivity is difficult to discern owing to the possible existence of multiple species and reaction pathways in these cases.[32–39] Progress in recent years has made a number of oxoiron(IV) complexes available that can be generated independently of H_2O_2 by artificial oxidants,[16] thus providing an opportunity to investigate their reactivity toward H_2O_2. Expanding upon investigations of the chemistry between high-valent intermediates found in heme enzymes and H_2O_2,[7,18,40–42] herein we present evidence of the direct and relatively rapid reaction of a mononuclear nonheme oxoiron(IV) complex, $[Fe^{IV}O(N4Py)]^{2+}$ (**1**; N4Py = *N*,*N*-bis(2-pyridylmethyl)-*N*-[bis(2-pyridyl)methyl]amine; Scheme 1),[43,44] with H_2O_2. To the best of our knowledge, this reaction demonstrates for the first time direct H_2O_2 reactivity of a terminal Fe^{IV}=O group of a nonheme iron complex.



1 **2**

Scheme 1. Structures of **1** and **2**.

Starting from $[Fe^{II}(N4Py)(CH_3CN)]^{2+}$ (**2**; Scheme 1), the generation of **1** was carried out with iodosylbenzene (PhIO),[43,44] which provided oxidation of the Fe^{II} center independently of H_2O_2. As shown in Figure 1 a, the characteristic absorption band of **1** ($\lambda_{max} = 692$ nm) disappeared upon the addition of H_2O_2 to a CH_3CN solution of **1** at −20 °C, indicating its direct reaction with H_2O_2. The nearly full decay (ca. 94 %) of **1** required 0.5 equiv of H_2O_2 with a half-life of

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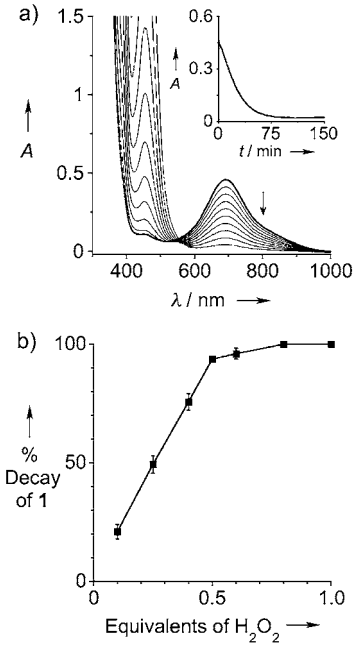


Figure 1. Reaction of **1** with H_2O_2. a) UV/Vis spectra of the reaction of 1.0 mM **1** in CH_3CN (bold line) with 0.5 equiv of H_2O_2 at −20 °C (path length, 1.0 cm). Inset: Time course of the reaction ($\lambda = 692$ nm). b) Percent decay of **1** for reactions with 0.1–1.0 equiv of H_2O_2 (based on the absorbance at 692 nm). The measurements were conducted in triplicate.

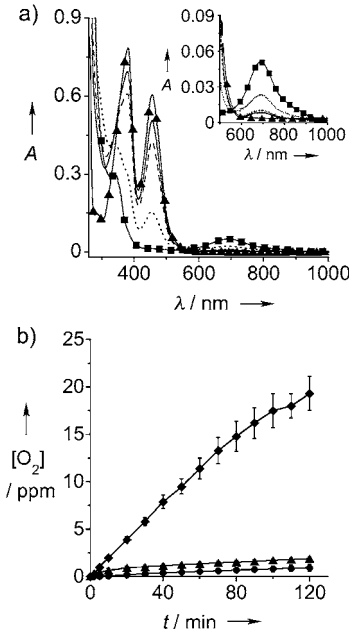


Figure 2. Determination of the iron-containing product and O_2 formation from the reaction of 1.0 mM **1** in CH_3CN with 0.5 equiv of H_2O_2 at −20 °C. a) UV/Vis spectra of 1.0 mM solutions of **1** (■) and **2** (▲) in CH_3CN and of the reaction mixture at about half (30 min, •••••) and nearly full consumption (100 min, -----) of **1** (path length, 0.1 cm). No further spectral changes were observed after 250 min (——). b) Time courses of the evolution of O_2 upon addition of 0.5 equiv of H_2O_2 to 1.0 mM solutions of **1** (◆) and **2** (▲) in CH_3CN and upon introduction of the same amount of H_2O_2 into CH_3CN (●).

about 20 min, suggesting a 2:1 stoichiometry for the reaction (Figure 1b). Following the decay of **1**, the reaction ultimately produced **2** in fairly high yield (ca. 85%, after 250 min) as determined from the absorption bands at 380 and 454 nm (Figure 2a; Supporting Information, Figure S1). The decomposition of **1** and formation of **2** were confirmed by electrospray ionization mass spectrometry (ESI MS) and ^{1}H NMR spectroscopy (Supporting Information, Figures S2, S3).

