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Water-soluble, Cationic Manganese and Vanadium Porphyrins as Biomimetic Models and Practical Oxidation Catalysts

Thomas Peter Umile

ABSTRACT

Synthetic metalloporphyrins are studied as biomimetic models of heme-containing oxygenase and peroxidase enzymes, such as cytochrome P450. In many instances, model compounds have not only informed our understanding of enzymatic activity but also yielded practical catalysts and processes. The focus of the present work is the reactivity of synthetic manganese and vanadium porphyrin complexes bearing cationically-charged $N$-methyl pyridyl and $N,N$-dimethyl imidazolyl meso-substituents.

Cationic manganese porphyrins are shown herein to rapidly and efficiently catalyze the conversion of aqueous solutions of sodium chlorite to chlorine dioxide, an industrially useful bleach and disinfectant. The more electron-withdrawing porphyrin ligands were found to produce the fastest catalysts for this process (ca. 0.4 turnovers s$^{-1}$), and a cationic manganese porphyrazine was even more active (> 30 turnovers s$^{-1}$). This process was investigated by stopped-flow spectroscopy and computationally-assisted kinetic simulations, and the proposed mechanism involves rate-limiting oxidation of manganese(III) by chlorite ion to afford an oxomanganese(V) intermediate. High-valent oxomanganese(V) and –(IV) subsequently oxidizes other chlorite ions to produce chlorine dioxide in a peroxidase-like manner. Promising results for directly applying this new technology in water treatment are also presented.

High-valent oxomanganese porphyrin species, known intermediates in a number of manganese porphyrin-catalyzed oxidations, were characterized by XAS and EXAFS.
Notably, the *trans*-dioxomanganese(V) porphyrin is shown to have two equivalent Mn-O bonds of 1.68 Å, consistent with that predicted by vibrational spectroscopy. This investigation also revealed two intriguing species worthy of further study: an oxomanganese(IV) with an extremely short Mn-O bond length of 1.63 Å and a manganese(V) with two anomalously long Mn-O/N bonds of 2.01 Å.

Finally, the synthesis and spectroscopic characterization of a family of cationic vanadyl (oxovanadium(IV)) porphyrins is presented. These complexes resist facile chemical and electrochemical oxidation, although under extremely alkaline conditions an irreversible electrochemical oxidation event is detectable. Observed pKₐ transitions and electrochemistry allow the prediction that a putative *trans*-dioxovanadium(V) porphyrin could break a C-H bond with a bond dissociation energy $> 99$ kcal mol$^{-1}$. These results suggest the potential of these novel vanadium porphyrins to be further developed as catalysts for alkane functionalization.
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PRIOR PRESENTATIONS AND PUBLICATIONS

Portions of this dissertation have been previously published and/or presented publically.

CHAPTER II & CHAPTER III

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CHAPTER V

Poster Presentation:

CHAPTER I

THE HEME PARADIGM: CYTOCHROME P450 AND SYNTHETIC METALLOPORPHYRINS
INTRODUCTION

The elements of the transition metal series play an important and often underappreciated role in biology. The lack of recognition for the importance of metals in living systems may be due to the fact that 95% (dry weight) of all life is composed of the so-called CHNOPS elements (carbon, hydrogen, nitrogen, oxygen, phosphorous, and sulfur). The non-metallic CHNOPS elements are the structural components of the carbohydrates, fats, proteins, and nucleic acids that are the stuff of living matter. However, many first-row transition metals including vanadium, manganese, iron, cobalt, nickel, copper, and zinc (as well as a few heavier metals like molybdenum and tungsten) are trace elements that are absolutely essential to proper metabolic function.

The ability of transition metals to exist in a variety of oxidation states, electronic structures, and bonding geometries may explain why evolution chose to use these elements as it did. Often, the roles filled by transition metals in biology (specifically within proteins) capitalize on their unique properties to form coordination complexes and participate in redox chemistry. Among the most abundant protein-metal interactions are the zinc fingers, in which zinc(II) ions coordinate with cysteine and histidine residues in proteins to impart structural features and increase their stability.\(^1\) A simple example of the redox involvement of metals in biology is the iron ion found in cytochrome c, which readily adopts both ferrous (Fe\(^{2+}\)) and ferric (Fe\(^{3+}\)) states and thereby allows cytochrome c to shuttle electrons through the electron transport chain during mitochondrial respiration.\(^2\) The ability of metals not only to adopt various oxidation states but to store multiple oxidation equivalents is another feature commonly exploited by biology. This is demonstrated by the multi-electron oxidation of water to oxygen (O\(_2\)) that is carried out
by a cluster of manganese, calcium and oxygen ions (Mn$_4$CaO$_5$) found in the oxygen evolving complex (OEC) of Photosystem II. Enzymes that employ metallic cofactors or prosthetic groups, like the manganese-containing OEC, are commonly called metalloenzymes.

One family of metalloenzymes that has been the focus of much attention is that of the cytochromes P450. These iron-containing enzymes are members of the larger family of oxygenases, and the P450s are specifically monooxygenases as they catalyze the transfer of a single oxygen atom from O$_2$ to a variety of substrates (S):

$$O_2 + 2 \text{e}^- + 2 \text{H}^+ + S \rightarrow \text{SO} + \text{H}_2\text{O}$$

This transformation is used to carry out the metabolism of xenobiotic compounds and plays a part in the biosynthesis of some secondary metabolites. The P450s have attracted interest from scientists in a wide diversity of fields. Studies range in scope from understanding the role of P450 in pharmacokinetics, drug metabolism, and biosynthesis to answering more fundamental chemical questions about how P450 enzymes activate O$_2$ in a controlled manner, generate a highly-reactive oxidizing species in the heart of a fragile protein matrix, and tune the reactivity of such species to carry out productive redox chemistry. This inspiring orchestration of oxygen activation and oxidation catalysis by P450s has been referred to as “the heme paradigm” and is the model to which most all oxygenase enzymes and oxidation catalysts are compared.

Apart from satisfying a scientific curiosity concerning how P450s work, an understanding of the chemical process of P450 (and other oxygenase) activity is of great practical relevance to the pharmaceutical, manufacturing, and energy industries. Synthetic model compounds designed to mimic P450 activity are studied not only to gain
insight into the enzymatic system but also to provide practical solutions to challenging chemical problems. This introductory chapter will provide an overview of how Cytochrome P450 activates and controls oxygen for productive purposes and then survey some advances that have been made using bio-inspired synthetic iron and manganese porphyrin catalysts.
CYTOCHROME P450

The cytochromes P450 (Figure 1) are a family of iron-containing enzymes that catalyze the transfer of an oxygen atom from O₂ to organic substrates, producing water as a by-product.⁹ Thousands of P450 enzymes are known and have been found in a range of organisms including bacteria, fungi, plants, insects, and mammals.¹⁰ In cells, P450 enzymes are commonly associated with membranes, especially the inner mitochondrial or endoplasmic reticulum membrane.⁵,¹¹ Although the various members of the P450 family share some structural similarities, by far the most important conserved feature in P450 is a cysteine-ligated heme (iron protoporphyrin IX, Figure 2).⁵-⁶ This heme prosthetic group is the active site of oxygen activation and O-atom transfer by P450. The intensely colored heme is also the source of the P450 name, as it produces a characteristic UV-Vis absorbance at 450 nm upon binding a molecule of carbon monoxide to its reduced form ("pigment 450").¹²-¹³

**Figure 1.** Structure of P450cam (PDB: 1DZ6)
Figure 2. Iron protoporphyrin IX, the active site of Cytochrome P450

Heme is a commonly encountered prosthetic group in nature. It is perhaps most well known as a component of the protein hemoglobin (Hb).\textsuperscript{14} In this role, heme is ligated not by a cysteine thiolate but by a histidine, and the iron center reversibly binds to \( \text{O}_2 \). Hence, Hb is the protein used to ferry \( \text{O}_2 \) throughout the circulatory system of most vertebrates. Heme can also be found in a number of other proteins and enzymes, including cytochrome \( c \) (cyt \( c \))\textsuperscript{2,15}, chloroperoxidase (CPO)\textsuperscript{16}, nitric oxide synthase (NOS)\textsuperscript{17}, horseradish peroxidase (HRP)\textsuperscript{5}, and chlorite dismutase (Cld)\textsuperscript{18-19}. As in hemoglobin, the heme iron in HRP and Cld is ligated by a histidine; however, the conserved cysteine thiolate found in P450 is similarly present in CPO, NOS, and the so-called unspecific fungal peroxygenases.\textsuperscript{20-21} As will be discussed below, this axial thiolate ligation is important to both oxygen activation and hydroxylation by P450.

P450s are remarkable because they catalyze the monooxygenation of a variety of substrates using \( \text{O}_2 \) as an oxidant. It is important, however, to note that when one oxygen atom from \( \text{O}_2 \) is passed to a substrate, the remaining oxygen atom is reduced to water by two protons and two electrons. The two electrons needed in order to carry out this
reduction are provided to P450 by a system of electron transport proteins and ultimately come from common NAD(P)H reducing equivalents.\textsuperscript{5} In most cases, this electron transport system consists of a flavoprotein (plus an iron-sulfur protein for mitochondrial and bacterial P450s) that shuttles electrons from NAD(P)H to P450. However, self-sufficient P450 enzymes are known (such as P450-BM3 from \textit{Bacillus mageterium}) which contain both the heme active site as well as an electron-shuttling flavin system in the same subunit.\textsuperscript{22-23}

\textbf{Catalytic Reactions}

As enzymes, P450s are key actors in chemical transformations crucial to both the metabolism of xenobiotics (e.g. pharmaceuticals, toxic agents) and the biosynthesis of secondary metabolites such as steroids.\textsuperscript{6,11} In drug metabolism, P450s carry out the bulk of Phase I metabolism, whereby a hydrophobic compound is oxidized or otherwise functionalized to impart increased water solubility (and bioavailability) and permit further downstream metabolism (including the eventual excretion of the compound).\textsuperscript{24-25} Such processing is also known to convert biologically inactive prodrugs into pharmaceutically active species.\textsuperscript{25} In some cases, the action of P450 is even tied to the \textit{toxicity} of certain drugs. For example, the common analgesic acetaminophen (Tylenol\textsuperscript{®}) is converted to the toxic arylating agent \textit{N}-acetyl-\textit{p}-benzoquinamine by a P450 enzyme (Figure 3).\textsuperscript{26}
Figure 3. P450 metabolism of acetaminophen produces $N$-acetyl-$p$-benzoquinone

From a synthetic perspective, the individual reactions catalyzed by P450 are extraordinary;\textsuperscript{27-28} as a specific example, the stereospecific hydroxylation of 6-deoxyerythronolide during erythromycin biosynthesis (Figure 4).\textsuperscript{29} For as much chemical finesse and selectivity as P450s demonstrate however, they can also remarkably catalyze transformations of incredibly strong C-H bonds. Recently, a non-covalently modified P450 enzyme (CYP102A1)* has been shown capable of accepting and oxidizing non-natural alkane substrates including methane to afford alcohols in high yield.\textsuperscript{30}

Figure 4. Hydroxylation of 6-Deoxyerythronolide B by the P450 enzyme EryF

* Specific Cytochrome P450 enzymes are abbreviated CYP, and the following numerals and letters identify the specific family and subfamily of the enzyme
On one hand, the controlled use of O₂ as a reagent is itself challenging. Although the reactions of O₂ with H₂ to produce water or hydrogen peroxide are both spontaneous reactions ($\Delta G^\circ = -54$ and $-25$ kcal mol⁻¹, respectively), O₂ is a kinetically-sluggish oxidant by itself.³¹-³² Moreover, once activated, aerobic oxidation can be difficult to control, and often results in over-oxidation or mixtures of products.³³ In spite of this, the regio- and stereo-selectivity demonstrated in P450 hydroxylation reactions displays a level of catalytic control that is the focus of many ongoing, synthetic research efforts.²⁷,³⁴-³⁵ Perhaps most tantalizing, however, is the insertion of O-atoms into strong, unactivated C-H bonds, especially in the presence of weaker or more reactive functionalities.³⁶

Although individual examples of P450 activity are numerous, most can be classified by a much smaller number of reaction types: hydroxylation, O-atom transfer (e.g., epoxidation, sulfoxidation), N-dealkylation, and desaturation.¹¹ The remainder of this section will focus on the unique hydroxylating reactivity of P450 using O₂, as it presents a rare example of the controlled breaking and subsequent functionalization of strong C-H bonds. The insights gained through an appreciation of the P450 catalytic mechanism (and related monooxygenases) have led to the development of practical catalysts that mimic the enzymatic oxidation of various substrates.

**Oxygen Activation by P450**

The catalytic mechanism of oxygen activation and substrate oxidation by Cytochrome P450 was an area of much active debate for many years.³⁷ However, it has
recently been confirmed by Rittle and Green that the reactive, hydroxylating intermediate in P450 catalysis is an oxoiron(IV) porphyrin cation radical (Compound I, for historical reasons; Figure 5). This interesting species is not unique to P450 but can be found as an active intermediate in other heme enzymes including CPO and HRP.

![Figure 5. Compound I in Cytochrome P450](image)

The consensus mechanism by which P450 activates O₂ to generate Compound I is given in Scheme 1. The resting state of P450 is a hexa-coordinate aquairon(III) species (1) that is characterized as being a low-spin, d⁵ doublet. Binding of a substrate displaces the aqua ligand, simultaneously resulting in the promotion of the iron(III) complex from low- to high-spin (2) and raising the reduction potential of the heme iron by as much as +300 mV. The penta-coordinate iron(III) species is then easily reduced by 1 electron donated from the associated electron transfer system to afford a ferrous iron(II) heme complex (3). That the binding of substrate results in a number of events that make this step (2 → 3) possible has been suggested to be a type of regulator, preventing the unproductive use of reducing equivalents in the absence of substrate. The ferrous complex then binds an oxygen molecule (O₂), as in Hb. As the ferrous heme is
experimentally a quintet and oxygen \((O_2)\) a triplet, this is formally a spin-forbidden reaction and is facilitated presumably via a low-lying triplet excited state of the ferrous heme.\(^{42}\)

**Scheme 1.** Mechanism of oxygen activation and O-atom transfer to substrate in Cytochrome P450 (Adapted from Reference 43)
The O₂/ferrous heme complex (4) can be written as a pair of redox tautomers (O₂-FeII or ‘O₂-FeIII). This complex is diamagnetic and EPR-silent, although Mössbauer spectroscopy and the weakened O-O stretching frequency suggest that the iron(III) complex more properly describes the intermediate. Regardless, the oxyheme complex is then further reduced by a second electron to afford a peroxoiron(III) complex (5). In addition to being an intermediate on the pathway of oxygen activation to generate Compound I, this peroxoiron(III) species has also been implicated as a nucleophilic oxidant that is active in some P450s (e.g. CYP19, CYP17) and involved in sterol biosynthesis (Scheme 2).

\[
\text{Scheme 2. (a) Deformylation of androgens by aromatase (CYP19) and its (b) mechanism involving nucleophilic peroxoiron(III) (Adapted from Reference 5)}
\]

Protonation of the peroxoion(III) intermediate affords a hydroperoxoiron(III) species (6, “Compound 0”) that was for a long time the last observable and isolable
intermediate in the P450 mechanism. This species can also be generated from activation of hydrogen peroxide by the resting ferric enzyme via the peroxide shunt pathway (Scheme 1). Heterolytic O-O bond cleavage of this hydroperoxoiron(III) is understood to proceed via the “push-pull” mechanism, proposed by Dawson, et al. (Figure 6). In histidine-ligated heme enzymes (such as HRP), the proximal histidine ligand is made more electron-donating by hydrogen-bonding to a nearby aspartate, which provides an inductive, electronic “push” to the iron. Simultaneously, distal histidine and arginine residues facilitate protonation of the distal oxygen atom of the hydroperoxoiron(III), affording a good leaving group (i.e. water) that “pulls” on the iron. The hydroperoxoiron(III) in P450s (which lack this distal machinery in their quite hydrophobic active site) are provided a so-called “big push” from the anionic thiolate ligand that is so conserved across the P450 family. This “big push” is believed to be assisted by a relay system of amino acids that provide protons from the medium in place of a “pull” effect.
Figure 6. “Push-pull” effect facilitating heterolysis of hydroperoxoiron(III) in (a) horseradish peroxidase and (b) Cytochrome P450 (Adapted from Reference 5)

Heterolytic cleavage of the hydroperoxy O-O bond affords an oxoiron species formally at the oxidation state of Fe$^V$. Numerous studies$^{38-40,48-51}$ have shown that the porphyrin ligand is non-innocent, and this oxoiron species is more properly an oxoiron(IV) antiferromagnetically coupled to a porphyrin cation radical (7, Compound I).$^{22,29-34}$ Compound I has been long known and observed in CPO$^{39}$ and HRP$^{40}$ and recently isolated and characterized in P450.$^{38}$ Using rapid freeze-quench techniques, Rittle and Green generated and isolated a Compound I species from the reaction of a P450 enzyme (CYP119) with $m$-chloroperoxybenzoic acid (mCPBA) and characterized it by UV-Vis, Mössbauer, and EPR.$^{38}$ That Compound I is the active oxidizing species in P450 catalysis is well accepted; however, the question remains: just how does an oxoiron(IV) porphyrin cation radical hydroxylate a substrate?
C-H Functionalization by P450

The mechanism for O-atom insertion into a C-H bond (i.e. hydroxylation) by Cytochrome P450 was first postulated by Groves, et al. in 1978. In what would become a landmark study, the authors proposed that a reactive, oxoiron species at the formal oxidation state of Fe^{V} abstracts a hydrogen atom from a C-H bond to afford a [HO-Fe^{IV}] intermediate and carboradical. Subsequent and fast radical recombinat or “rebound” was suggested to result in the formation of the alcohol product. The radical rebound mechanism (Scheme 3) has withstood the test of time, both experimentally and theoretically.

