Rapid, Reversible Oxygen Atom Transfer between an Oxomanganese(v) Porphyrin and Bromide: A Haloperoxidase Mimic with Enzymatic Rates**

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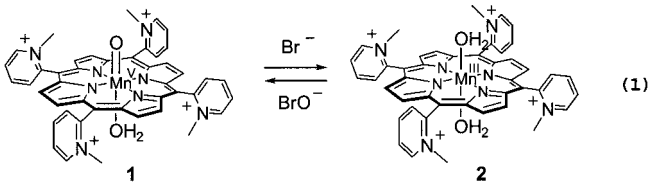
Synthetic manganese porphyrins and related systems have been used extensively in chemical modeling of biological monooxygenation reactions catalyzed by heme proteins.[1] They are also versatile catalysts for the oxygenation of alkanes, alkenes, and nitrogen- and sulfur-containing compounds using oxygen donors such as iodosylbenzene, sodium hypochlorite, alkyl, aryl, and hydrogen peroxide, amine *N*-oxides, and molecular oxygen.[2] Only recently has the key oxomanganese(v) intermediate been well characterized.[3] Here we report that the oxomanganese(v)-5,10,15,20-tetrakis(*N*-methyl-2-pyridyl)porphyrin (**1**) can efficiently transfer its oxo ligand to bromide ion, and that this oxo transfer is rapid and reversible. [Eq. (1)]



(1)

The forward reaction mimics the halide oxidation reaction catalyzed by haloperoxidases,[4] while the reverse reaction is the catalyst activation step in substrate oxygenation by manganese porphyrins. This well-behaved equilibrium allows the assignment of a free energy change for the reaction depicted in Equation (1).

OxoMn^{V}TM-2-PyP (**1**) has unusual stability in aqueous solution compared to other oxoMn^{V} porphyrin intermediates.[3b] It can be generated by the stoichiometric reaction of Mn^{III}TM-2-PyP[5] (**2**) with oxidants such as HSO_5^-, *m*-CPBA (chloroperoxybenzoic acid), and OCl^-. We have found that hypobromite, a weaker oxidant,[6] is also able to generate **1** smoothly. Figure 1a shows the reaction between 5 μM **2** and 50 μM HOBr/OBr$^-$[7] at pH 8.5 monitored by stopped-flow spectrophotometry.[3a,b] Clear isosbestic points were observed at 392, 444, and 558 nm. Remarkably, the identical isosbestic behavior was also found for the reverse reaction, oxoMn^{V} + Br$^-$ at higher bromide concentration. Typical spectral changes observed for the oxo-transfer reaction from

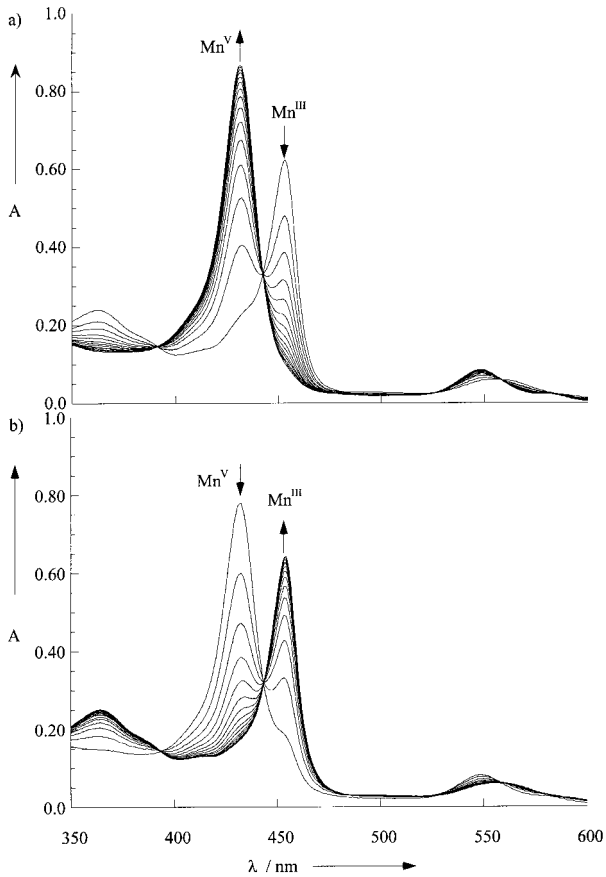


Figure 1. Time-resolved UV/Vis sepctra for the reaction of a) 5 μM Mn^{III}TM-2-PyP (**2**) and 50 μM HOBr/OBr$^-$; b) 5 μM Mn^{V}TM-2-PyP (**1**) and 50 mM Br$^-$ at pH 8.5 (10 mM $Na_2B_4O_7/H_2SO_4$ buffer). For both reactions there were 60 scans in 120 ms. Every fourth scan is shown.