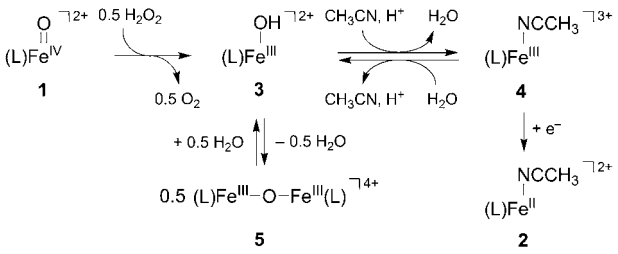
With the 2:1 stoichiometry for **1** and H_2O_2 established, a plausible mechanism for the initial phase of the reaction involves two hydrogen atom transfer (HAT) steps from H_2O_2 to **1** to produce 0.5 equiv of O_2 with respect to **1** [Equations (3) and (4), L = N4Py].[45,46] To verify O_2 generation, concentrations were measured by an optical probe throughout the reaction of 1 mM **1** with 0.5 equiv of H_2O_2 (Figure 2b; Supporting Information, Figure S4). Indeed, (19 ± 1) ppm of O_2 was detected upon decay of **1** (0.47 mM O_2, ca. 95% yield based on two HATs from H_2O_2). Both the stoichiometric production of O_2 and the 2:1 ratio of the reactants (Figure 1b) support the mechanism described by Equations (3) and (4). The 2:1 ratio also implies that O_2 was not formed (or with only minimal contribution) from the reaction of the Fe^{III} complex $[Fe^{III}(N4Py)(OH)]^{2+}$ (**3**) with the hydroperoxy radical (•OOH) [Eq. (5)], because this would require a 1:1 ratio.

$$[(L)Fe^{IV}{=}O]^{2+} + H_2O_2 \rightarrow [(L)Fe^{III}{-}OH]^{2+} + {}^{\bullet}OOH \quad (3)$$

$$[(L)Fe^{IV}{=}O]^{2+} + {}^{\bullet}OOH \rightarrow [(L)Fe^{III}{-}OH]^{2+} + O_2 \quad (4)$$

$$[(L)Fe^{III}{-}OH]^{2+} + {}^{\bullet}OOH \rightarrow [(L)Fe^{II}{-}OH_2]^{2+} + O_2 \quad (5)$$

Complex **3** would then be the expected iron product from the reaction of **1** with H_2O_2 [Scheme 2, Eqs. (3) and (4)]. Although direct evidence for **3** was not obtained by UV/Vis spectroscopy, the lag phase for the formation of **2**, along with an initial isosbestic point at 536 nm (Supporting Information, Figure S1), suggested the formation of an intermediate that does not absorb light in this region.[47,48] To probe potential iron-containing species prior to the production of **2**, the reaction of **1** with 0.5 equiv of H_2O_2 was investigated by electron paramagnetic resonance (EPR) spectroscopy (Supporting Information, Figure S5). The spectrum of a sample of the reaction mixture frozen after the disappearance of **1** exhibited an EPR signal ($g = 2.40$, 2.14, and 1.94) corresponding to **3** (Supporting Information, Figure S5).[47,49] In comparison to an authentic sample of **3** prepared in acetone,



Scheme 2. Proposed mechanism for the reaction of **1** with 0.5 equiv of H_2O_2 (L = N4Py).

however, the signal was very weak, indicating that this complex was not present in a substantial amount. The low accumulation of **3** in the reaction of **1** with 0.5 equiv of H_2O_2 could be due to its fast conversion to $[Fe^{III}(N4Py)(CH_3CN)]^{3+}$ (**4**) and self-decay to **2** in CH_3CN or to the possible equilibrium between **3** and the oxo-bridged dimer $[\{Fe^{III}(N4Py)\}_2(\mu\text{-}O)]^{4+}$ (**5**), which is EPR silent (X-band, perpendicular mode) and does not absorb in the visible region (Scheme 2).[47,48]