![Scheme 3](image)

**Scheme 3.** Radical rebound mechanism

Large, primary kinetic isotope effects (>10) have been repeatedly observed for catalytic hydroxylations by P450 as well as for the direct reaction between hydrocarbons and authentic Compound I (and model compounds). Furthermore, the isotope effects observed for P450 hydroxylations and N-dealkylations are surprisingly well-mimicked by those observed in authentic hydrogen atom abstractions by the tert-butoxy radical. (Meyer has discussed at length the resemblance of hydrogen atom abstraction
by metal oxos to that by organic radicals.\textsuperscript{56} The observed isotope effects support the proposal of a rate-determining C-H bond-breaking during the hydroxylation mechanism.

Hydrogen atom abstraction from a substrate by Compound I generates a substrate (carbon-centered) radical and an iron(IV) species. The existence of this radical has been inferred by such observations as the P450-catalyzed hydroxylation of exo-exo-exo-exo-tetradeuteronorbornane that afforded exo-alcohol while retaining the tetradeuterium label (Figure 7).\textsuperscript{52} Radical clock substrates, which rearrange at known rates upon hydrogen atom abstraction, have also been used to time the lifetime of the incipient radical formed in the P450 active site (ca. 1 – 250 ps).\textsuperscript{57-62}

![Figure 7](image)

**Figure 7.** Exo-alcohols formed by the hydroxylation of exo-exo-exo-exo-tetradeuteronorbornane by P450

The oxoiron species generated upon 1-electron reduction of Compound I (generated by hydrogen atom abstraction from a substrate) is commonly referred to as Compound II. Compound II itself has been the focus of much recent exploration and discovery. Central to these studies was the question “Is Compound II protonated?” The motivation for answering that question arises from two astute observations: \textit{first}, the driving force for a hydrogen-atom abstraction (i.e. the enthalpic difference between the C-H bond broken and O-H bond formed) is a crucial mediator of reactivity by hydrogen atom transfer;\textsuperscript{56,63} and \textit{second}, the bond dissociation enthalpy (BDE) of the H-O bond in
a metal hydroxide is truly a function of the acidity (pK\textsubscript{a}) of the hydroxide and the 1-electron reduction potential (ΔE\textsuperscript{o}) of the complex (Figure 8).\textsuperscript{63-65} This latter point therefore suggests that, redox potentials being equal, more basic metal oxos will be able to break stronger C-H bonds.\textsuperscript{66} (\textit{N.B.} This analysis makes the assumption that entropic effects of homolytic bond dissociation are negligible, a point which is not always accurate.)

\[ \Delta G_{o1} = -nF\Delta E^o \]

\[ \Delta G_{o2} = -RT \ln(K_a) \]

\[ \text{BDFE} = \Delta G_{o1} + \Delta G_{o2} + C \]

\textbf{Figure 8.} Bond Dissociation Free Energy is linearly related to the free energy of reduction and subsequent protonation of a metal oxo (adjusted by a constant C)

An analysis of observed Fe-O bond lengths for authentic Compound II species in a variety of heme enzymes revealed that the ferryl species in HRP is indeed a true oxoiron(IV), whereas in CPO and P450 the oxoiron(IV) species is basic and might be
better described as a hydroxoiron(IV). The origin of this difference may arise from the axial thiolate ligation found in the “protonated Compound II” enzymes. The stronger electron-donating nature of the thiolate (relative to the histidine found in the other heme proteins) weakens the trans-axial Fe-O bond. The increased basicity of Compound II in the thiolate-bound hemes implies that these enzymes can more easily participate in hydrogen-atom abstraction reactions; indeed, P450 and CPO (but not HRP) can catalyze hydroxylation reactions.\textsuperscript{5, 70}

The axial cysteine thiolate is also suggested to slightly stabilize the high-valent iron(IV) species, reducing its redox potential by ca. 300 mV.\textsuperscript{71} Although this technically should attenuate reactivity, that effect is somewhat offset by the increase in Compound II basicity. In fact, the combination of a slightly stabilized iron(IV) coupled to a more basic oxo can be understood to result in an enzyme that is tuned for productive C-H abstraction chemistry over simple electron transfer (peroxidase) reactivity.\textsuperscript{71}

The recombination of the substrate radical and Compound II proceeds to afford the functionalized alcohol product and the resting Fe\textsuperscript{III} enzyme. Theoretical modeling has shown that the kinetics of this step depend on which of two nearly-degenerate spin-state reaction surfaces is traversed. Compound I, a ground state doublet, has a nearly degenerate quartet excited state (Figure 9).\textsuperscript{47, 72}
Figure 9. Qualitative orbital diagram for doublet ($S = 1/2$) and quartet ($S = 3/2$) Compound I

In this so-called “Two State Reactivity” model, the recombination of radical and hydroxoiron(IV) is barrierless on the doublet spin surface (Figure 10)$^{72}$. However, the slightly excited quartet Compound I ($+0.1$ kcal mol$^{-1}$) encounters a ca. 10 kcal mol$^{-1}$ barrier. This phenomenon has been reviewed in detail elsewhere,$^{72}$ but is understood to arise from the requirement (on the quartet surface) for the carboradical electron to fill a high-energy $\sigma^*$ orbital during radical rebound. On the doublet manifold, this electron fills a lower-energy $\pi^*$ or ligand-based $a_{2u}$ orbital.
**Figure 10.** Simplified reaction coordinate diagram for the “Two State Reactivity” model for hydroxylation of RH (phenylmethylcyclopropane) byCompound I (energy in kcal mol$^{-1}$). n.b. the lowest energy intermediate is calculated as formally an iron(III) porphyrin cation radical species (Adapted from reference 72)

**Intermezzo**

The preceding has provided a brief overview of the remarkable reactions and chemistry that are involved in the catalytic activity of Cytochrome P450. This discussion has thus far neglected to comment on the mature field of biomimetic model compounds. Many of the insights we now have on P450 chemistry including the role of electronic configuration, redox potential, pKₐ/s, and charge effects would have been impossible to
uncover without the extensive body of literature concerning reactive (and unreactive), synthetic models. However, as our understanding of P450 and other monooxygenases becomes more mature, the focus of model studies is shifting towards adapting the tools and “tricks” of biology for practical, catalytic systems. The next section will survey a few key findings from the study of synthetic, P450-inspired metalloporphyrins that have provided both fundamental understanding, as well as practical, applied technologies.
METALLOPORPHYRIN MODEL COMPOUNDS

Our understanding of the factors that influence the reactivity of Cytochrome P450 were in large part elucidated through the use of synthetic model compounds. These models allowed researchers to explore chemistry incompatible with classical enzymology, such as working in nonaqueous solvents or at pH extremes. Although P450 and other heme enzymes employ ligands based on the porphyrin motif, model studies and biomimetic compounds have been explored not only with synthetic porphyrins but also related porphyrinoid ligands such as phthalocyanines, corroles, and corrolazines and also non-heme ligand motifs.

As these studies have progressed, it has become apparent that biomimetic model compounds are not merely useful tools for probing the mechanisms and reactivity of metalloenzymes; the lessons learned from model studies have also informed discussions about fundamental catalytic reactivity. In some cases, the study of model compounds has resulted in the direct development of commercially useful and practical new technologies.

Indeed, these early successes using biomimetic catalysts hold much promise for the future of the energy and manufacturing sectors. With the advantage of billions of years of evolution on its side, nature has been able to control and tune the reactivity of a myriad of catalysts (i.e. metalloenzymes) that employ naturally-abundant, cheap first-row transition metals. In many cases, the same chemical transformations that biology carries out with iron or manganese (e.g. hydrocarbon functionalization and water splitting) cannot be replicated by chemists without the use of rare, expensive heavier transition elements (e.g. platinum and iridium). With this in mind, we turn now to some interesting
examples of fundamental advances concerning both oxygen activation by and oxygen atom transfer from biomimetic metalloporphyrin model compounds.

**Iron Porphyrins**

Groves, *et al.* were the first to recognize the ability of synthetic iron porphyrin compounds to catalyze the oxidation of organic substrates. Iron tetraphenyl porphyrin (FeTPP) was found to catalyze oxygen transfer from iodosobenzene to cyclohexane, affording cyclohexenol and cyclohexene oxide.\(^{54}\) Using the slightly more sterically-hindered iron tetramesityl porphyrin (FeTMP), the first model oxoiron(IV) porphyrin cation radical was prepared at –77 °C and characterized by NMR, UV-Vis, and Mössbauer spectroscopies.\(^{85}\) When this species was prepared in the presence of \(\text{H}_2^{18}\text{O}\), 99% of the product incorporated the oxygen label. This demonstrated that the high-valent, oxoiron complex, and not a porphyrin adduct of the oxidant, was the O-transfer reagent. Since then, other Compound I analogues\(^{86}\) have been prepared and characterized, although none quite as reactive as the authentic oxoiron(IV) porphyrin cation radical species generated by P450.\(^{38}\)

More recently, preparations of water-soluble oxoiron(IV) porphyrin cation radical compounds were accomplished using both anionic (tetra-mesityl-disulfonato, TMPS) and cationic (tetra-\(N\)-methyl-4-pyridyl, TM4PyP) porphyrins (Figure 11) and mCPBA as an oxidant.\(^{54, 87}\) The Compound I model generated in the TMPS ligand was remarkably stable, permitting its *room temperature* characterization by \(^1\text{H}\) NMR. The oxoiron(IV)TM4PyP cation radical, conversely, was highly unstable. However, the TM4PyP complex was also highly reactive; for example, it could epoxidize the water-
soluble olefin carbamazepine with a second-order rate constant of $2.0 \times 10^5 \text{M}^{-1} \text{s}^{-1}$, nearly five orders of magnitude faster than the TMPS Compound I.

![Structure of Compound I models based on TM4PyP and TMPS porphyrin ligands](image)

Figure 11. Structures of Compound I models based on TM4PyP and TMPS porphyrin ligands

Clearly, the charge and electron-donating characters of the porphyrin ligand have a dramatic effect on the reactivity of these Compound I analogues. Here, the argument was made that TM4PyP ligand itself has a higher porphyrin ring oxidation potential owing to the electron-withdrawing $N$-methyl pyridyl pendants. Moreover, the cationic porphyrin is a poor $\sigma$-donor ligand, lowering the energy of a vacant $d_{x^2-y^2}$ orbital and allowing access to a reactive, higher-spin configuration.$^{54}$

The oxoiron(IV)TM4PyP cation radical was found to be a stupendous hydroxylating agent much like authentic Compound I. As a catalyst, FeTM4PyP was prone to bleaching and decay.$^{87}$ However, the TM4PyP Compound I intermediate demonstrated that it could break C-H bonds as strong as 90 kcal mol$^{-1}$ with first-order rate constants ($k_{\text{app}}$) ranging from $10^4 \text{M}^{-1} \text{s}^{-1}$ to $10^6 \text{M}^{-1} \text{s}^{-1}$. $^{54}$ The O-H bond dissociation

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enthalpy (BDE) in hydroxoiron(IV)TM4PyP was predicted to be 100 kcal mol⁻¹, and so stronger C-H bonds should be accessible. An interesting aspect of this work was the observation that reactivity (i.e. rate) correlated linearly with C-H BDE for hydroxylation reactions but not with O-H BDE in hydrogen atom abstractions from alcohols; this suggests an important role for entropic effects such as solvation in oxometal catalysis and hydrogen-atom abstraction kinetics in a broader sense.⁸⁷

**Manganese Porphyrins**

The history of synthetic manganese porphyrins as biomimetic catalysts has evolved almost simultaneously along with analogous iron porphyrins. Colloquially, manganese porphyrins have outperformed iron porphyrins as catalysts, particularly being less sensitive to bleaching during turnover. Independently, Groves and Hill both showed that a synthetic manganese porphyrin (MnTPP) could catalyze a variety of hydrocarbon oxidations,⁸⁸-⁸⁹ including epoxidation, hydroxylation, and chlorination. This seminal discovery laid the groundwork for over 30 years of research into manganese porphyrins, and related complexes, as biomimetic oxidation catalysts.³⁴,⁹⁰-⁹²

The Compound I analogue in manganese porphyrins is the oxomanganese(V) species (Figure 12). The existence of this species was first inferred as a product of the reaction of manganese(III)TMP with mCPBA.⁹³ Soon thereafter, an oxoMn⁵TMP was prepared by oxidizing Mn³⁺TMP at –78 °C.⁹⁴ This EPR-silent compound was shown to differ markedly in its reactivity from the more-stable oxoMn⁴⁺TMP, as the former compound oxidized cis-styrene to its epoxide with retention of configuration (unlike oxoMn⁴⁺).
Figure 12. a) oxomanganese(V) porphyrin; b) qualitative crystal field splitting for low-spin d² oxomanganese(V) porphyrins

Although early work showed that oxoMn⁴⁺ porphyrins were accessible and isolable (further discussed in Chapter 4), the oxoMn⁵⁺ porphyrin remained elusive. Still, stopped-flow spectroscopic techniques could be used to observe an intermediate with a sharp, blueshifted Soret that was assigned as oxoMn⁵⁺TM₄PyP (tetra-N-methyl-4-pyridyl porphyrin). It was later discovered that subtle ligand modifications had dramatic changes in the reactivity of oxoMn⁵⁺ (Figure 13). For example, oxoMn⁵⁺TM₂PyP (tetra-N-methyl-2-pyridyl porphyrin) was reported to be more stable than the oxoMn⁵⁺TM₄PyP isomer by more than 2 orders of magnitude (based on decay t₁/₂), allowing its characterization by ¹H NMR as a ground state diamagnetic d² singlet. The inner pyrrole protons of freebase H₂TM₂PyP are more acidic than in H₂TM₄PyP (pKₐ –0.9 and 1.4, respectively), and thus the oxoMn⁵⁺ species is apparently stabilized by the more electron-withdrawing ligand. The reader should note that this is the opposite of the trend.
seen for the synthetic, iron Compound I analogues discussed above (for reasons explained below).

Figure 13. Structures of oxomanganese(V)TM4PyP and TM2PyP porphyrins

Under alkaline conditions, the normally unstable and reactive oxoMnV porphyrins were found to be remarkably stable, even at room temperature. This permitted their characterization by NMR and also vibrational techniques (resonance Raman and IR). The vibrational spectroscopy revealed that under these alkaline conditions, oxoMnV porphyrins exist as trans-dioxomanganese(V) species, the first known trans-dioxo transition metal complexes in the first row. The oxidation of a highly electron-deficient N,N-dimethylimidazolyl manganese porphyrin (MnTDMImP) by hydrogen peroxide to afford this trans-dioxo species was recently reported to proceed via the intermediacy of a hydroperoxomanganese(III) (a Compound 0 analogue) which undergoes a pH-independent O-O heterolysis akin to the “push-pull” effect seen in Cytochrome P450 (Figure 14).
The existence of trans-dioxomanganese(V) porphyrins was suggested\textsuperscript{100} some eight years earlier and later corroborated by observations concerning the kinetics of O-atom transfer from water-soluble oxoMn\textsuperscript{V} porphyrins to bromide ion (Br\textsuperscript{−}).\textsuperscript{101}-\textsuperscript{102} The rate of this reaction was found to be highly dependent on pH, resulting in the speculation that protons were required to activate a stable dioxomanganese species (Figure 15). Moreover, this O-atom transfer to Br\textsuperscript{−} was found to be rapid and reversible. From a measurement of the forward and backward rates of reaction, the free energy change for this reaction was calculated, allowing oxomanganese(V) porphyrins to be placed on an absolute energy scale for the first time. This analysis suggested that, at acidic pH, the redox potential of oxoMn\textsuperscript{V}/Mn\textsuperscript{III} comes tantalizingly close to that of H\textsubscript{2}O, and thus water oxidation by manganese porphyrin catalysts is a viable target for future research efforts. (The relationship between oxomanganese(V) redox potential and pH is further discussed in Chapter 2.)
Chapter I

It is now generally accepted that hydroxylations catalyzed by manganese porphyrins proceed by the same radical rebound mechanism seen in P450.\textsuperscript{34, 80, 103} Hydrogen atom abstraction by oxoMn\textsuperscript{V} has been investigated using radical clocks with known rearrangement rates, and the lifetime of the incipient radical produced from hydrogen atom abstraction by oxoMn\textsuperscript{V} has been measured to be in the range of ca. 10-500 ps.\textsuperscript{104} This range correlates well with radical lifetimes observed for P450s. Using the generation of a radical species to its advantage, a biphasic manganese porphyrin system has recently been developed that catalyzes the selective chlorination of strong C-H bonds using hypochlorite.\textsuperscript{80} The mechanism of this process is believed to be a variation on the classic rebound process; an oxoMn\textsuperscript{V} species abstracts a hydrogen atom, affording a carboradical that, rather than rebounding normally, encounters a hypochloritoMn\textsuperscript{IV} which chlorinates the carboradical (Scheme 4).