1 to Br$^-$ are shown in Figure 1b. The generation of hypobromite, which is favored by excess bromide and lower pH, was confirmed by observing the diagnostic bromination reaction of phenol red.[8]

The pH dependence of the rate of oxo transfer to bromide was examined between pH 5.2 and 9.0 (I = 0.25 M $NaClO_4$). The reaction was found to be first-order in both oxoMn^{V} and Br$^-$, and independent of the buffer concentration. Kinetic profiles were obtained by monitoring oxoMn^{V} (**1**) at 433 nm. Pseudo-first-order fitting of the kinetic data to a single exponential was carried out with at least six concentrations of Br$^-$ at each pH value. The apparent second-order rate constant k_{app} was calculated from the slope of the linear plot of k_{obs} versus C_{Br^-}. Our results show that **1** was nearly as effective an oxygen donor to Br$^-$ ($3.8 \times 10^5\,M^{-1}s^{-1}$ at pH 7.0) as myeloperoxidase compound I ($1.1 \times 10^6\,M^{-1}s^{-1}$ at pH 7.0),[9] and much more effective than vanadium bromoperoxidase (estimated to be $2.78 \times 10^3\,M^{-1}s^{-1}$ at pH 7.9 and $1.75 \times 10^5\,M^{-1}s^{-1}$ at pH 4.0) and related functional mimics.[10] The pH dependence of k_{app}, spanning five orders of magnitude, is plotted in Figure 2.

To explain this profound pH dependence, *two* proton transfers must be involved. We propose that **1** exists as a dioxo species [O=Mn^{V}–O$^-$] at high pH,[11] which is inert to the nucleophilic attack of Br$^-$. Two fast acid–base equilibria

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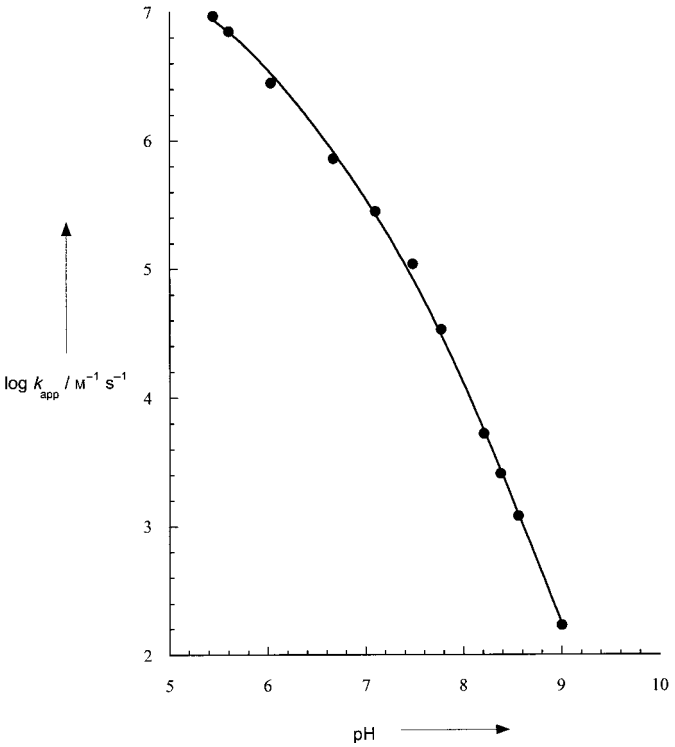


Figure 2. Plot of the second-order rate constants ($\log k_{app}$) as a function of pH for the reaction between oxoMn^{V}TM-2-PyP (**1**) and Br$^-$ (25 °C, I = 0.25 M $NaClO_4$). Compound **1** was generated by the reaction of 5 μM Mn^{III}TM-2-PyP (**2**) and 5 μM potassium peroxymonosulfate (see ref. [3b]). Bromide concentrations ranged from 100 μM to 50 mM depending on pH. The plotted curve was computed on the basis of best-fit parameters obtained from nonlinear least-squares analysis of the data points using Equation (2).

between the dioxo Mn^{V} [O=Mn^{V}–O$^-$], oxo–hydroxo Mn^{V} [O=Mn^{V}–OH], and oxo–aqua Mn^{V} [O=Mn^{V}–OH_2] species must be required to fully activate the oxoMn^{V} species prior to oxo-transfer reaction.[12] The experimental points fit well to Equation (2) ($R = 0.9993$) which was deduced from this reaction model (Scheme 1).

$$\text{kapp} = \frac{k_{int} a_H^2}{a_H^2 + a_H k_{a1} + k_{a1} k_{a2}} \qquad (2)$$

From the data in Figure 2, we determined the pK_{a_2} of the oxo–aqua Mn^{V} species to be 7.7 ± 0.1. The pK_{a_1} must be less than 5 and beyond the range of the data. The intrinsic or pH-independent rate constant for the reaction between the doubly protonated oxo–aqua Mn^{V} species and bromide ion, k_{int}, could be estimated to be around $10^8\,M^{-1}s^{-1}$.