To determine kinetic parameters, the reaction of **1** with H_2O_2 was studied under pseudo-first-order conditions. The observed rate constant (k_{obs}) for the decomposition of **1** increased linearly with the concentration of H_2O_2 (Figure 3a), whereas no significant change in k_{obs} values was observed for varying concentrations of **1** (Supporting Information, Figure S6). This behavior indicated a bimolecular reaction with a k_2 value of $(0.80 \pm 0.02)\,L\,mol^{-1}\,s^{-1}$.[50] Thermodynamic parameters were determined from an Eyring plot for experiments in the temperature range from −40 to −10 °C, affording an enthalpy of activation $\Delta H^{\neq}$ of $(30.8 \pm 0.7)\,kJ\,mol^{-1}$ and an entropy of activation $\Delta S^{\neq}$ of $(-158 \pm 2)\,J\,mol^{-1}\,K^{-1}$ (Figure 3b). In contrast to the reaction of **1** with 0.5 equiv of H_2O_2, the absorption spectra of reactions with an excess of H_2O_2 showed an additional feature at 532 nm that is indicative of $[Fe^{III}(N4Py)(OOH)]^{2+}$ (**6**), which was confirmed by EPR spectroscopy (Supporting Information, Figure S7). These spectroscopic data were consistent with those previously reported for **6**.[47,51] In the H_2O_2 reaction of **1**, complex **6** may be expected to form from the reaction of **3** with H_2O_2 [Eq. (6)], but pathways involving other Fe^{III} or Fe^{II} species and $^{\bullet}OOH$ or excess H_2O_2 are also possible (Supporting Information, Scheme S1).[47]

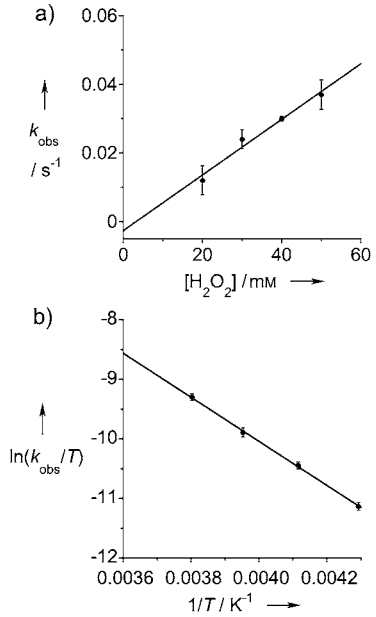


Figure 3. Kinetic and thermodynamic results for the reaction of 1.0 mM **1** in CH_3CN with H_2O_2. a) Plot of the pseudo-first-order rate constant k_{obs} versus $[H_2O_2]$ (20–50 mM) to determine the second-order rate constant k_2 at −20 °C. b) Eyring plot for the reaction of **1** with 20 equiv of H_2O_2 (T = 233–263 K).

$$[(L)Fe^{III}\text{–}OH]^{2+} + H_2O_2 \rightarrow [(L)Fe^{III}\text{–}OOH]^{2+} + H_2O \quad (6)$$

For comparison, the reaction of another oxoiron(IV) complex, $[Fe^{IV}O(tmc)(CH_3CN)]^{2+}$ (**7**; tmc = 1,4,8,11-tetramethyl-1,4,8,11-tetraazacyclotetradecane),[52] with H_2O_2 was investigated. The reactivity of this complex in the same solvent (CH_3CN) was significantly lower than that of **1**, yielding a k_2 value of $(3.5 \pm 0.2) \times 10^{-2}\,L\,mol^{-1}\,s^{-1}$ at 25 °C (Supporting Information, Figure S8). This result is consistent with the lower reactivity of **7** (compared to **1**) previously observed in oxidation reactions of organic substrates.[16,43] On the other hand, the H_2O_2 reactivity of **1** is comparable to that of the oxoruthenium(IV) complex $[Ru^{IV}O(bpy)_2(py)]^{2+}$. When the reaction of **1** with an excess of H_2O_2 was carried out at 25 °C, a k' value of about $8\,L\,mol^{-1}\,s^{-1}$ was obtained ($k' = k_{obs}/[H_2O_2]$), while a similar value had been reported for the Ru complex, albeit in a different solvent ($k' = (12.7 \pm 1.3)\,L\,mol^{-1}\,s^{-1}$ (25 °C, H_2O, pH 7.92)).[28b] Previous reactivity studies of nonheme oxoiron(IV) complexes have largely been focused on organic substrates. For C–H bond activation mediated by nonheme oxoiron(IV) complexes (including **1**), the bond dissociation energy (BDE) and k_2' typically are inversely correlated ($k_2' = k_2/n$, where n is the number of available protons for hydrogen atom transfer).[16] Complex **1** showed a higher reactivity toward H_2O_2 ($k_2' = (0.40 \pm 0.01)\,L\,mol^{-1}\,s^{-1}$, −20 °C) than in C–H bond activation reactions ($k_2' = 4.6 \times 10^{-6}$–$0.037\,L\,mol^{-1}\,s^{-1}$, 25 °C, BDE = 81–99 kcal mol^{-1}),[43] even though the BDE of the O–H bond in H_2O_2 (89.5 kcal mol^{-1})[53] falls in the range of the C–H BDEs of the hydrocarbon substrates used. The divergence from the correlation of BDE and k_2' is consistent with the greater reactivity of O–H bonds over C–H bonds in HAT mechanisms.[46] Taken together, complex **1** exhibits significantly greater H_2O_2 reactivity than **7** and is also more reactive toward H_2O_2 than in hydrocarbon C–H bond activation.