\textbf{Figure 15.} Prototropy between dioxo-, oxohydroxo-, and oxoaqua-manganese(V) porphyrins is required to activate the stable dioxo species
The significant stability of trans-dioxomanganese(V) porphyrins\textsuperscript{98} and the origin of the differences in reactivity observed in the TMPyP isomers\textsuperscript{96} have also been explored computationally. Key to understanding the effects that dictate oxoMn\textsuperscript{V} reactivity is the often under-appreciated role of spin in metal-mediated oxidations.\textsuperscript{105} Oxygen-atom transfer from oxoMn\textsuperscript{V} to Br\textsuperscript{−} formally involves a spin-state crossing from singlet manganese(V) to quartet manganese(III).\textsuperscript{96} Furthermore, hydrogen-atom abstraction by a metal-oxo more properly occurs via a “metal oxyl” moiety, with spin density placed on the oxo ligand, which is only formally possible for a triplet (or higher) oxomanganese(V) species.\textsuperscript{83, 106-108}

DFT calculations\textsuperscript{108} revealed two factors that mediate the spin state behavior of oxomanganese(V) porphyrins: first, the protonation of trans-dioxomanganese(V) leads to
a dramatic lowering in energy of excited triplet and quintet states (Figure 16); and

second, more electron-withdrawing porphyrin meso-substituents stabilize the porphyrin

\(a_2u\) orbital and increase the singlet-quintet (S-Q) energy gap. Ergo, both unprotonated

\(trans\)-dioxoMn\(^V\) as well as oxoMn\(^V\) in more electron-withdrawing ligands should have a

more difficult time accessing the higher-spin excited states necessary to either cross from

the singlet manifold\(^{109}\) or to attain metal-oxyl character\(^{108}\). Such effects are revealed

experimentally by the relative ease of isolation of \(trans\)-dioxonanese(V)\(^{98}\) as well as

the differing reactivity of oxoMn\(^V\)TM2PyP and TM4PyP isomers.\(^{96}\) In the TMPyP

isomers, the \(trans\)-dioxoMn\(^V\)TM2PyP is experimentally less basic, the \(N\)-methyl-2-

pyridyl substituents more electron-withdrawing, and the oxoMn\(^V\) complex more stable.
Figure 16. Effect of trans-axial oxo protonation on the relative energies of the singlet (S), triplet (T), and quartet (Q) spin states in oxomanganese(V) porphyrins (Adapted from Reference 109)
RECAPITULATION AND ONWARD

This introduction has described the truly inspiring reactions carried out by the Cytochromes P450 and explored the chemistry of oxygen activation, hydrogen atom abstraction, and oxometal reactivity used by these enzymes. We have seen also how biomimetic model compounds based on iron and manganese porphyrins have not only assisted in understanding P450 reactivity, but have also provided new insights that are of much use for the future of biomimetic and practical catalysis. In the following pages, we will detail further studies with model porphyrin compounds of manganese and vanadium.

The focus of Chapter 2 is the discovery and mechanistic elucidation of a novel, catalytic generation of the disinfectant chlorine dioxide using water-soluble manganese porphyrins that could form the basis of a new, practical method for producing ClO₂. A number of vignettes concerning the advantages and possible applications of this manganese porphyrin/chlorine dioxide system are then described in Chapter 3, highlighting potential benefits and routes for future basic and applied study. Chapter 4 archives data collected in a study of oxomanganese poprhyrins by X-ray Absorption Spectroscopy and an Extended X-ray Absorption Fine Structure analysis that answers some questions and raises new ones about reactive, oxomanganese intermediates. To close, Chapter 5 describes the synthesis and characterization of novel oxovanadium porphyrins and provides early and promising signs for their future as catalysts in hydrocarbon functionalization.
REFERENCES


CHAPTER II

MECHANISM OF CATALYTIC CHLORINE DIOXIDE FORMATION FROM SODIUM CHLORITE BY CATIONIC MANGANESE PORPHYRINS AND PORPHYRAZINES
ABSTRACT

Chlorine dioxide, an industrially important biocide and bleach, is produced rapidly and efficiently from chlorite ion in the presence of water-soluble, manganese porphyrins and porphyrazines at neutral pH under mild conditions. The electron-deficient manganese(III) tetra-(N,N-dimethyl)imidazolium porphyrin (MnTDMImP), tetra-(N,N-dimethyl)benzimidazolium (MnTDMBImP) porphyrin and manganese(III) tetramethyl-2,3-pyridinium porphyrazine (MnTM23PyPz) were found to be the most efficient catalysts for this process. The more typical manganese tetra-4-N-methylpyridium porphyrin (Mn-4-TMPyP) was much less effective. Rates for the best catalysts were in the range of 0.24-32 TO/s with MnTM23PyPz being the fastest. The kinetics of reactions of the various ClO\textsubscript{x} species (e.g. chlorite ion, hypochlorous acid and chlorine dioxide) with authentic oxomanganese(IV) and dioxomanganese(V)TDMImP intermediates were studied by stopped-flow spectroscopy. Rate-limiting oxidation of the manganese(III) catalyst by chlorite ion via oxygen atom transfer is proposed to afford a trans-dioxomanganese(V) intermediate. Both trans-dioxomanganese(V)TDMImP and oxoaqua-manganese(IV)TDMImP oxidize chlorite ion by 1-electron, generating the product chlorine dioxide with bimolecular rate constants of $6.30 \times 10^3 \text{ M}^{-1} \text{s}^{-1}$ and $3.13 \times 10^3 \text{ M}^{-1} \text{s}^{-1}$, respectively, at pH 6.8. Chlorine dioxide was able to oxidize manganese(III)TDMImP to oxomanganese(IV) at a similar rate, establishing a redox steady-state equilibrium under turnover conditions. Hypochlorous acid (HOCl) produced during turnover was found to rapidly and reversibly react with manganese(III)TDMImP to give dioxoMn(V)TDMImP and chloride ion. The measured equilibrium constant for this reaction ($K_{eq} = 2.2$ at pH 5.1) afforded a value for the oxoMn(V)/Mn(III) redox
couple under catalytic conditions (E' = +1.35 V vs. NHE). In subsequent processes, chlorine dioxide reacts with both oxomanganese(V) and oxomanganese(IV)TDMImP to afford chlorate ion. Kinetic simulations of the proposed mechanism using experimentally measured rate constants were in agreement with observed chlorine dioxide growth and decay curves, measured chlorate yields, and the oxoMn(IV)/Mn(III) redox potential (E' = +1.03 V vs. NHE). This acid-free catalysis could form the basis for a new process to make ClO$_2$. 
INTRODUCTION

Chlorine dioxide (ClO₂) is an oxidizing gas used as an alternative to chlorine (Cl₂) in paper manufacturing, disinfection, and water treatment.¹ In many respects, ClO₂ is preferred to Cl₂ because of the former’s reduced tendency to produce chlorinated by-products, better biocidal activity, and insensitivity to environmental factors like pH.¹ Unfortunately, ClO₂ is prone to detonation and cannot be stored or shipped easily. Therefore, it must be generated on-site, adding an extra burden to its implementation. This chapter will describe a new, promising, catalytic method for generating ClO₂. First, however, we will briefly introduce chlorine dioxide as a reagent and describe the various methods currently practiced to produce ClO₂.

Chlorine Dioxide

Chlorine dioxide (ClO₂) was first discovered in 1811.¹ It is a bent triatomic molecule with chlorine in an unusual +4 oxidation state.² It is the only metastable chlorine oxide radical species (ClOₓ) known.³ An unpaired electron resides in a π* orbital, and ClO₂ acts as a strong 1-electron oxidant (ΔE° = +1.06 V vs. NHE).⁴

\[ \text{ClO}_2 + 1 \text{ e}^- \rightarrow \text{ClO}_2^- \]

The boiling point of ClO₂ is 11 °C, and so it is a gas at standard handling temperatures. It solidifies at −59 °C and dimerizes to a diamagnetic species at −84 °C.⁵ In concentrated or pure form, ClO₂ is thermally unstable, and it detonates spontaneously upon exposure to light:¹⁻²,⁶

\[ 2 \text{ ClO}_2 + \text{hv} \rightarrow \text{Cl}_2 + 2 \text{ O}_2 \quad \Delta H^\circ = -23 \text{ kcal mol}^{-1} \]
Even relatively dilute mixtures of the gas (10% in air) are potentially explosive;\(^7\) therefore, it is more safely handled in dilute aqueous solution. ClO\(_2\) is remarkably soluble in water at 20 °C (70 g/L).\(^1\) Solutions of ClO\(_2\) have a yellow-green color and produce a characteristic UV-Vis spectrum, often with some degree of vibronic fine structure.\(^8\) Unlike elemental chlorine, ClO\(_2\) does not hydrolyze appreciably in water,\(^1\) and so its reactivity as an oxidant is not affected by factors such as pH. Although it is volatile, dilute aqueous solutions are stable if protected from heat and light, and solutions of ClO\(_2\) can be frozen for prolonged storage.\(^1\)

Practical interest in ClO\(_2\) has grown in recent times due to its implication as a participant in ozone depletion and also its remarkable efficiency as an alternative to chlorine (Cl\(_2\), HOCl) as an oxidant and disinfectant.\(^1,9,10\) In the latter role, ClO\(_2\) is a broad-spectrum biocide that is effective even against chlorine-resistant pathogens such as *Giardia* and *Cryptosporidium*.\(^1,11\) Compared to traditional paper and pulp bleaching, ClO\(_2\) produces whiter, brighter paper products.\(^1,12\) Furthermore, the disinfection of wastewater with ClO\(_2\), compared to chlorine, produces fewer halogenated by-products from side reactions with any organic matter present.\(^1,9,13\) This is attributable to chlorine dioxide’s greater tendency to act simply as a 1-electron oxidant. ClO\(_2\) has also begun to be used industrially to treat and recycle wastewater produced from hydraulic fracturing.\(^14\)

In spite of the benefits, widespread use of ClO\(_2\) remains highly attenuated. Although it produces fewer halogenated by-products, ClO\(_2\) treatment can produce large quantities of inorganic by-products such as chlorites (ClO\(_2^{-}\)) and chlorates (ClO\(_3^{-}\)), which are themselves regulated pollutants.\(^15\) Even more burdensome is the inability of
ClO₂ to be stored for long periods and in high concentrations.¹ Thus, ClO₂ must be generated on-site, immediately prior to application for it to be successfully employed in an industrial operation.

**Industrial Methods of ClO₂ Production**

ClO₂ was first prepared from the acidification of potassium chlorate (KClO₃) with hydrochloric acid¹⁰,¹⁶. This reaction leads to the formation of mixtures of ClO₂ and Cl₂. Obviously, the presence of Cl₂ negates any “non-halogenating” benefits of ClO₂, and therefore a primary concern for ClO₂ generation is the purity of the product. In addition to Cl₂, other common impurities found in ClO₂ product streams include chlorites (ClO₂⁻) and chlorates (ClO₃⁻).¹¹,¹⁵ Often, these result from an incomplete conversion of starting material. A variety of industrial methods have been developed for the gas and/or solution-phase generation of ClO₂, and the careful balance of feedstock costs, reaction efficiency, and product purity dictate which of the various methods is employed.

By far the most common use of ClO₂ is by the paper manufacturing community.¹ The preferred method for generating ClO₂ in this capacity uses sodium chlorate (NaClO₃) as starting material.¹² Under extremely acidic conditions (4 – 8 N), NaClO₃ is chemically reduced to ClO₂ by such reagents as hydrogen peroxide, hydrochloric acid, sulfur dioxide, or methanol.¹²

*Hydrogen peroxide:* 2 NaClO₃ + H₂O₂ + H₂SO₄ → 2 ClO₂ + O₂ + Na₂SO₄ + 2 H₂O

*Hydrochloric acid:* 2 NaClO₃ + 4 HCl → 2 ClO₂ + Cl₂ + 2 NaCl + 2 H₂O

*Sulfur dioxide:* 2 NaClO₃ + SO₂ + H₂SO₄ → 2 ClO₂ + 2 NaHSO₄

*Methanol:* 6 NaClO₃ + CH₃OH + 4 H₂SO₄ → 6 ClO₂ + CO₂ + 2 Na₃H(SO₄)₂ + 5 H₂O
In practice, the reagents of choice are sulfuric acid and methanol as their use avoids the generation of Cl\(_2\) impurities. However, this route is not terribly efficient (e.g. 30-40% of the methanol is not consumed), results in the formation of a so-called “acid salt cake” by-product (sodium sesquisulfate, Na\(_3\)H(SO\(_4\))\(_2\)), and often generates formic acid from the partial oxidation of methanol.\(^{12,17}\)

ClO\(_2\) can also be produced industrially from chlorite ion (ClO\(_2^−\)).\(^1\) In practice, NaClO\(_2\) is itself produced from NaClO\(_3\),\(^{17}\) and so the ultimate precursor in all ClO\(_2\) systems is truly NaClO\(_3\). However, as a practical matter, it is easier to control the purity of the ClO\(_2\) product in the chlorite reactor systems,\(^{10}\) and this is the more preferred method in smaller-scale operations such as water treatment. Three broad reaction types describe the conversion of chlorite ion to ClO\(_2\): acidification of NaClO\(_2\), chemical oxidation of ClO\(_2\) by Cl\(_2\), and electrochemical oxidation of NaClO\(_2\). Of these reaction possibilities, six commonly practiced methods for the production of ClO\(_2\) from NaClO\(_2\) have emerged (Table 1).\(^{1,18}\)

One of the more simple methods for producing ClO\(_2\) from NaClO\(_2\) involves the acidification of solutions of chlorite (the so-called “acid-chlorite” method), and can achieve production rates of up to 100 lbs/day.\(^1\) At very acidic pH (< 2), NaClO\(_2\) is converted to chlorous acid (HClO\(_2\)), which decomposes rapidly to produce a number of products, including ClO\(_2^−\).\(^{1,18-19}\) The yield, purity, and speed of ClO\(_2\) production are dependent on factors such as the concentration of HClO\(_2\), pH, and presence of Cl\(^−\).\(^{18-21}\) The use of concentrated sulfuric acid in this method results in a very endothermic reaction that leads to off-gassing of the volatile ClO\(_2\) product. A more common variation of this process employs instead hydrochloric acid and leads to much higher yields of
product (77%). Weak acids such as citric acid are used in some ClO₂-generating products designed for personal-use to disinfect small volumes of water; however, the effect of pH and acid strength on the speed and yield of ClO₂ by the acid-chlorite method is reflected in these small-scale devices by the requirements of long reaction times (on the order of hours) or highly concentrated solutions (10-30%). To meet large-scale, industrial needs, concentrated strong acids are a necessity, the inherent risks of which offset the relative simplicity of the acid-chlorite method.

<table>
<thead>
<tr>
<th>Method</th>
<th>Reaction(s)</th>
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| Acid-chlorite solution | 4 NaClO₂ + 2 H₂SO₄ → 2 ClO₂ + 2 Na₂SO₄ + HCl + HClO₃ + H₂O  
|                              | or 5 NaClO₂ + 4 HCl → 4 ClO₂ + 5 NaCl + 2 H₂O       |
| Chlorine solution-chlorite solution | 2 ClO₂⁻ + HOCl + H⁺ → 2 ClO₂ + Cl⁻ + H₂O          |
| Acid-chlorine-chlorite solution | 2 NaClO₂ + NaOCl + 2 HCl → 2 ClO₂ + 3 NaCl + H₂O  
|                              | or (1) HOCl + HCl → Cl₂ + H₂O  
|                              | (2) Cl₂ + 2 NaClO₂ → 2 ClO₂ + 2 NaCl               |
| Chlorine gas-chlorite solution | Cl₂(g) + 2 NaClO₂(aq) → 2 ClO₂(g) + 2 NaCl(aq)      |
| Chlorine gas-solid chlorite   | Cl₂(g) + 2 NaClO₂(s) → 2 ClO₂(g) + 2 NaCl(s)        |
| Electrochemical               | 2 ClO₂⁻ + 2 H₂O → 2 ClO₂ + 2 −OH + H₂              |

Table 1. Industrial routes to convert chlorite feedstocks into chlorine dioxide (Adapted from reference 1)
Chlorite ion can also be oxidized directly to ClO$_2$ by chlorine gas/hypochlorous acid, and this reaction is the basis for a number of preparative methods of ClO$_2$ in either aqueous solution or the gas phase. These methods are characterized by yields higher than 90% using a slight excess of chlorine$^{22}$, and can, in practice, produce up to 250 lbs/day ClO$_2$. In addition to the requirement and drawbacks of using reactive Cl$_2$, HOCl, or NaOCl as reagents, the solution-based processes also operate at low pH (< 4), and, in the case of a ternary acid/chlorine/chlorite system, require the precise metering and control of three separate, reactive, chemical feed stocks.$^1$

Direct electrochemical oxidation of NaClO$_2$ is yet a third method for producing ClO$_2$ from chlorite ion, and current reactors can generate 50-150 lb/day.$^1$ The benefits of this method are its relatively simplicity; all that is required are solutions of NaClO$_2$ and some electrical power source. However, this method is currently limited in its ability to compete with other methods based on its lower rate of production and reduced efficiency arising due to impurities collecting on the electrodes.$^9$ Additionally, in practice this method produces undesired by-products including H$_2$, NaClO$_3$, HCl, and/or NaOH.$^1$.$^9$

The common theme in all of these currently-practiced ClO$_2$-generating methods is the need for added acids or oxidizing/reducing agents, often in concentrated form. In some cases (e.g. the ternary acid-chlorine-chlorite method), the precise and careful metering and mixing of reagents is required to produce a pure product in high yield. These needs are even larger complicating factors when one considers that these processes must be carried out on-site, at the point of use.