This fast, reversible oxo-transfer reaction provides an unusual opportunity to determine the equilibrium constant K_{oxo} for Equation (1) and to define the free energy of the high-valent oxoMn^{V} intermediate. K_{oxo} was calculated from the ratio of the forward and reverse rate constants,[13] k_f/k_r, at a given pH. Alternatively, K_{oxo} was determined directly by finding the concentration ratio C_{BrO^-}/C_{Br^-} that caused no UV/Vis spectral changes in a Mn^{V}–Mn^{III} solution (2.5 μM in each) upon stopped-flow mixing with a BrO$^-$–Br$^-$ solution. K_{oxo} was found to vary from 3.5 at pH 7.3 to 2.9×10^{-5} at pH 9.6. The data could be extrapolated to other pH regions with the Nernst equation from the known Mn^{V} and Mn^{III} pK_a values.[11] Figure 3 shows a potential versus pH diagram for the oxoMn^{V}/Mn^{III}TM-2-PyP system and those for the BrO$^-$/Br$^-$ and ClO$^-$/Cl$^-$ redox pairs. Interestingly, this relationship predicts that chloride oxidation should become accessible near pH 5. Indeed, the reaction of Cl$^-$ with oxoMn^{V}TM-2-PyP (**1**) to afford hypochlorite, as monitored by the chlorination of methyl orange, was found to occur efficiently at pH < 5. The chlorination yields were significantly lower at higher pH[14] in good agreement with the predicted behavior.

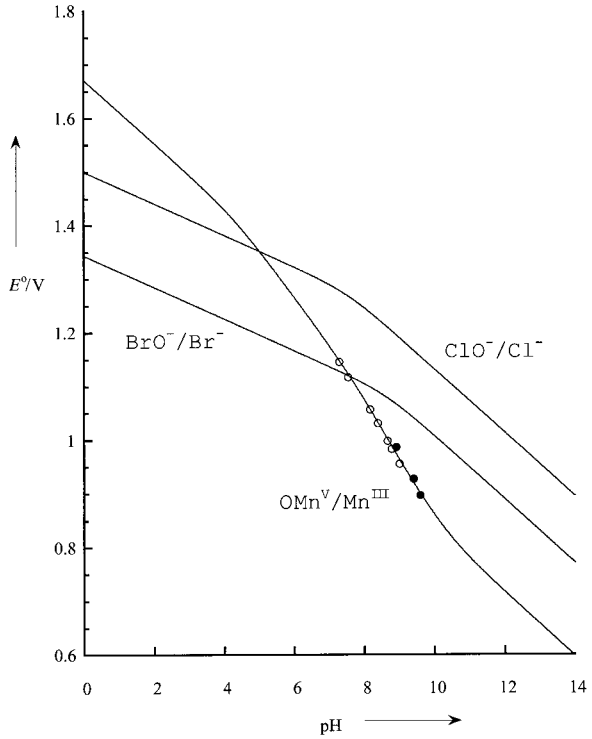
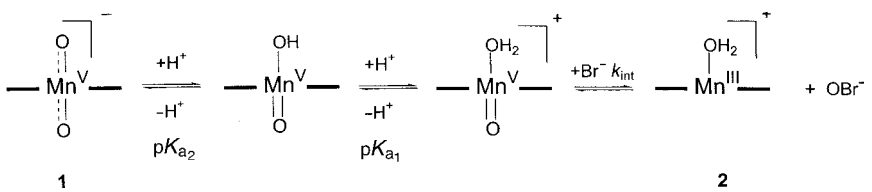


Figure 3. Standard electrode potential (E°) versus pH diagram for the oxoMn^{V}/Mn^{III}TM-2-PyP system along with BrO$^-$/Br$^-$, ClO$^-$/Cl$^-$ redox pairs. Equilibrium constants were either measured directly (solid circles), or calculated from the ratio of forward and reverse reaction rate constants k_f/k_r (empty circles). E° was then calculated from equilibrium constants and known BrO$^-$/Br$^-$ potentials.[6] The oxoMn^{V}/Mn^{III} curve was extrapolated to other pH regions with the Nernst equation using the experimental data and the following pK_a values: Mn^{III}$(OH_2)_2$, $pK_{a_1} = 9.6$, $pK_{a_2} = 10.7$; oxoMn^{V}(OH_2) $pK_{a_1} < 5$ $pK_{a_2} = 7.7$, (see ref. [11]). Crossings of the BrO$^-$/Br$^-$ and ClO$^-$/Cl$^-$ lines are at pH 5.1 and 7.6, respectively.