The nature of the reaction of the nonheme oxoiron(IV) complex **1** with H_2O_2 presents a new view of iron redox chemistry and potentially of ROS detoxification and/or production. In the context of reactive metal-oxygen intermediates and also the oxidation of organic substrates catalyzed by metalloenzymes or synthetic metal complexes, H_2O_2 is commonly an oxidant.[6,7,15,16,18] In our study with a nonheme iron model complex, we have demonstrated that H_2O_2 can also function as a reductant resulting in O_2 production. The reaction is related to the HAT mechanism proposed for catalase compound I; however, both mechanisms differ in the ratio of oxoiron(IV) reactant to O_2 product; that is, 2:1 versus 1:1 [Scheme 2, Eqs. (3) and (4)].[5–7] Our results indicate that the reactivity of oxoiron(IV) species with H_2O_2 or $^{\bullet}OOH$ within the active site of mononuclear nonheme iron enzymes may be feasible and could be involved in the detoxification or generation of ROS (O_2 versus $^{\bullet}OOH$ production) depending on the environmental conditions. Although H_2O_2 reactivity has not been documented for oxoiron(IV) intermediates in nonheme iron enzymes, to the best of our knowledge, it is intriguing to

consider this interaction as a possible mechanism in biological systems.

In conclusion, the direct reaction of H_2O_2 with a nonheme oxoiron(IV) complex, generated independently of H_2O_2, was investigated and found to be relatively rapid. Nearly full decay of **1** was achieved with 0.5 equiv of H_2O_2 (2:1) resulting in the formation of an Fe^{II} complex, **2**, and O_2 (Scheme 2). The 2:1 stoichiometry and O_2 generation are consistent with a mechanism involving two HAT steps from H_2O_2 to the Fe^{IV}=O group of **1**. Determination of the bimolecular rate constant under pseudo-first-order conditions revealed that the reaction of **1** with H_2O_2 was more facile than its previously reported reactions with hydrocarbon substrates. In the presence of an excess of H_2O_2, the hydroperoxoiron(III) complex **6** was formed in the course of the reaction, but this was not observed for low equivalents of H_2O_2. The overall observations described herein indicate that if H_2O_2 (and/or $^{\bullet}OOH$) can directly react with nonheme oxoiron(IV) intermediates in enzymes, it may be converted into the hydroperoxy radical or O_2 [Eqs. (3) and (4)] and could thus either disrupt or contribute to ROS homeostasis. Therefore, our findings on the interaction of a nonheme oxoiron(IV) complex with H_2O_2 may provide new insight into the reactivity of ROS with high-valent iron centers in both biomimetic complexes and metalloenzymes.

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2.13, and 1.96), which is possibly attributable to $[Fe^{III}(N4Py)(CH_3CN)]^{3+}$ (**4**) or $[Fe^{III}(N4Py)(H_2O)]^{3+}$ (Scheme 2; Supporting Information, Figure S5). Furthermore, Fe^{III} species present upon oxidation of **2** with PhIO ($g = 4.3$) may be due to incomplete formation of **1**. See: a) H. Kotani, T. Suenobu, Y.-M. Lee, W. Nam, S. Fukuzumi, *J. Am. Chem. Soc.* **2011**, *133*, 3249; b) A. Decker, J.-U. Rohde, E. J. Klinker, S. D. Wong, L. Que, Jr., E. I. Solomon, *J. Am. Chem. Soc.* **2007**, *129*, 15983.

[50] Reactions of **1** with an excess of deuterium peroxide (D_2O_2) showed no dependence of k_{obs} on the D_2O_2 concentration; thus a kinetic isotope effect (KIE) could not be determined. When 0.5 equiv of D_2O_2 was added to a 1.0 mM solution of **1** in CH_3CN at −20 °C, a circa 70% decay of **1** was observed.

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