This chapter describes a catalytic method using manganese porphyrins that converts stable solutions of sodium chlorite into ClO$_2$ under ambient conditions, at near-
neutral pH, with no need for added reagents. This catalysis is intriguing not only for its practical applicability in ClO₂ generation, but also to add to our understanding of the myriad reactions of chlorite ion with heme enzymes and synthetic metalloporphyrins. The focus of the present work is the mechanistic elucidation of this novel, ClO₂-forming process.

This discovery could represent the first generation of a new catalytic method for producing ClO₂ that mitigates the risks inherent in the need for concentrated strong acids, oxidants, and reducing agents found in the currently practiced routes. Therefore, this chapter reports a complete mechanistic elucidation of this catalytic reaction in order to best understand those catalyst properties that most influence the process, lead to the generation of by-products, and would need to be controlled for any practical implementation.
RESULTS

Chlorite ion ($\text{ClO}_2^-$) is known to react with heme-containing enzymes as well as synthetic iron and manganese porphyrins, normally as an oxidant.$^{23-29}$ Notably, however, $\text{ClO}_2^-$ is efficiently consumed by the heme-containing chlorite dismutase (Cld) enzyme and converted to $\text{O}_2$ and $\text{Cl}^-$. Synthetic iron porphyrin analogues of Cld have also been shown to catalyze this novel transformation. We have found that under similar aqueous conditions ($< \text{pH 8}$), cationic manganese porphyrins (Figure 1) also rapidly consume $\text{ClO}_2^-$, but they instead dramatically produce the water-soluble gas chlorine dioxide ($\text{ClO}_2$) (Figure 2).$^{30-31}$ This section details experiments focused on elucidating the mechanism of this process.

![Diagram of manganese porphyrins and porphyrazine studied as catalysts](image)

**Figure 1.** Manganese porphyrins and porphyrazine studied as catalysts
Figure 2. Time resolved UV-vis spectra of ClO$_2$ (359 nm) generation when 10 µM MnTDMImP (445 nm) is mixed with 1.9 mM NaClO$_2$ (260 nm) at pH 4.7 (100 mM acetate buffer) and T = 25 °C. The reaction time shown is 240 s, scanning every 10 s.

Catalytic Generation of ClO$_2$ from ClO$_2^-$

Several cationic, water-soluble manganese porphyrins and a similar porphyrazine complex catalyzed the rapid production of chlorine dioxide (ClO$_2$) from chlorite ion (ClO$_2^-$) under mild conditions (Figure 1). The evolved ClO$_2$ could be monitored from the appearance of the characteristic chromophore at 359 nm (Figure 3).$^{32}$ During turnover, the visible spectra of the Mn$^{III}$ catalysts remained largely unchanged, demonstrating the resistance of the catalyst to bleaching as well as providing mechanistic insight ($vide infra$). The turnover rates for each of the studied catalysts are reported in Table 2. Extremely high activity (32 TO/s) was observed for the manganese(III) porphyrazine, MnTM23PyPz. However, unlike the porphyrin catalysts, the porphyrazine complex was unstable under the reaction conditions and completely bleached within seconds of turnover.
Figure 3. (a) Rapid appearance of ClO$_2$ (359 nm) from the MnTDMImP-catalyzed disproportionation of ClO$_2^-$ (10 mM) at pH 6.8 with 0.1 mol% catalyst (445 nm) showing the first 30 s of reaction, 1 scan/s. (b) Similar reaction with MnTM23PyPz (9 mM sodium chlorite, 10 mM MnTM23PyPz, pH 4.7 100 mM acetate buffer) showing the first 30 s of reaction, 1 scan/s
<table>
<thead>
<tr>
<th>Catalyst</th>
<th>Initial TOF (s(^{-1}))</th>
<th>mol % cat.</th>
</tr>
</thead>
<tbody>
<tr>
<td>MnTM4PyP</td>
<td>0.01(^a)</td>
<td>0.25</td>
</tr>
<tr>
<td>MnTM2PyP</td>
<td>0.24(^a)</td>
<td>0.25</td>
</tr>
<tr>
<td>MnTDMImP</td>
<td>0.40(^a)</td>
<td>0.25</td>
</tr>
<tr>
<td>MnTDMBImP</td>
<td>0.48(^a)</td>
<td>0.50</td>
</tr>
<tr>
<td>MnTM23PyPz</td>
<td>32.0(^b)</td>
<td>0.27</td>
</tr>
</tbody>
</table>

\(T = 25 \, ^\circ\)C

\(a\), pH 6.8, 50 mM phosphate buffer, 2.0 mM NaClO\(_2\)

\(b\), pH 4.7, 50 mM acetate buffer, 1.8 mM NaClO\(_2\)

**Table 2.** Turnover frequencies (TOF) of ClO\(_2\) generation

The appearance of ClO\(_2\) using MnTDMImP was studied in 100 mM acetate buffer (pH 4.7 and 5.7) (Figure 2) and 100 mM phosphate buffer (pH 6.8) (Figure 3). Initial turnover frequencies observed under these conditions (25 °C, 2 mM ClO\(_2\), 10 μM Mn\(^{III}\)TDMImP) were 1.00, 1.03, and 0.42 at pH 4.7, 5.7, and 6.8, respectively. However, the turnover frequency was influenced more by buffer composition than by pH. Increasing either buffer concentration or ionic strength (using sodium perchlorate) was found to inhibit the MnTDMImP-catalyzed reaction by a factor of 5 in the range 5 mM – 100 mM (Figure 4) while at a constant buffer concentration, the turnover frequency did not change from pH 5.9 to 7.01 (Figure 5).
Figure 4. Effect of added anions (phosphates, perchlorates) on turnover frequency of ClO$_2$-evolution from MnTDMImP. Black squares: phosphate buffer concentration effect at constant pH = 6.9 (5 µM MnTDMImP, 1 mM NaClO$_2$, T = 25 °C) Red circles: Sodium perchlorate effect at pH = 6.8 (5 µM MnTDMImP, 0.85 mM NaClO$_2$, T = 25 °C, 5mM phosphate buffer).

Figure 5. a) Effect of pH on the turnover frequency of ClO$_2$-evolution from MnTDMImP in constant 25 mM phosphate buffer. Experimental conditions: 5 µM MnTDMImP, 0.85 mM NaClO$_2$, T = 25 °C. b) ClO$_2$ growth and decay from 2 mM NaClO$_2$ and ca. 10 µM MnTDMImP at pH 4.7, 5.7, and 6.8.
When ClO$_2^-$ was rapidly mixed with Mn$^{III}$TDMImP at 25 °C, the spectroscopically-observed concentration of ClO$_2$ produced rose quickly and reached a plateau within 3 min. The product ClO$_2$ absorbance subsequently decayed in a slower process over the course of about 15 min. The maximum ClO$_2$ concentration was not affected by temperature (Figure 6), although the reaction rate increased markedly from 5 to 35 °C (Figure 7).

**Figure 6.** Effect of temperature on the evolution of ClO$_2$ upon mixing 2.0 mM ClO$_2^-$ and 10 µM Mn$^{III}$TDMImP. ClO$_2$ concentration is measured by the change in absorbance at 359 nm. Subtle changes in the catalyst spectrum at this wavelength over very long reaction times resulted in an overestimate of ClO$_2$ decay at 35 °C. Experimental conditions: 100 mM pH 6.8 phosphate buffer.
Figure 7. a) Arrhenius plot of $k_{\text{app}}$ as a function of temperature for the reaction of ClO$_2^-$ with Mn$^{\text{III}}$TDMImP. b) Effect of temperature on the initial rate of oxo-transfer from ClO$_2^-$ to Mn$^{\text{III}}$TDMImP formation as a function of ClO$_2^-$ concentration (vide infra)

**Reduction of oxoMn$^V$ and oxoMn$^{IV}$TDMImP by ClO$_2^-$**

The reactions of both oxoMn$^V$TDMImP and oxoMn$^{IV}$TDMImP with ClO$_2^-$ were studied by double-mixing, stopped-flow spectroscopy. In the first push, authentic oxoMn$^V$ or oxoMn$^{IV}$ was generated by mixing Mn$^{\text{III}}$TDMImP with 1 equiv of oxone or tBuOOH, respectively, in 10 mM pH 8.0 phosphate buffer.$^{33-35}$ In the second push, oxoMn$^V$ or oxoMn$^{IV}$ was mixed with an excess of ClO$_2^-$ in 100 mM acetate or phosphate buffer (pH 4.7 and 6.8, respectively). This pH jump experiment permitted observation of the reaction between each of the high-valent manganese species with ClO$_2^-$ at lower pH values.

The decay of oxoMn$^V$ (as measured by the characteristic Soret absorbance at 425 nm$^{34}$) was pH-dependent and followed pseudo-first order kinetics when mixed with an excess of ClO$_2^-$. This decay was fitted to a modeled exponential decay curve in order to obtain the observed pseudo-first order rate constant ($k_{\text{obs}}$), which was plotted as a
function of [ClO$_2^-$]. The apparent second-order rate constants ($k_{app}$) calculated from the slope of $k_{obs}$ vs. [ClO$_2^-$] are $6.8 \times 10^5$ and $6.3 \times 10^3$ M$^{-1}$ s$^{-1}$ at pH 4.7 and 6.8, respectively.

At pH 4.7, the 1-electron reduction of oxoMn$^V$ by ClO$_2^-$ was fast and allowed the direct observation of the reduced oxoMn$^{IV}_{\text{TDIImP}}$ catalyst (Figure 8, panel a) with a single isosbestic point between the Soret bands at 437 nm. By contrast, at pH 6.8 the reaction of oxoMn$^V$ with ClO$_2^-$ afforded Mn$^{III}$ (Figure 8, panel b). The presence of two time-separated isosbestic points (436 nm and 432 nm) during this reaction demonstrated the intermediacy of oxoMn$^{IV}$ in the reaction between oxoMn$^V$ and ClO$_2^-$. Under these conditions, the apparent second-order rate constant $k_{app}$ was determined by first obtaining a pseudo-first order rate constant ($k_{obs}$) during the first phase of the reaction (where only the 436 nm isosbestic was observed).
Figure 8. a) Time-resolved UV-vis spectra of the reaction between 5 μM oxoMnV-TDMImP and 125 μM ClO2− in 100 mM pH 4.7 acetate buffer over 0.3 s; b) Time-resolved spectra of the reaction between 10 μM oxoMnV-TDMImP and 1.0 mM ClO2− in 100 mM pH 6.8 phosphate buffer over 3 s. Insets: Plots of pseudo-first order rate constant (kobs) of the decay of versus initial [ClO2−]. *n.b.*, Self-decay of MnV and MnIV to MnIII results in non-zero intercepts.
Figure 9. a) Time-resolved UV-vis spectra of the reaction between 10 µM oxoMn$^{IV}$TDMImP and 1.0 mM ClO$_2^−$ in 100 mM pH 4.7 acetate buffer over 3 s. b) Time-resolved UV-vis spectra of the reaction between 10 µM oxoMn$^{IV}$TDMImP and 1.0 mM ClO$_2^−$ in 100 mM pH 6.8 phosphate buffer over 1.5 s. Insets: Plot of pseudo-first order rate constant ($k_{obs}$) of the decay of oxoMn$^{IV}$ versus initial [ClO$_2^−$].

The reaction between oxoMn$^{IV}$ and an excess of ClO$_2^−$ was similarly found to result in a pseudo-first order decay of oxoMn$^{IV}$ to Mn$^{III}$ (as measured by the characteristic Soret absorbance of oxoMn$^{IV}$ at 422nm) at pH 4.7 and 6.8 (Figure 9). A single isosbestic
point was observed at 433 nm between the Soret bands of oxoMn$^{IV}$ and Mn$^{III}$. The apparent second-order rate constants measured for the reduction of oxoMn$^{IV}$ by ClO$_2^-$ are 6.0 x 10$^3$ and 3.1 x 10$^3$ M$^{-1}$ s$^{-1}$ at pH 4.7 and 6.8, respectively.

**Measurement of $k_{\text{app}}$ for oxo-transfer between HOCl and Mn$^{III}$**

The reaction between hypochlorous acid (HOCl) and Mn$^{III}$TDMImP was studied by single-mixing stopped-flow spectrometry. At pH 6.8, the reaction resulted in complete formation of oxoMn$^V$ in <100 ms (Figure 10). An apparent second-order rate constant for the reaction was determined by measuring $k_{\text{obs}}$ of oxoMn$^V$ appearance (425 nm) as a function of [HOCl] (Figure 11). The calculated rate constant ($k_4$) at pH 6.8 was 1.7 x 10$^6$ M$^{-1}$ s$^{-1}$. An initial-rate analysis of oxoMn$^V$ formation gave a slower rate constant of 1.47 x 10$^5$ M$^{-1}$ s$^{-1}$.

At pH 4.7, complete formation of oxoMn$^V$ could not be elicited with even a 50-fold excess of HOCl (Figure 10, panel c). Nevertheless, the apparent second-order rate constant ($k_4 = 6.6 x 10^4$ M$^{-1}$ s$^{-1}$) under these conditions was calculated from a plot of $k_{\text{obs}}$ for the decrease in Mn$^{III}$ absorbance (444 nm) versus [HOCl] (Figure 11). A similar analysis using the method of initial rates provided a lower calculated $k_4 = 4.22 x 10^3$ M$^{-1}$ s$^{-1}$. After a quick reaction time (< 0.1 s at pH 4.7), the concentrations of Mn$^{III}$ and oxoMn$^V$ reached an apparent steady-state equilibrium. This steady-state observation was better demonstrated at slightly less-acidic conditions of pH 5.1 (Figure 10, panel b).
Figure 10. UV-Vis spectral changes for the reaction of Mn\textsuperscript{III}TDMImP with HOCl at (a) pH 6.8, (b) 5.1, and (c) 4.7. Buffer conditions: pH 6.8, 100 mM phosphate buffer; pH 4.7 and 5.1, 100 mM acetate buffer. Reaction conditions: (a) 10 µM Mn\textsuperscript{III}, 5 equiv HOCl; (b) 5 µM Mn\textsuperscript{III}, 20 equiv HOCl; (c) 5 µM Mn\textsuperscript{III}, 50 equiv HOCl
Figure 11. Determination of the apparent second-order rate constant of oxo-transfer from HOCl to Mn$^{III}$-TDMImP. The slope of each line is equal to the product of $k_{app}$ and the concentration of Mn$^{III}$ ($k_{app\,4.7} = (6.64 \pm 0.23) \times 10^4 \text{ M}^{-1} \text{s}^{-1}$, $k_{app\,6.8} = (1.71 \pm 0.27) \times 10^6 \text{ M}^{-1} \text{s}^{-1}$)

Reaction of ClO$_2$ with Mn$^{III}$-TDMImP

In order to account for the observed decay of ClO$_2$ (Figure 6), the reaction of ClO$_2$ with Mn$^{III}$-TDMImP was investigated. The ClO$_2$ absorbance at 360 nm quickly disappeared in the presence of Mn$^{III}$ TDMImP at pH 6.8, but not pH 4.7 (Figure 12). In both cases, the only porphyrin oxidation state observed in solution was Mn$^{III}$. 
Figure 12. Comparison of the reaction of ClO$_2$ with 5 µM Mn$^{III}$TDMImP at pH 6.8 and 4.7. The ClO$_2$ concentration was measured at 359 nm.

Electron transfer between oxoMn$^{IV}$, oxoMn$^{V}$ and ClO$_2$

Stopped-flow techniques were again used to observe how oxoMn$^V$TDMImP and oxoMn$^{IV}$TDMImP reacted with ClO$_2$. Both oxoMn$^V$ and oxoMn$^{IV}$ reacted with ClO$_2$ via 1-electron transfer. Although oxoMn$^{IV}$ did not build up in the reaction of oxoMn$^V$ with ClO$_2$, the observation of two, time-separated isosbestic points indicated the intermediacy of oxoMn$^{IV}$ in the reaction. The product of 1-electron oxidation of ClO$_2$ is formally a chlorine(V) species, shown below to be chlorate (ClO$_3^-$). The apparent second-order rate constants ($k_{app}$) for the reduction of oxoMn$^V$ by ClO$_2$ were $1.6 \times 10^4$ M$^{-1}$ s$^{-1}$ and $2.2 \times 10^4$ M$^{-1}$ s$^{-1}$ at pH 6.8 and 4.7, respectively (Figure 13, panel a). Calculated $k_{app}$ for the reduction of oxoMn$^{IV}$ by ClO$_2$ are $1.2 \times 10^4$ M$^{-1}$ s$^{-1}$ and $7.9 \times 10^3$ M$^{-1}$ s$^{-1}$ at pH 6.8 and 4.7, respectively (Figure 13, panel b).
Figure 13. a) Pseudo-first order analysis of the reaction between oxoMn$^V$ and ClO$_2$. The observed first-order decay of oxoMn$^V$ ($k_{obs}$) is plotted versus ClO$_2$ concentration. b) Pseudo-first order analysis of the reaction between oxoMn$^{IV}$ and ClO$_2$. The observed first-order decay of oxoMn$^{IV}$ ($k_{obs}$) is plotted versus ClO$_2$ concentration.
**ClO$_3^-$ Determination by HPLC**

The observation that ClO$_2$ itself acted as a 1-electron reductant of oxoMn$^{IV}$TDMImP and oxoMn$^{V}$TDMImP suggests that some chlorine(V) species was produced. An indirect UV-detection HPLC method$^{36}$ was therefore employed to confirm and quantify the production of chlorate ion (Table 3). In brief, a solution of 4-aminosalicylic acid, which absorbs at 320 nm, was used as the mobile phase. As anions eluted from the ion chromatography column, the baseline absorbance at 320 nm decreased and created “valleys” (rather than peaks).