Scheme 1. Reaction model for the oxo-transfer reaction.

These results show that bromide and chloride are readily oxidized to the corresponding hypohalite by an oxoMn^{V} porphyrin (**1**). The reversibility of this process has placed this oxoMn^{V} intermediate on a

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thermochemical energy scale.[15] The more reactive oxoiron porphyrin systems[16] are under current investigation.

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[8] A buffered (pH 7.0, 25 mM phosphate buffer) solution of 10 μM (**2**), 5 mM NaBr, and 50 μM phenol red had 100 μM HSO_5^- added, resulting in production of bromophenol blue (yield 30%, $\lambda_{abs} = 592$ nm) within 1 s. In the absence of catalyst, control experiments showed less than 1% production of bromophenol blue within 1 min.

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[13] In the pH region investigated, the reverse reaction rate constant, k_r was found to be first order in both $c_{Mn^{III}}$ and c_{OBr^-+HOBr}.

[14] In a typical experiment, 1M NaCl (1 mL), **2** (1 mL), 50 mM pH 5 phosphate buffer solution (2 mL), 1 mM methyl orange (1 mL) were mixed prior to the addition of a 10 mM oxone (HSO_5^-; 100 μL) solution. After two minutes, this was extracted with *n*-heptane. GC-MS analysis revealed monochlorodimethylaniline (*m*/*z* 154) and dichlorodimethylaniline (*m*/*z* 188), matching with calibration experiments using methyl orange and authentic hypochlorite. The estimated yields of OCl^- per mole of oxone were 85% at pH 5, 5.5% at pH 7.6, and 0.3% at pH 9.0.

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The High-Valent Compound of Cytochrome P450: The Nature of the Fe–S Bond and the Role of the Thiolate Ligand as an Internal Electron Donor**

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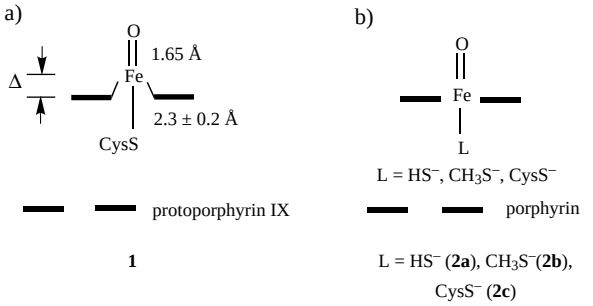
Recently, Schlichting et al.[1] have used time-lapse X-ray crystallography to "photograph" the hydroxylation pathway of camphor by cytochrome $P450_{cam}$, which includes the elusive, high-valent iron-oxene species (**1** in Scheme 1a). In response to this exciting work, we present here an extensive density functional theoretical (DFT) investigation of iron



Scheme 1. Selected X-ray diffraction data for a) the high-valent P450 iron oxene species **1**. Δ indicates the protrusion of the iron center from the porphyrin plane. b) Model systems **2a–c**.

oxene (**2a–c**, Scheme 1b) with emphasis on geometry, electronic structure, and unusual features of the Fe–S bonding. Thus, while the X-ray diffracting species[1] qualitatively fits iron oxene, its precise geometric data are less certain. For example, the distance between the iron and the proximal ligand, $r_{Fe\text{-}S}$, appears quite short but the value 2.3 ± 0.2 Å has a significant uncertainty. Another uncertainty, discussed by the authors,[1] is the possible contamination by an additional species. Theory[2] itself has not as yet settled on a value for this distance, which appears to vary between 2.37–2.69 Å for different models systems and computational levels.[2] An associated issue is the theoretical characterization of the flexibility of the Fe–S linkage in **1** and the role of the thiolate ligand as an internal electron donor.[3] A still uncertain feature of P450 iron oxene is whether it involves a porphyrin cation radical, as in the analogous Compound I species of horseradish peroxidase[4a] and synthetic models,[4b] or, rather, does it possess a sulfur radical situation,[2a,e] or perhaps a resonance hybrid of these forms.[3, 5] A related question concerns the spin-state identity; high-spin as in some Compound I species,[4a,b] or low

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