Buffer, catalyst, ClO$_2^-$, and ClO$_2$ stock solutions were free of ClO$_3^-$ prior to reaction. A measured amount of either ClO$_2^-$ or ClO$_2$ was added to solutions of MnTDMImP and stirred for ca. 1 h before being analyzed. Because the excess buffer anions co-eluted with chloride anion, the quantification of chloride produced by the catalytic decomposition of ClO$_2^-$ could not be accomplished.

<table>
<thead>
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<th>Added substrate</th>
<th>[ClO$<em>3^-$]$</em>{obs}$ (mM)</th>
<th>[ClO$<em>3^-$]$</em>{pred}$ (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 mM ClO$_2$</td>
<td>0.7</td>
<td>0.8</td>
</tr>
<tr>
<td>1 mM ClO$_2^-$</td>
<td>0.6</td>
<td>0.7</td>
</tr>
<tr>
<td>2 mM ClO$_2^-$</td>
<td>1.7</td>
<td>1.4</td>
</tr>
</tbody>
</table>

Conditions: 10 µM MnTDMImP, pH 6.8, 100 mM phosphate buffer [ClO$_3^-$]$_{pred}$ calculated from kinetic model of the proposed mechanism (*vide infra*).

*Table 3. Measured concentrations of ClO$_3^-$ produced*
Air Stripping of the Porphyrin/Chlorite Reaction

ClO₂ gas generated from the catalytic decomposition of ClO₂⁻ could be removed and isolated via efficient sparging (“air stripping”) of a side arm-equipped reaction tube charged with pH 6.8 phosphate buffer, ClO₂⁻, and the manganese catalyst (MnTDMImP). Helium was bubbled through the reaction vessel at a high rate via a fritted glass sparging tube to promote the outgassing of generated ClO₂. This effluent was then bubbled into chilled, distilled water, which took on the characteristic color of dilute aqueous ClO₂ and could be confirmed by UV-Vis spectroscopy.

Because the collection vessel was also prone to outgassing of ClO₂ due to the nature of the sparging method, the transferred ClO₂ could not be easily quantified by this method. However, by sparging the reactor’s effluent into a chilled solution of concentrated potassium iodide (KI), the evolved ClO₂ could be “trapped” by iodide ion (Figure 14). Iodide ion is readily oxidized to I₂ by ClO₂, and the thus-evolved I₂ could be quantified by titrimetry.
The method for quantifying the amount of ClO$_2$ successfully removed from the reactor is based on a known method$^{37}$ and takes into account the possibility for Cl$_2$ impurities in the ClO$_2$ product stream. Both ClO$_2$ and Cl$_2$ react with iodide ion to generate I$_2$.

Reaction A: \[ 2 \text{ClO}_2 + 2 \Gamma^- \rightarrow 2 \text{ClO}_2^- + \text{I}_2 \]

Reaction B: \[ \text{Cl}_2 + 2 \Gamma^- \rightarrow 2 \text{Cl}^- + \text{I}_2 \]

It is important to note that the products of Reaction A and B are ClO$_2^-$ and Cl$^-$, respectively. After the catalytic reaction and sparging are complete, the trapping solution is titrated (e.g. with thiosulfate) to determine the amount of I$_2$ present. Then, concentrated sulfuric acid is added, which activates the ClO$_2^-$ by-product of Reaction A to afford more I$_2$:

Reaction C: \[ \text{ClO}_2^- + 4 \text{H}^+ + 4 \Gamma^- \rightarrow \text{Cl}^- + 2 \text{H}_2\text{O} + 2 \text{I}_2 \]
The solution is now titrated a second time to determine the amount of I\textsubscript{2} generated by Reaction C. The amount of I\textsubscript{2} generated by reaction C is directly related to the amount of ClO\textsubscript{2}\textsuperscript{−} produced by Reaction A (and therefore the amount of ClO\textsubscript{2} transferred). Any I\textsubscript{2} measured in the first titration step (from Reactions A and B) that cannot be attributed to ClO\textsubscript{2} is then assigned as a product of Cl\textsubscript{2} oxidation.

Using this procedure, 46.6 µmoles of ClO\textsubscript{2} were recovered from a 25 °C reaction of 98.4 µmoles of Na ClO\textsubscript{2} with 0.1 µmoles of MnTDMImP (0.1 mol %) at pH 6.8, representing a 47% isolated yield. A small amount of iodide oxidation attributable to Cl\textsubscript{2}/HOCl (3.7 µmol) was also detected.

**Chlorination of Methyl Orange**

In order to indirectly test for the generation of hypochlorite (ClO\textsuperscript{−}) during turnover, methyl orange (MO, 4-dimethylaminoazobenzene-4'-sulfonic acid sodium salt) was added to the reaction of chlorite (ClO\textsubscript{2}\textsuperscript{−}) and MnTDMImP at pH 4.7. MO reacts with chlorinating oxidants such as ClO\textsuperscript{−}, producing dichlorodimethylaniline (DDA) (Scheme 1).\textsuperscript{38} DDA can be extracted from the aqueous medium with heptane and observed by GC-MS (m/z = 188).

![Scheme 1. Generation of dichlorodimethyl anilines (DDA) from methyl orange (MO)](image)

As controls, the reaction of MO with ClO\textsubscript{2}\textsuperscript{−}, ClO\textsuperscript{−}, and chlorine dioxide (ClO\textsubscript{2}) was tested. ClO\textsubscript{2}\textsuperscript{−} did not react at all with methyl orange at pH 4.7, whereas the other two
oxidants quickly bleached the methyl orange chromophore (464 nm). ClO\(^-\) produced dichlorodimethylaniline (DDA), detectable by GC-MS. No DDA was ever observed in reactions of MO with ClO\(_2\). However, we did observe that ClO\(_2\) quickly oxidized away authentic DDA to unknown products (Figure 15). Of course, the absence of observable DDA in reactions of MO and ClO\(_2\) does not prove that DDA is not transiently produced.

When MO was added to a solution of MnTDIImP before the addition of ClO\(_2^-\), a very small amount DDA (ca. 2 µmol) could be observed by GC-MS in heptane extracts of the reaction medium (1 mM ClO\(_2^-\), 1 mol\% catalyst, 100 µmol MO). When MO was added to the catalytic reaction after 1 and 5 minutes, less DDA was observed (Figure 16). Because DDA is consumed by ClO\(_2\) which is being generated during turnover, heptane extraction of the reaction mixture was done 1 minute after MO was added. Further, when MO was added to completed reactions of MnTDIImP/ClO\(_2^-\) (reaction time = 10-30 min), no DDA was observed. This qualitative experiment therefore asserts that a species capable of chlorinating MO is produced during turnover.
Figure 15. GC-MS chromatogram of authentic DDA and the extract of the reaction of DDA reacted with ClO$_2$.

Figure 16. GC-MS chromatogram of DDA generated when MO was present during the turnover reaction of MnTDMImP and ClO$_2^-$.
Chapter II

Formation of nitridoMn$^V$ by chlorite and ammonia

The presence of as little as 1-10 equiv ammonium chloride relative to manganese porphyrin during turnover conditions inhibits ClO$_2$ formation, but not NaClO$_2$ consumption, and results in the partial or complete conversion of the Mn$^{III}$TDMI$m$P (444 nm) to a new species (Figure 17) with a Soret at 414 nm (Q-bands 537 nm, 573 nm) that we assign as a nitridoMn$^V$ complex (Figure 18). Owing to the high sensitivity of this reaction to ammonia/ammonium ion, we were able to detect even trace ammonium impurities in some unpurified buffer salts (Figure 17, 0 equiv).

![Absorbance vs Wavelength](image)

**Figure 17.** Spectra of the MnTDMI$m$P catalyst (ca. 10 μM) after complete consumption of 2 mM NaClO$_2$ in the presence of 0, 1, 5, and 10 equiv NH$_4$Cl (conditions: pH 6.8, 100 mM KPi)
Nitridomanganese(V) porphyrins are known and have been characterized as diamagnetic, d² species. The addition of NaClO₂ to solutions of MnTDMImP containing an excess of ammonium chloride or acetate afforded a single new manganese compound with a diamagnetic ¹H NMR spectrum (Figure 19). The ¹H NMR spectrum shows the inequivalence of the N-methyl protons (δ 3.68, δ 3.64) on the TDMImP ligand consistent with the assignment of a C₄ᵥ nitridomanganese porphyrin species (Figure 18). The inequivalent but nearly isochronous imidazolyl protons also give rise to a slightly broadened signal at ca. δ 8.08. The porphyrin pyrrole protons give rise to a sharp singlet at δ 9.07.

**Figure 18.** Side-on structure of nitridoMn^V TDMImP, showing the chemical inequivalence of the two N-methyl and two imidazolyl protons
The nitridoMn\textsuperscript{V} complex could be generated by the addition of either NaClO\textsubscript{2} or NaClO (but not oxone) to a solution of MnTDMImP in the presence of NH\textsubscript{4}\textsuperscript{+} (Figure 20). Oxone instead generated the oxoMn\textsuperscript{V} complex. HOCl (but not ClO\textsubscript{2} or ClO\textsubscript{2}\textsuperscript{−}) is known to react with ammonia in solution to generate chloramines\textsuperscript{15,40-42}, and chloramines have shown to efficiently produce nitridoMn\textsuperscript{V} from Mn\textsuperscript{III} porphyrins.\textsuperscript{43} Therefore, we interpret these data as indirect evidence for the generation of HOCl during turnover of the porphyrin/NaClO\textsubscript{2} system.
Figure 20. Oxidation of Mn$^{III}$TDMImp in 100 mM ammonium acetate (pH 6.9) with NaOCl, NaClO$_2$, and oxone.
DISCUSSION

We have studied the catalytic oxidation of ClO$_2^-$ to chlorine dioxide by water-soluble manganese porphyrins and a manganese porphyrazine. Large amounts of chlorine dioxide, representing many turnovers of the catalysts, were observed within seconds for the tetra-dimethylimidazolium porphyrin (MnTDMImP), the corresponding benzimidazolium porphyrin (TDMBImP), and the cationic manganese porphyrazine (MnTM23PyPz). This latter catalyst proved to react so rapidly with chlorite ion (32 TO/s) that it was difficult to analyze in detail and is the target of future research. By contrast, the reaction of chlorite ion with MnTDMImP was particularly well behaved, allowing a complete kinetic deconvolution of the primary steps in this catalysis. Given the industrial significance of ClO$_2^-$, the need for better methods for its production and the variety of differing known heme- and porphyrin-mediated reactions of chlorite ion, it is of interest to understand the mechanisms of these reactions and to elucidate the catalyst properties that favor each of these specific reactivities.

General Mechanism for Manganese Catalyzed ClO$_2$ Generation

We initially propose the mechanism shown in Scheme 2 to account for the appearance of ClO$_2$ in these reactions. The key initiating step in this catalytic cycle is suggested to be oxygen atom transfer from chlorite to the Mn$^{III}$ catalyst to form trans-dioxoMn$^V$TDMImP. The thermodynamic driving force for oxo-transfer from chlorite ion is only slightly less than that of hypobromite ion (BrO$^-$) ($\Delta\Delta G^\circ = +16.7$ kJ mol$^{-1}$) and hypobromite is a very facile oxo-transfer agent in its reaction with Mn$^{III}$ porphyrins.
Moreover, reactive oxoMn\textsuperscript{V} intermediates have been implicated in the manganese porphyrin-catalyzed oxidations of cyclohexane by NaClO\textsubscript{2}\textsuperscript{28}

\begin{center}
\begin{tikzpicture}
\node [circle, draw] (mnn) at (0,0) {$\text{Mn}^{\text{III}}$};
\node [circle, draw] (mnnv) at (0,1.5) {$\text{Mn}^{\text{V}}$};
\node [circle, draw] (mnniv) at (0,-1.5) {$\text{Mn}^{\text{IV}}$};
\node [circle, draw] (cl2) at (1,0) {$\text{ClO}_2^-$};
\node [circle, draw] (hocl) at (1,1.5) {$\text{HOCl}$};
\node [circle, draw] (cl2h) at (1,-1.5) {$\text{ClO}_2^-$};
\draw [->, thick] (mnn) edge[bend left] node [above] {$k_1$} (hocl);
\draw [->, thick] (mnnv) edge[bend left] node [above] {$k_2$} (cl2h);
\draw [->, thick] (mnniv) edge[bend left] node [above] {$k_3$} (cl2);
\draw [->, thick] (cl2) edge[bend left] node [below] {$k_4$} (cl2h);
\end{tikzpicture}
\end{center}

\textbf{Scheme 2.} Proposed mechanism for ClO\textsubscript{2} evolution from ClO\textsubscript{2}\textsuperscript{-}

For the reaction with chlorite ion, the fact that the Mn\textsuperscript{III} oxidation state of the catalyst persisted during turnover (Figure 3) requires that any change in the porphyrin oxidation state from Mn\textsuperscript{III} be slow relative to Mn\textsuperscript{III}-forming reactions. Therefore, we conclude that the oxidation of Mn\textsuperscript{III} by ClO\textsubscript{2}\textsuperscript{-} must be the rate-determining step of the overall cycle. Accordingly, a slower 2-electron oxidation of Mn\textsuperscript{III} by ClO\textsubscript{2}\textsuperscript{-}, generating HOCl and \textit{trans}-dioxoMn\textsuperscript{V} is consistent with these observations. The generation of HOCl during turnover is indirectly suggested by the characteristic generation of chloroanilines from methyl orange. Furthermore, the addition of NH\textsubscript{4}\textsuperscript{+} during turnover resulted in the formation of nitridoMn\textsuperscript{V}, which can be independently synthesized by reacting MnTDMImP with HOCl in the presence of NH\textsubscript{4}\textsuperscript{+} salts. Presumably, this occurs via an \textit{in situ} formation of chloramine, which oxidizes the manganese(III) complex.
Interestingly, the pH-independence of the turnover rate (Figure 5) implies that the oxygen transfer step to afford the Mn\textsuperscript{V} species is pH-independent and occurs at about the same rate as the analogous heterolysis of HOO-Mn\textsuperscript{III}TDMImP, which also affords Mn\textsuperscript{V}.\textsuperscript{35}

As can be seen in the data in Table 1, higher turn-over rates were observed for porphyrins with the most electron-withdrawing meso-substituents (TDMImP and TDMBImP). Although porphyrin electronics could potentially influence an initial binding of chlorite ion to the Mn\textsuperscript{III} center, porphyrin substituent effects have been shown to have little affect on the normally fast axial ligand exchange rate.\textsuperscript{45} However, electron-withdrawing substituents on porphyrins have been shown to increase the Mn\textsuperscript{III}/Mn\textsuperscript{IV} redox potential, to stabilize trans-dioxomanganese(V) porphyrins by decreasing the basicity of the terminal oxo-groups, and to lower the energy of the porphyrin $a_{2u}$ HOMO.\textsuperscript{34, 46-47} If oxidation of the manganese(III) catalyst to oxoMn\textsuperscript{V} is the rate-determining step of this reaction, the greater accessibility and stability of this intermediate may be the reason the more electron-deficient porphyrins are faster catalysts.

The observed reduction of oxoMn\textsuperscript{V} and oxoMn\textsuperscript{IV}TDMImP by chlorite ion ($E^0 = +1.068 \text{ V}$)\textsuperscript{48} to afford ClO\textsubscript{2} is highly analogous to the well-studied reduction of high-valent manganese phophyrins by nitrite ion ($E^0 = +1.04 \text{ V}$).\textsuperscript{46} The similar reduction of Compounds I and II of horseradish peroxidase by chlorite ion to generate ClO\textsubscript{2} is also known.\textsuperscript{26} The measured $k_{\text{app}}$ for reduction of oxoMn\textsuperscript{V}TDMImP by chlorite ion at pH 6.8 ($6.30 \pm 0.62 \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$) is significantly slower than those previously reported\textsuperscript{46} for the related cationic N-methylpyridinium porphyrins, oxoMn\textsuperscript{V}TM4PyP and oxoMn\textsuperscript{V}TM2PyP with nitrite ($1.5 \times 10^7$ and $2 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$, respectively) at pH 7.4. The rate of chlorite
oxidation by oxoMn\textsuperscript{V}TDMImP increased by approximately 2 orders of magnitude over a ~2 pH range, indicating that a single protonation of the \textit{trans}-dioxomanganese(V) species is required for activation.\textsuperscript{33,49}

Hypochlorous acid is implicated as the product of the initial chlorite-Mn(III) reaction (Scheme 2). In this regard, the results for the reaction of Mn\textsuperscript{III}TDMImP with HOCl were very revealing. At pH 6.8, the reaction generated oxoMn\textsuperscript{V} completely within 300 ms (\(k_{\text{app} \ 6.8} = (2.23 \pm 0.11) \times 10^5 \text{ M}^{-1} \text{s}^{-1}\)) (Figure 6). However, at pH 4.7 and 5.1, Mn\textsuperscript{III}TDMImP and \textit{trans}-dioxoMn\textsuperscript{V}TDMImP were apparently in rapid, reversible equilibrium (Scheme 2, reaction 1), similar to the behavior of bromide/hypobromite with this porphyrin.\textsuperscript{34} The significance of this observation with respect to the proposed mechanism is that HOCl and \textit{trans}-dioxoMn\textsuperscript{V}TDMImP are observed to be present together over the entire period of the measurement. Thus, hydrogen abstraction from H-OCl by O=Mn\textsuperscript{V}=O to afford the chloroxy radical and oxoMn\textsuperscript{IV} must be slow or thermodynamically unfavorable.

As an aside, the equilibrium between HOCl/Mn\textsuperscript{III} and Cl\textsuperscript{-}/dioxoMn\textsuperscript{V} (Figure 6, panels b and c) allows us to directly calculate \(K_{\text{eq}}\) for the reversible oxo-transfer from dioxoMn\textsuperscript{V} to chloride ion. This steady state could be observed at pH 4.7, 5.1, and 5.6 and the concentrations of both porphyrin species could be directly measured from the steady-state UV-vis spectrum. From these values and the known HOCl and chloride ion concentrations, an equilibrium constant \(K_{\text{eq}}\) was calculated for this oxo-transfer reaction (Table 4). This value allowed a further determination of the redox potential \(E(\text{OMn}^\text{V}/\text{Mn}^\text{III})\), for oxo-transfer from oxoMn\textsuperscript{V}TDMImP. The calculated potentials as a function of pH are plotted in Figure 10.\textsuperscript{34} As can be seen, there is a pronounced pH effect
on the thermodynamics of halide oxygenation, with bromide ion oxygenation being facile at neutral pH and chloride ion oxidation becoming accessible at pH 5. The facile oxygenation of chloride ion observed here also suggests that the in situ generation of hypochlorite may be useful in other manganese porphyrin catalyzed reactions, such as in the unusual methylene-selective C-H chlorination we have recently reported. Interestingly, Figure 10 indicates that water oxidation could become accessible at pH 3.

<table>
<thead>
<tr>
<th>pH</th>
<th>$K_{eq}$</th>
<th>$\Delta E$ (mV)</th>
<th>$E(Mn^{V}/Mn^{III})$ (V, vs NHE)</th>
</tr>
</thead>
<tbody>
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<td>4.7</td>
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<td>-68 ± 1</td>
<td>+ (1.411 ± 0.001)</td>
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<tr>
<td>5.1</td>
<td>$(2.2 \pm 0.7) \times 10^{-1}$</td>
<td>-20 ± 5</td>
<td>+ (1.352 ± 0.005)</td>
</tr>
<tr>
<td>5.6</td>
<td>12.0</td>
<td>+32</td>
<td>+1.285</td>
</tr>
</tbody>
</table>

Errors represent standard deviation from multiple experiments with varying [HOCl], except for pH 5.6 where equilibrium was observed only under one set of conditions tested (50 µM HOCl, 5 µM MnTDMImP)

**Table 4.** Calculated thermodynamic parameters ($K_{eq}$, $E$) for the reversible oxo-transfer from HOCl to Mn$^{III}$TDMImP
Figure 21. Plot of calculated MnTDMImP $E_{\text{OMn(V)/Mn(III)}}$ redox potential as a function of pH from HOCl equilibrium data (black squares) and HOBr equilibrium data (black circles, from reference 34). Nernst equation plots for HOBr/Br$^-$ (blue), HOCl/Cl$^-$ (green) and H$_2$O$_2$/H$_2$O (black) are superimposed for reference.

As shown in Figure 3, ClO$_2$ is generated in an initial fast reaction upon mixing solutions of ClO$_2^-$ with the catalysts MnTDMImP and MnTM23PyPz. The ClO$_2$ concentration was observed to rise quickly to a plateau and then to decrease with minimal catalyst bleaching in a slower subsequent phase of the reaction.

To account for the further decay of ClO$_2$ during the slower second phase of this catalysis, the reactions of ClO$_2$ with authentic oxoMn$^V$, oxoMn$^IV$, and Mn$^{III}$TDMImP were examined. Traditional stopped-flow spectroscopy indicated 1-electron redox chemistry between ClO$_2$ and both oxoMn$^V$ and oxoMn$^IV$ (Figure 13) to produce ClO$_3^-$.

Indeed, chlorate ion was detected as a product by indirect ion chromatography HPLC.
(Table 2). Chlorine dioxide also decomposed in the presence of aqua-hydroxoMn\textsuperscript{III}-TDMImP at pH 6.8 (Figure 12). The Mn\textsuperscript{IV}/Mn\textsuperscript{III} redox potential could be observed by square wave voltammetry to be 1.03 V vs. NHE at this pH. Thus, the reduction potential for oxoMn\textsuperscript{IV}/Mn\textsuperscript{III}-TDMImP is either equal to or slightly less than that of ClO\textsubscript{2}/ClO\textsubscript{2}\textsuperscript{−} in the vicinity of pH 6.8, confirming that ClO\textsubscript{2} should be capable of oxidizing Mn\textsuperscript{III}. Of course, this is the reverse reaction of the observed oxidation of ClO\textsubscript{2}\textsuperscript{−} by oxoMn\textsuperscript{IV}, and we conclude therefore that this step is reversible during catalysis near neutral pH.

By accounting for the new results described here, we propose a more complete mechanism of this manganese porphyrin-chlorite reaction as shown in Scheme 3. All but two of the elementary steps of this catalytic cycle have been observed and analyzed directly by stopped-flow methods. The endergonic oxidation of Mn\textsuperscript{III} by ClO\textsubscript{2}\textsuperscript{−} is the rate-limiting step for this system (k\textsubscript{1}) and cannot be directly observed due to the rapid subsequent steps. Similarly, the oxidation of Mn\textsuperscript{III} to oxoMn\textsuperscript{IV} by ClO\textsubscript{2} (k\textsubscript{−3}) could not be directly observed, as the reverse reaction (reduction of oxoMn\textsuperscript{IV} by ClO\textsubscript{2}\textsuperscript{−}) is shown to progress fully under the conditions studied.

Although we could not directly observe the 2-electron oxidation of Mn\textsuperscript{III} by ClO\textsubscript{2}\textsuperscript{−}, the rate constant of this step was inferred by monitoring the first-order decay of ClO\textsubscript{2}\textsuperscript{−} ion during turnover. However, a steady-state approximation for the mechanism in Scheme 1 can be used to infer the relationship between the rate of ClO\textsubscript{2}\textsuperscript{−} decay and the rate of the RDS. From the mechanism in Scheme 1, the rate of ClO\textsubscript{2}\textsuperscript{−} consumption/generation is:

$$\frac{d[\text{ClO}_2^-]}{dt} = -(k_1[Mn^{III}][\text{ClO}_2^-] + k_2[\text{oxoMn}^{IV}][\text{ClO}_2^-] + k_3[\text{oxoMn}^{IV}][\text{ClO}_2^-] - k_3'[Mn^{III}][\text{ClO}_2^-])$$
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Assuming that [HOCl], [oxoMn\textsuperscript{V}], and [oxoMn\textsuperscript{IV}] all remain small and constant,

\[
\frac{d[\text{HOCl}]}{dt} = k_1[\text{ClO}_2^-][\text{Mn}^{\text{III}}] - k_4[\text{HOCl}][\text{Mn}^{\text{III}}] = 0
\]

\[
[\text{HOCl}] = \frac{k_1[\text{ClO}_2^-]}{k_4}
\]

\[
\frac{d[\text{oxoMn}^{\text{V}}]}{dt} = k_1[\text{ClO}_2^-][\text{Mn}^{\text{III}}] + k_4[\text{HOCl}][\text{Mn}^{\text{III}}] - k_2[\text{ClO}_2^-][\text{oxoMn}^{\text{V}}] = 0
\]

\[
[\text{oxoMn}^{\text{V}}] = \frac{k_1[\text{ClO}_2^-][\text{Mn}^{\text{III}}] + k_4[\text{HOCl}][\text{Mn}^{\text{III}}]}{k_2[\text{ClO}_2^-]} = 2 \frac{k_1}{k_2} [\text{Mn}^{\text{III}}]
\]

\[
\frac{d[\text{oxoMn}^{\text{IV}}]}{dt} = k_2[\text{ClO}_2^-][\text{oxoMn}^{\text{V}}] + k_3[\text{ClO}_2^-][\text{Mn}^{\text{III}}] - k_4[\text{ClO}_2^-][\text{oxoMn}^{\text{IV}}] = 0
\]

\[
[\text{oxoMn}^{\text{IV}}] = \frac{2k_1[\text{ClO}_2^-][\text{Mn}^{\text{III}}] + k_3[\text{ClO}_2^-][\text{Mn}^{\text{III}}]}{k_3[\text{ClO}_2^-]}
\]

therefore,

\[
\frac{d[\text{ClO}_2^-]}{dt} = -(k_1[\text{Mn}^{\text{III}}][\text{ClO}_2^-] + 2k_1[\text{Mn}^{\text{III}}][\text{ClO}_2^-] + 2k_1[\text{Mn}^{\text{III}}][\text{ClO}_2^-]) = 5k_1[\text{Mn}^{\text{III}}][\text{ClO}_2^-]
\]

with \( k_{\text{obs}} = 5k_1[\text{Mn}^{\text{III}}] \).

The above derivation suggests that the decomposition of chlorite ion is first-order in both chlorite and manganese porphyrin and is confirmed by experiment. Furthermore, the pseudo-first order rate constant \( k_{\text{obs}} \) for ClO\textsubscript{2}\textsuperscript{-} consumption is equal to \( 5k_1[\text{Mn}^{\text{III}}] \), where \( k_1 \) is the apparent second-order rate constant for oxo-transfer from ClO\textsubscript{2}\textsuperscript{-} to Mn\textsuperscript{III}. At pH 6.8, the first-order conversion of ClO\textsubscript{2}\textsuperscript{-} by 8.6 \( \mu \)M MnTDMImP is constant ((2.92
and predicts a \( k_1 \) equal to \( 68 \pm 4.4 \text{ M}^{-1} \text{s}^{-1} \). This value is consistent with an initial rate analysis of the same data (Figure 22).

**Figure 22.** Determination of the apparent second-order rate constant (kapp) between 10 uM Mn^{III}TDMImP and ClO$_2^-$ by the method of initial rates. The initial rate of ClO$_2$ appearance was divided by four (see text) and plotted as a function of initial [ClO$_2^-$]. The slope of each line is equal to the product of kapp and the concentration of Mn^{III}TDMImP (k$_{\text{app}}$ 4.7 = 250 ± 13 M$^{-1}$ s$^{-1}$, k$_{\text{app}}$ 6.8 = 59.2 ± 1.8 M$^{-1}$ s$^{-1}$).
Scheme 3. Mechanism of ClO$_2^-$ dismutation by MnTDMImP. The bold arrows indicate steps contributing to ClO$_2$ formation. Due to the pH range of this study (4.7-6.8), hypochlorite is written in protonated form.

<table>
<thead>
<tr>
<th>Reaction</th>
<th>$k_{app}$ (M$^{-1}$ s$^{-1}$)</th>
<th>pH 4.7</th>
<th>pH 6.8</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_1$</td>
<td>Mn$^{III}$ + ClO$_2^-$ → oxoMn$^{V}$ + ClO$^-$</td>
<td>(2.50 ± 0.13) x 10$^2$</td>
<td>(6.80 ± 0.14) x 10$^1$</td>
</tr>
<tr>
<td>$k_2$</td>
<td>oxoMn$^{V}$ + ClO$_2^-$ → oxoMn$^{IV}$ + ClO$_2$</td>
<td>(6.77 ± 0.08) x 10$^5$</td>
<td>(6.30 ± 0.62) x 10$^3$</td>
</tr>
<tr>
<td>$k_3$</td>
<td>oxoMn$^{IV}$ + ClO$_2^-$ → Mn$^{III}$ + ClO$_2$</td>
<td>(6.03 ± 0.06) x 10$^3$</td>
<td>(3.13 ± 0.12) x 10$^3$</td>
</tr>
<tr>
<td>$k_{-3}$</td>
<td>Mn$^{III}$ + ClO$_2$ → oxoMn$^{IV}$ + ClO$_2^-$</td>
<td>n.d.</td>
<td>(4.59 ± 2.37) x 10$^2$ *</td>
</tr>
<tr>
<td>$k_4$</td>
<td>Mn$^{III}$ + Cl$^-$ → oxoMn$^{V}$ + ClO$^-$</td>
<td>(6.64 ± 0.23) x 10$^4$</td>
<td>(1.71 ± 0.27) x 10$^6$</td>
</tr>
<tr>
<td>$k_{-4}$</td>
<td>oxoMn$^{V}$ + Cl$^-$ → Mn$^{III}$ + ClO$^-$</td>
<td>1.30 x 10$^7$ †</td>
<td>1.42 x 10$^2$ †</td>
</tr>
<tr>
<td>$k_5$</td>
<td>oxoMn$^{V}$ + ClO$_2$ → oxoMn$^{IV}$ + ClO$_3^-$</td>
<td>(2.24 ± 0.14) x 10$^4$</td>
<td>(1.61 ± 0.12) x 10$^4$</td>
</tr>
<tr>
<td>$k_6$</td>
<td>oxoMn$^{IV}$ + ClO$_2$ → Mn$^{III}$ + ClO$_3^-$</td>
<td>(7.90 ± 0.53) x 10$^3$</td>
<td>(1.19 ± 0.06) x 10$^4$</td>
</tr>
</tbody>
</table>

All errors represent standard errors of linear fit analyses as described in the text; For simplicity, both HOCl and ClO$^-$ are represented in reactions 1, 4, and -4 as ClO$^-$; $^*$ $k_{-3}$ reported as the average of two values determined from computational fit of mechanism to data (see text); † $k_{-4}$ calculated from measured $k_4$ and $K_{eq}$; n.d., not determined.

Table 5. Collected apparent second-order rate constants for the elementary steps in the decomposition of ClO$_2^-$ by MnTDMImP.
Kinetic Simulation of the Mechanism

To evaluate the proposed scheme (and the reversibility of oxoMn$^{IV}$ reduction by ClO$_2^-$), the mechanism in Scheme 3 was modeled by kinetic simulation using the Berkeley-Madonna software platform. Experimentally-determined rate constants for all known reactions were used (Table 5) and the model was fitted to ClO$_2$ growth and decay curves such as those in Figure 6 and Figure 12. The rate constant $k_{-3}$, corresponding to the oxidation of Mn$^{III}$ by ClO$_2$ was left as an unconstrained parameter. Two computational fits of the model to the data provided calculated $k_{-3}$ values of $3.04 \times 10^2$ or $6.64 \times 10^2$ M$^{-1}$ s$^{-1}$ (Figure 23). The predicted $k_{-3}$ value (when taken with the experimentally observed $k_3$) allows calculation of a $K_{eq}$ to be $\sim 7.5$, in agreement with our prediction of a reversible step near pH 6.8. The Mn$^{IV}$/Mn$^{III}$ reduction potential that can be calculated from these equilibrium values is between + 1.0 V and + 1.13 V, depending upon which of several standards is used for the oxidation potential of chlorite ion,\textsuperscript{51} essentially the same as the measured value (1.03 V) and those reported for other cationic manganese porphyrins.\textsuperscript{52-54} Thus, the Mn$^{IV}$/Mn$^{III}$ reduction potential of MnTDMImP is well matched to that of chlorite ion such that Mn$^{IV}$ does not accumulate during catalysis.
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Figure 23. Modeling data for the mechanism in Scheme 3. a) Data from Figure 6, 25 °C. Model calculates $k_{3} = 6.64 \times 10^{2}$ M$^{-1}$ s$^{-1}$. b) Data from Figure 12. Model calculates $k_{3} = 3.04 \times 10^{2}$ M$^{-1}$ s$^{-1}$

**Activation Parameters for Oxo-transfer from ClO$_{2}^{-}$ to Mn(III)TDMImP**

Using the data from Figure 6, it was possible to extract second-order rate constants for the oxidation of Mn$^{\text{III}}$ by ClO$_{2}^{-}$ at pH 6.8 over the temperature range 5 – 35 °C (Figure 7). Using these values, thermodynamic activation parameters could be determined for the initial oxo-transfer reaction; $\Delta H^{\ddagger} = + 44.4$ kJ mol$^{-1}$ and $\Delta S^{\ddagger} = - 60.7$ J mol$^{-1}$ K$^{-1}$.

Positive, moderate $\Delta H^{\ddagger}$ values and negative $\Delta S^{\ddagger}$ values have been associated with heterolytic O-O bond cleavage of iron,$^{55}$ chromium,$^{56}$ and manganese peroxides,$^{35}$ leading to metal-oxo formation. The calculated DH$^{\ddagger}$ value is similar to that reported for oxygen atom transfers to phosphites and phosphonites from dioxomolybdenum and dioxotungsten complexes (+34 to + 58 kJ mol$^{-1}$). Heterolysis of the O-Cl bond in a chloritomanganese(III) adduct leading to o xoMn$^{\text{V}}$ would be analogous to the concerted and entropically-unfavorable O-O cleavage in HOO-Mn$^{\text{III}}$TDMImP (Scheme 4).$^{35}$ A
negative activation entropy term would also arise from the bimolecular nature of the proposed manganese porphyrin oxidation by chlorite ion. Another similarity between these two heterolytic reactions is the pH-independence that is observed, suggesting a similar push-pull protonation-deprotonation scenario in both mechanisms.

Scheme 4. Proposed mechanism for pH-independent O-X heterolysis for the formation of trans-dioxomanganese(V)

Chlorite as a 1- or 2-electron Oxidant

The kinetic data reported here argues that ClO$_2^-$ reacts with Mn$^{III}$ via oxo-transfer to generate dioxoMn$^V$TDMImP and HOCl. Further, the observed steady-state equilibrium shown in Figure 10 shows that HOCl does not reduce dioxoMn$^V$TDMImP at a detectable rate. Another conceivable pathway from an initial chloritomanganese(III) adduct would lead via Cl-O bond homolysis to an oxoMn$^{IV}$ intermediate and chloroxy radical (ClO$^*$), as suggested by Abu-Omar et al.$^{23}$ A similar homolysis mechanism has been implicated computationally for chloritoiron(III) porphyrins.$^{58}$ Chlorite is known to act as both a 1- and 2-electron oxidant with transition metal systems.$^{59}$ As discussed above, the rationale
for our proposal of $\text{ClO}_2^-$ as a 2-electron oxidant is the similar $\text{DG}^\circ$ for oxo-transfer from both $\text{ClO}_2^-$ and hypobromite ($\text{BrO}^-$),\textsuperscript{44} the latter a known oxo-transfer reagent in manganese porphyrin chemistry. Further, $\text{ClO}_2^-$ has been demonstrated to be an oxo-transfer “shunt” oxidant for manganese porphyrins in organic solvents, generating presumed oxoMn$^\text{V}$ intermediates.\textsuperscript{28} Substrate oxygenations and oxygen production were reported in that case.

One can consider the two proposed oxidations of Mn$^{\text{III}}$ by ClO$_2^-$ as competing homolysis and heterolysis of the O-Cl bond in an inferred chloritomanganese(III) adduct (Figure 24). A chloritoferron(III) complex has been detected in an iron-catalyzed oxidation of chlorite ion at very low pH.\textsuperscript{51} Although both of the plausible reactions are endergonic for Mn$^{\text{III}}$TDMImP, a thermochemical consideration can be used to consider the driving force for homolysis versus heterolysis.
Figure 24. Hypothetical equilibrium between oxoMn$^{IV}$ and oxoMn$^{V}$ as a result of heterolytic or homolytic cleavage of the O-Cl bond from a chloritomanganese(III) adduct.

It is possible to consider the hypothetical equilibrium (Figure 24) as the sum of redox half-reactions. Although we have not measured directly the redox potential between trans-dioxomanganese(V) and manganese(IV), it can be determined using thermodynamics of other measured reactions. First, the redox potential for the two-electron reduction of trans-dioxomanganese(V) to give manganese(III) can be determined from the previously reported equilibrium of oxo-transfer between oxomanganese(V) and bromide ion at pH 6.8.$^{34}$ Under those conditions, $K_{eq}$ for the oxidation of Mn$^{III}$ by HOBr is given as 2.9, corresponding to a pH 6.8 redox potential $E^{'}_{\text{Mn(V)/Mn(III)}} = +1.117$ V vs. NHE. Next, the redox potential for the one-electron
reduction of oxomanganese(IV) to give manganese(III) can be determined via square wave voltammetry at pH 6.8 to be \( E'_{\text{Mn(IV)/Mn(III)}} = +1.03 \text{ V vs. NHE.} \) From these two values (\( E'_{\text{Mn(V)/Mn(III)}} \) and \( E'_{\text{Mn(IV)/Mn(III)}} \)), the pH 6.8 reduction potential for the couple oxoMn\( ^{V} \)/oxoMn\( ^{IV} \) can be calculated as \( E'_{\text{Mn(V)/Mn(IV)}} = +1.20 \text{ V} \).

In regards to the other half reaction, the oxidation of ClO\( ^{-} \), the value of \( E^\circ(\text{ClO}^{\cdot}/\text{ClO}^{-}) \) has been measured as \(+1.41 \text{ vs. NHE.}^{60} \) Correcting this pH-independent value to the relevant \( E^\circ(\text{ClO}^{\cdot}/\text{ClOH}) \) using the \( pK_a \) of hypochlorous acid gives \( E^\circ(\text{ClO}^{\cdot}/\text{ClOH}) = +1.86 \text{ V} \) (Figure 25) and therefore at pH 6.8 \( E'_{\text{ClO}/\text{ClOH}} = +1.46 \text{ V vs. NHE.} \)

![Figure 25. Derivation of \( E^\circ(\text{ClO}^{\cdot}/\text{ClOH}) \) using \( E^\circ(\text{ClO}^{\cdot}/\text{ClO}^{-}) \) and \( pK_a \) of hypochlorous acid.](image)

From these two values (\( E'_{\text{ClO}/\text{ClOH}} \) and \( E'_{\text{Mn(V)/Mn(IV)}} \)), \( \Delta E \) for the equilibrium in Figure 24 can be calculated as \( \Delta E = -0.26 \text{ V.} \) Therefore, the hypothetical oxidation of HOCl by oxoMn\( ^{V} \) is unfavorable by ca. 25 kJ mol\(^{-1} \) (consistent with Figure 10) and one would predict a heterolytic O-Cl bond cleavage in the oxidation of Mn\(^{III} \) by ClO\(_{2}^{-}\).
CONCLUSIONS

We have investigated the mechanism of a manganese porphyrin-catalyzed chlorine dioxide production from chlorite ion. This process involves rate-limiting oxidation of the manganese(III) catalyst by ClO$_2^-$ to afford high-valent manganese species. We argue on kinetic, electrochemical and thermodynamic grounds that this initial intermediate is the same trans-dioxoMn$^V$TDMImP species that we have previously observed in reactions of this manganese porphyrin with hypobromite$^{34}$ and hydrogen peroxide.$^{35}$ Both oxoMn$^V$ and oxoMn$^IV$ oxidize ClO$_2^-$ directly to ClO$_2$. Interestingly, both the oxidation of ClO$_2^-$ by oxoMn$^IV$ and the oxo-transfer from HOCl to Mn$^{III}$ were found to be fast and reversible near pH 6.8. The ClO$_2$ evolved from this catalysis is itself further oxidized to ClO$_3^-$ by oxoMn$^V$ and oxoMn$^IV$ in a slower subsequent phase of the reaction. The entire catalytic process can be modeled via kinetic simulation, in good agreement with empirical ClO$_2$ growth and decay curves and the measured ClO$_3^-$ that is produced. According to this mechanism (4 ClO$_2$ produced per 5 NaClO$_2$), the 47% isolated yield of ClO$_2$ measured by air-stripping of the catalytic reaction represents ca. 500 turnovers.

The fast and efficient evolution of ClO$_2$ catalyzed by MnTDMImP and MnTM23PyPz suggest that a viable, scalable process could be developed for chlorine dioxide production using these catalysts. The mechanism of this process now suggests methods for greatly enhancing the rate of ClO$_2$-generation under mild, neutral conditions, for instance, by focusing on electron-withdrawing porphyrin or other ligands that facilitate the oxo-transfer, rate-determining step. Of even more interest is understanding what causes the record-setting porphyrazine catalyst to degrade; a manganese
porphyrazine with the same activity as observed here that withstands the reaction conditions would be a powerful catalyst.
ACKNOWLEDGMENTS

Dr. Dong Wang completed the synthesis of MnTDMImP and the square-wave voltammetry experiments described in this chapter, and is recognized as a co-author of reference 31. Christina Chang, ’12 is thanked for experimental assistance concerning the effect of ionic strength.
EXPERIMENTAL

Reagents

Sodium chloride, sodium chlorate, \( t \)-butyl hydroperoxide (70% aqueous solution), and Oxone were purchased from Aldrich and used as received. Sodium chlorite was obtained from Aldrich as >80% technical grade and recrystallized twice from ethanol/water (>95% final). All oxidant stock solutions were prepared fresh daily and standardized by iodometric titration before use. Dilute (0.5-10.0 mM) chlorite solutions were standardized spectrophotometrically (\( \varepsilon_{260 \text{nm}} = 154 \text{ cm}^{-1} \text{ M}^{-1} \)). Buffers were prepared fresh each day using either acetic acid/sodium acetate (pH = 4.7, 5.7) or potassium phosphate (monobasic)/potassium phosphate (dibasic) (pH = 6.8, 8.0) and pH-adjusting no more than 0.1 units using perchloric acid or sodium hydroxide. ClO\(_2\) was prepared from ClO\(_2\)\(^-\) using a previously reported procedure. Briefly, a 2.5% w/w solution of NaClO\(_2\) was acidified with sulfuric acid under an argon flow in the dark. The evolved gas was carried by the argon flow through a gas scrubbing tower containing a 2.5% w/w solution of NaClO\(_2\) and bubbled through deionized water in a chilled amber bottle. Aliquots of the resulting solution were frozen at -30 °C for prolonged storage. The concentration of ClO\(_2\) was checked immediately prior to use (\( \varepsilon_{359 \text{nm}} = 1230 \text{ cm}^{-1} \text{ M}^{-1} \)). Leftover ClO\(_2\) from all experiments was neutralized with sodium iodide before being disposed of in general waste. Mn\(^{III}\)TDMImP was synthesized as the chloride salt using reported procedures, and MnTDMBImP was prepared analogously from N-methyl-2-benzimidazolone-carboxaldehyde. MnTM2PyP and MnTM4PyP were purchased from Mid Century and purified by double precipitation.
following the method of Wöhrle. Briefly, manganese diacetate (170 mg) and pyridine-2,3-dicarbonitrile (500 mg) were heated in an unsealed reaction tube to 200 °C with mechanical stirring for 4 h. The deep blue solid product (a mixture of isomers) was washed with acetone, isolated by filtration, suspended in 50 mL DMF, and tetra-methylated using excess dimethyl sulfate at 120 °C for 12 h. Product was precipitated with acetone and purified by double precipitation.

**Instrumentation**

UV-Vis spectroscopic measurements were taken using a Hewlett-Packard 8453 diode array spectrophotometer equipped with a temperature-controlled cell housing, VWR 1140 thermostat bath and a Hi-Tech SFA Rapid Kinetics Accessory. Stopped-flow experiments for fast reactions were carried out using a Hi-Tech SF-61 double-mixing instrument with a 1 cm path length equipped with an ISOTEMP 1016 S thermostat bath. Ion chromatography was accomplished with an HPLC system consisting of a Waters 600 controller, Hamilton PRP X-100 column, and Waters 996 photodiode array detector.

**Stopped-flow Experiments**

Reactions of ClO$_2^-$ with Mn$^{III}$ porphyrins were studied using traditional UV-vis and rapid mixing techniques. Solutions of catalyst and ClO$_2^-$ were prepared in buffered solutions and mixed 1:1. All rate calculations were based on final concentrations resulting from this dilution. The oxidations of ClO$_2^-$ and ClO$_2$ by oxoMn$^V$TDMImP and oxoMn$^IV$TDMImP were studied in double mixing mode using diode array detection. Solutions of Mn$^{III}$TDMImP and oxidant (oxone, tBuOOH) were prepared in weak pH =
8.0 phosphate buffer (10 mM) and mixed 1:1 in a first push. After a short aging time to ensure complete conversion to the high-valent species (2-150 s, fine tuned for each experiment), the porphyrin solution was mixed 1:1 with the substrate (ClO$_2^-$ or ClO$_2$) prepared in a higher strength buffer (100 mM) at the pH to be studied (4.7 or 6.8). All concentrations used in subsequent rate calculations accounted for the 4-fold and two-fold dilutions inherent to the double-mixing technique. Each reaction was run in duplicate or triplicate at T = 25 °C, and most errors in measured rate constants were < ± 2.0%. In many cases in the text, the error bars in the reported data are smaller than the data point marker. Averaging of the runs and analysis of the data was accomplished using the KinetAsyst 3 software package. Kinetic simulations of the overall mechanism were performed with the Berkeley-Madonna software package.

**Ion Chromatography Experiments**

Aliquots of ClO$_2^-$ or ClO$_2$ solutions were added to buffered solutions of Mn$^{III}$TDMImP under mechanical stirring at ambient temperatures. ClO$_3^-$ was quantified using an indirect ion chromatography method$^{36}$. In brief, an aqueous solution of 4-aminosalicylic acid (4 mM) was pH adjusted to pH = 6.0 and employed as the mobile phase. Reaction samples were injected directly without any modification. The eluent was monitored at 320 nm, and a decrease in absorbance was observed as anions were eluted. Concentration of analyte was calculated directly from total area of the peak using a concentration curve prepared daily using prepared standards.

**Temperature-dependence on ClO$_2$ Evolution**
Buffered solutions (100 mM) of Mn$^{III}$TDMImP and ClO$_2^-$ were mixed 1:1 and monitored in the UV-Vis. The cell holder and mixing accessory were equilibrated to the set temperature for 30 min before the measurement.
REFERENCES


37. Sampling and analytical methods: Chlorine and chlorine dioxide in workplace atmospheres (id-126sgx). OSHA, U., Ed. Industrial Hygiene Chemistry Division OSHA Salt Lake Technical Center: Salt Lake City, UT.


60. Huie, R. E.; Clifton, C. L.; Neta, P., Equilibria of the carbonate and sulfate radical anions. 1991, 477.


CHAPTER III

APPLICATIONS OF \textit{IN SITU} CHLORINE DIOXIDE FORMATION BY 
CATIONIC MANGANESE PORPHYRINS AND PORPHYRAZINES: 
OXIDATION OF ORGANIC MATTER AND BACTERIAL DISINFECTION
ABSTRACT

An *in situ* method of water treatment involving the catalytic generation of chlorine dioxide by a manganese porphyrin (MnTDMImP) and sodium chlorite was tested for its efficacy in chemically oxidizing organic pollutants and disinfecting solutions of *E. coli*. The chlorite/porphyrin system was twice as effective at oxidizing trichlorophenol than was authentic chlorine dioxide, although it was only *as* effective as chlorine dioxide at oxidizing phenol and the NSAID diclofenac. In the case of diclofenac, however, authentic chlorine dioxide produced chlorinated by-products whereas the chlorite/porphyrin system did not and instead generated diclofenac-derived dimers. We interpret these results to suggest that *in situ* chlorine dioxide formation by manganese porphyrins and chlorite operate by recycling “spent” chlorine dioxide (i.e. chlorite ion) and prevent substrate chlorination by intercepting any chlorinating species (e.g. hypochlorous acid) generated by chlorine dioxide. Furthermore, the chlorite/porphyrin system was effective at killing *E. coli*, but only at chlorite ion concentrations one order of magnitude higher than the minimum chlorine dioxide dose. Regardless, this method suggests a simple way to chemically treat contaminated drinking water without the need for chemical reactors or the pre-mixing of reagents.
INTRODUCTION

Chlorine dioxide (ClO\textsubscript{2}) is a water-soluble, oxidizing gas that is finding increased use as an alternative bleaching agent and disinfectant to traditional chlorine and chlorine bleach (Cl\textsubscript{2}/HOCl). Interest in using ClO\textsubscript{2} in the paper industry is driven by its ability to produce a brighter and stronger paper fiber and afford fewer organohalogen by-products. ClO\textsubscript{2} is additionally useful in water treatment; it can oxidize metal contaminants (Mn\textsuperscript{II}, Fe\textsuperscript{II}) to insoluble oxides, destroy off-odors and –tastes caused by phenolic compounds, and act a broad-spectrum biocide.\textsuperscript{1} ClO\textsubscript{2} use in water treatment produces fewer toxic organohalogen compounds from side reactions with humic substances or other common contaminants such as pharmaceutical wastes, compared to traditional chlorine (Cl\textsubscript{2}).\textsuperscript{2} However, the instability of ClO\textsubscript{2} offsets many of these benefits, requiring it to be generated immediately prior to use, which is both risky and expensive. The common methods used to produce ClO\textsubscript{2} were presented in Chapter 2.

Generally, ClO\textsubscript{2} reacts purely as a 1-electron oxidant, and is most active towards oxidizing phenols, benzylic positions, olefins, and amines (although it does not generate chloramine).\textsuperscript{1,3-6} It is not a chlorinating agent, although it can lead to the generation of halogenated by-products through the downstream production of HOCl.\textsuperscript{6} However, in water treatment, ClO\textsubscript{2} produces far fewer of halogenated by-products (especially trihalomethanes) than traditional chlorine, and that remains one of the strongest arguments making ClO\textsubscript{2} a contender to replace chlorine.\textsuperscript{1,6-7}

In addition to the costs and risks of ClO\textsubscript{2} use in water treatment, however, a concern with its implementation is the generation of chlorite (ClO\textsubscript{2}\textsuperscript{−}) and chlorate (ClO\textsubscript{3}\textsuperscript{−}) anions as by-products of ClO\textsubscript{2} use.\textsuperscript{7} Chlorite, in addition to being a by-product of ClO\textsubscript{2}
generation, is practically unavoidable as it is the direct product of the 1-electron reduction of ClO\(_2\) (i.e. ClO\(_2\)’s mode of action). Chlorite residuals in water treatment have a maximum allowable limit ("Maximum Containment Limit," MCL) post-treatment of 1.0 mg L\(^{-1}\) but can be relatively easily removed by common water treatment methods such as the addition of ferrous iron or sulfites.\(^1\)\(^8\) Chlorates, on the other hand, have no MCL stipulated by the EPA; however, the World Health Organization quotes a lethal human dose as low as 230 mg kg\(^{-1}\).\(^9\) In addition to being a common impurity generated during ClO\(_2\) production, ClO\(_3\)^\(-\) is also a photodecomposition product of ClO\(_2\). Unlike ClO\(_2^+\), ClO\(_3^+\) cannot be easily removed from treated water, and so its generation is generally avoided wherever possible.

As described in detail in the Chapter 2, we have discovered that cationic, water-soluble manganese porphyrins and porphyrazines are remarkably efficient catalysts for completely converting stable solutions of sodium chlorite (NaClO\(_2\)) to chlorine dioxide (ClO\(_2\)) at near-neutral pH in minutes. We also showed that this system generates ClO\(_3^+\) as the ultimate product of ClO\(_2^-\) consumption, and that ClO\(_2\) is more properly an intermediate in the system (Scheme 1).
Scheme 1. Mechanism of NaClO₂ consumption by manganese porphyrins, generating ClO₂ as an intermediate

The catalytic process can be thought of as two separate, sequential reactions:

\[
\begin{align*}
\text{ClO}_2 \text{forming:} & \quad 5 \text{NaClO}_2 + 4 \text{H}^+ \rightarrow 4 \text{ClO}_2 + \text{Cl}^- + 5 \text{Na}^+ + 2 \text{H}_2\text{O} \\
\text{ClO}_3^- \text{forming:} & \quad \text{NaClO}_2 + 4 \text{ClO}_2 + 2 \text{H}_2\text{O} \rightarrow 4 \text{ClO}_3^- + \text{NaCl} + 4 \text{H}^+
\end{align*}
\]

In each step, one NaClO₂ acts as an “engine” to drive the oxidation of Mn^{III} porphyrin. The other four substrate molecules (NaClO₂ or ClO₂) are each oxidized by 1-electron by high-valent Mn (to ClO₂ or ClO₃⁻, respectively). In essence, this analysis says that ClO₃⁻ formation is a direct result of the efficiency in removing or using the ClO₂ intermediate before it is itself oxidized. More practically, however, it reminds us that efficient application of this catalytic system would require engineering such that ClO₂ production
is maximized, but that the thus-formed ClO₂ is removed from the catalyst before it is, in essence, wasted and converted into a troublesome by-product.

In considering ways in which the catalytic method developed using manganese protoporphyrins might be implemented on a practical scale, we envisioned three possibilities. In one way, the manganese catalyst could be used in place of some other reagent (acid or oxidant) in a chlorite batch reactor. Air stripping of the reactor, a common practice for ClO₂ capture¹, might then be used to transfer the gaseous ClO₂ product to another vessel, perhaps water to be treated or some other container for short-term, temporary storage.

A second method would be a solid-phase cartridge¹⁰ impregnated with the catalyst over which one would flow a sodium chlorite solution. Properly engineered, such a device could produce ClO₂ solutions in as large or small quantities as the situation desired. In the batch reactor case (above), one avoids the risks of adding strong acids or oxidants to a reactor; in this latter, flow-through case, the need for a reactor is avoided altogether!

A third way in which to implement the catalytic process would be to use the catalyst \textit{in situ} in the very water to be treated (Figure 1). The addition of chlorite and catalyst (perhaps bound to a solid support,¹⁰ for simple removal post-treatment) to a volume of polluted or otherwise-contaminated water would then produce ClO₂ \textit{in situ} to bring about water treatment. This manner of implementation would lend itself to readily portable, recreational small-scale water treatment as well as providing access to clean drinking water in isolated, impoverished regions of the world.
Figure 1. *In situ* generation of chlorine dioxide by manganese porphyrins as a method of simple and portable disinfection and water treatment

Given the novelty of such an *in situ* method for generating and using ClO$_2$ in water treatment, a more thorough investigation is warranted. This chapter presents preliminary experiments designed to test and observe the efficacy of *in situ* ClO$_2$ generation for chemically oxidizing organic matter and disinfecting a model wastewater. The goal of this work was to determine if *in situ* generation of ClO$_2$ using manganese porphyrins is as effective as treatment with authentic ClO$_2$. 
RESULTS

The method for generating chlorine dioxide (ClO$_2$) using manganese porphyrins and porphyrazines discussed in Chapter 2 demonstrated a method for producing ClO$_2$ under ambient conditions. A possible use for using the porphyrin system would be the \textit{in situ} generation of ClO$_2$, as a method for the purposes of drinking water disinfection and decontamination. This section describes preliminary experiments to assess this \textit{in situ} generation of ClO$_2$ in model polluted waters containing trichlorophenol, the NSAID diclofenac, phenol, and \textit{E. coli}.

\textbf{Trichlorophenol}

Trichlorophenol (TCP) was used as a model to study the efficacy of the manganese porphyrin/chlorite system in oxidizing phenolic pollutants in wastewater. TCP is easily oxidized by peroxidases and 1-electron oxidants to dichloroquinone (DCQ) (Figure 2), and the reaction can be easily followed by UV-Vis spectroscopy, owing to the latter’s intense and characteristic chromophore at 272 nm ($\varepsilon_{272} = 10^{4.16} \text{ M}^{-1} \text{ cm}^{-1}$).

\begin{figure}
\centering
\includegraphics[width=0.5\textwidth]{fig2}
\caption{Oxidation of trichlorophenol produces dichloroquinone.}
\end{figure}

To aqueous, unbuffered, and stirring solutions of ca. 0.75 mM trichlorophenol was added either authentic ClO$_2$ alone or first 5 $\mu$M MnTDMImP followed by NaClO$_2$. Various amounts of oxidants (ClO$_2$ or NaClO$_2$) were added in the range of 0.2 – 0.8 mM,
in order to assess the stoichiometry between oxidant and quinone product. The reactions were allowed to proceed until completion (ca. 30 minutes), diluted 1:20 into cuvettes, and analyzed by UV-Vis to determine the amount of quinone product formed. As can be seen in Figure 3, the reaction of authentic ClO$_2$ with trichlorophenol proceeds with a ca. 0.5:1 stoichiometry of product to oxidant, as would be expected from the oxidation of a phenol to a quinone by a 1-electron oxidant. The ClO$_2$–generating MnTDMImP/NaClO$_2$ system, on the other hand, proceeds with a 1:1 stoichiometry of quinone product to added oxidant.

![Graph showing stoichiometry of TCP consumption by ClO$_2$ and MnTDMImP/NaClO$_2$](image)

**Figure 3.** Consumption of TCP by either ClO$_2$ or MnTDMImP/NaClO$_2$

**Diclofenac**

The ability of the manganese porphyrin/chlorite ion system to oxidize the generic NSAID diclofenac (Figure 4) was tested. Diclofenac is known to readily react with ClO$_2$, although the products of such reaction are unknown.$^3$ Given its known reactivity with
ClO$_2$, we employed diclofenac as a test of the porphyrin/chlorite system against common pharmaceutical, wastewater contaminants. Solutions of the drug compound were reacted with authentic ClO$_2$, chlorite ion, oxone, a combination of chlorite ion and oxone (which produces ClO$_2$ \textit{in situ}), or chlorite ion in the presence of $\mu$M amounts of manganese catalyst. Diclofenac consumption was followed using LC-MS.

![Structure of diclofenac](image)

\textbf{Figure 4.} Structure of diclofenac (C$_{14}$H$_{11}$Cl$_2$NO$_2$)

Diclofenac is completely consumed by ClO$_2$, and we have further observed that the molecule is also easily decomposed by direct reaction with oxone and a combination of oxone and chlorite ion (Figure 5). ClO$_2$ is readily produced \textit{in situ} from the combination of oxone and chlorite ion. Chlorite ion alone, however, did not have a pronounced reaction with diclofenac. In spite of this, the presence of MnTDMImP and MnTM23PyPz lead to almost quantitative consumption of the compound by chlorite ion (Figure 6), in agreement with a catalytic conversion of chlorite ion to ClO$_2$ and the known sensitivity of diclofenac (and other phenolic and anilinic compounds) to ClO$_2$.\textsuperscript{6}
Figure 5. Consumption of diclofenac (10 mM) in 70:30 water:acetonitrile by 1 equiv added oxidant (oxone and/or sodium chlorite) with or without Mn catalyst (+/-10% error)
The products of the reaction of diclofenac with both authentic ClO₂ and the MnTDMImP/chlorite system were analyzed by reverse phase LC-MS (Figure 7). As not all products produced strong ions by LC-MS, the reaction was monitored using the instrument’s UV detector. An internal standard (2,6-dichloropyridine) was used to calibrate the chromatograms thus obtained. Five new peaks are observed in the LC-MS analysis. Products 1 and 2 appear in both reaction mixtures, 3 is only produced by the ClO₂ treatment, and 4 and 4’ are made only by the porphyrin/NaClO₂ system. These new compounds were isolated by semi-preparative HPLC and subjected to high-resolution ESI-MS analysis for identification (Table 1).
Figure 7. LCMS traces of the reaction mixture of 10 mM diclofenac with ClO₂ or 5 μM MnTDMImP and NaClO₂
<table>
<thead>
<tr>
<th>Product</th>
<th>m/z</th>
<th>Calc’d Formula</th>
<th>Δ m/z</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, 1’</td>
<td>312.0041</td>
<td>C_{14}H_{12}Cl_{2}NO_{3}</td>
<td>0.0153</td>
<td>Hydroxylation (+ 1 O)</td>
</tr>
<tr>
<td></td>
<td>310.0025</td>
<td>C_{14}H_{10}Cl_{2}NO_{3}</td>
<td>0.0013</td>
<td>Iminoquinone (+1 O, −2 H)</td>
</tr>
<tr>
<td>2</td>
<td>282.0089</td>
<td>C_{13}H_{10}Cl_{2}NO_{2}</td>
<td>0.0000</td>
<td>?? (−2 H, −1 C)</td>
</tr>
<tr>
<td>3</td>
<td>345.9778</td>
<td>C_{14}H_{11}Cl_{3}NO_{3}</td>
<td>0.0027</td>
<td>Hydroxylation/chlorination (+1 Cl, +1 O, −1 H)</td>
</tr>
<tr>
<td>4, 4’</td>
<td>589.0211</td>
<td>C_{28}H_{21}Cl_{4}N_{2}O_{4}</td>
<td>0.0044</td>
<td>dimer</td>
</tr>
<tr>
<td></td>
<td>591.0232</td>
<td>C_{28}H_{23}Cl_{4}N_{2}O_{4}</td>
<td>0.0180</td>
<td>?? (dimer + 2H)</td>
</tr>
</tbody>
</table>

**Table 1.** Observed m/z of products (M+H) of diclofenac oxidation by ClO$_2$ and MnTDMImP/NaClO$_2$

Product 3, which appears to have been both hydroxylated and chlorinated according to the MS analysis, was also characterized by $^1$H NMR (Figure 8). The spectrum shows a single major species. This compound has three aromatic resonances at $\delta$ 7.23 (d, $J = 8.0$ Hz, 2H), $\delta$ 6.77 (t, $J = 8.0$ Hz, 1H), and $\delta$ 6.71 (s, 2H) as well as an upfield signal at $\delta$ 3.69 (s, 2H). The aromatic doublet-triplet pattern suggests the dichlorinated ring in diclofenac has been untouched in this compound. The remaining aromatic ring must therefore be the site of disubstitution, and the remaining two hydrogens are presumably isochronous. The upfield shift at $\delta$ 3.69 suggests the methylene protons from the parent compound remain intact. A proposed structure for this species, as well as some other observed products, is given in Figure 9.
Figure 8. $^1$H NMR spectrum of 3 in CD$_3$OD
Figure 9. Structures of diclofenac and possible structures of products of chemical oxidation 1, 1', 3, and 4

Phenol

ClO₂ is known to react with phenol to afford quinones and, by downstream pathways involving the intermediacy of hypochlorous acid, chlorinated quinones. In our hands the reaction of 1 mM phenol with 1 equiv ClO₂ resulted in a net consumption of 86% of the starting phenol, as determined by LC-MS. Strikingly, in the presence of MnTDMImP, the consumption was quantitative (Table 2).

Chlorite ion by itself did not react appreciably with phenol under these conditions. However, in the presence of MnTDMImP, chlorite ion was rapidly consumed, along with 75% of the initial phenol, presumably due to the generation of ClO₂. Intriguingly, the addition of 10 equiv NaNO₂ to this reaction resulted in the formation of 2- and 4-nitrophenol in ca. 50% yield, as monitored by LC-MS.
Table 2. Oxidation of phenol by ClO$_2$ and MnTDMImP/NaClO$_2$

<table>
<thead>
<tr>
<th>Ox.</th>
<th>Cat</th>
<th>NaNO$_2$</th>
<th>% Cons.</th>
<th>$[4\text{-NO}_2]$ (uM)</th>
<th>$[2\text{-NO}_2]$ (uM)</th>
<th>2- NO$_2$:4- NO$_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>ClO$_2$</td>
<td>-</td>
<td>-</td>
<td>86%</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>+</td>
<td>50%</td>
<td>0</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>-</td>
<td>100%</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>+</td>
<td>100%</td>
<td>86</td>
<td>99</td>
<td>1.16 : 1</td>
</tr>
<tr>
<td>NaClO$_2$</td>
<td>-</td>
<td>-</td>
<td>0%</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>+</td>
<td>10%</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>-</td>
<td>75%</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>+</td>
<td>83%</td>
<td>211</td>
<td>271</td>
<td>1.28 : 1</td>
</tr>
</tbody>
</table>

Cat, 10 µM MnTDMImP, [phenol] = [Ox.] = 1 mM; [NaNO$_2$] = 10 mM

E. coli

Efficacy of *in situ* generation of ClO$_2$ against bacteria was investigated using a homogeneous, fluorometric cell-viability assay. In brief, the blue indicator dye resazurin is added to treated samples of cells. Viable and metabolically-active cells reduce blue resazurin to generate the red, fluorescent reporter molecule resorufin (Figure 10). Nonviable cells, conversely, do not reduce resazurin, and therefore the production of resorufin can be used to estimate the metabolic activity (and viability) of treated cells.

![Figure 10. Metabolism of resazurin to resorufin by viable cells](image-url)
The viability of *E. coli* to tolerate chlorite ion, authentic ClO$_2$, and MnTDMImP was assessed using the above-described resazurin method. *E. coli* were grown in sterile culture tubes in Miller’s LB, inoculated from single colonies. They were subsequently centrifuged, washed, and resuspended in pH 6.8 100 mM KPi. At high OD values (ca. 3), simply by eye one can see that *E. coli* exposed to either the catalyst or chlorite ion remained metabolically active, whereas the *E. coli* samples exposed to authentic ClO$_2$ or a co-treatment of MnTDMImP and chlorite ion were not active (Figure 11).

![Figure 11](image)

**Figure 11.** Annotated photograph showing viable (red) and non-viable (blue) *E. coli* cells treated with MnTDMImP, NaClO$_2$, ClO$_2$, and NaClO$_2$/MnTDMImP.

We screened over a range of catalyst, NaClO$_2$, and cell concentrations under these conditions (pH 6.8 100mM KPi) (Figure 12). The fluorescence of the red resazurin dye (indicative of *E. coli* viability) was measured using a microplate reader and normalized to similar measurements for untreated controls to calculate cell viability. At an OD$_{600}$ of 0.15 (ca. 1.2 x 10$^8$ cells mL$^{-1}$), authentic ClO$_2$ was effective at a concentration of 1.7 µM (ca. 0.1 ppm). To achieve the same efficacy with
MnTDMImP/NaClO$_2$ required a catalyst concentration of 5 µM and NaClO$_2$ concentration of 17 µM, an order of magnitude more than authentic ClO$_2$. At this OD, all catalyst concentrations up to 50 µM were tolerated by the cells, and NaClO$_2$ itself was only toxic at concentrations above 170 µM.

**Figure 12.** Percent viability of *E. coli* (OD600 = 0.15) treated with ClO$_2$ or MnTDMImP/NaClO$_2$
DISCUSSION

The catalytic generation of ClO₂ from NaClO₂ by manganese porphyrins under non-acidic, ambient conditions (Chapter 2) presented us with a practical alternative to ClO₂ generation methods currently employed in industry. In addition to the possibility of using this method to simply produce pure ClO₂ solutions (e.g. air stripping from a batch reactor, a solid-phase cartridge system) for further normal application downstream, we were intrigued by the possibility that an *in situ* method for ClO₂ generation based on the manganese porphyrin catalysts might be an even simpler method for using chlorine dioxide. This approach has been realized for cyanide remediation, affording the slightly less-toxic cyanate ion.¹⁵ A series of preliminary studies were carried out to assess the advantages and disadvantages of such an *in situ* method for both the oxidation of chemical water contaminants and the disinfection of model wastewaters.

“Recycling” of chlorite

The oxidation of trichlorophenol to afford dichloroquinone is a common method for assaying peroxidase (1-electron oxidation) activity. The results of our tests comparing the efficacy of ClO₂ versus the *in situ* ClO₂-producing NaClO₂/Mn system show that on a molar basis (of oxidant), the NaClO₂/Mn system results in more than *twice* as much oxidation of trichlorophenol (Figure 3). This is striking because, NaClO₂ (chlorine(III)) is more reduced than ClO₂ (chlorine(IV)). On a molar basis, NaClO₂ can ultimately “accept” fewer electrons:

\[
\begin{align*}
\text{ClO}_2^- + 4 \text{e}^- + 4 \text{H}^+ & \rightarrow \text{Cl}^- + 2 \text{H}_2\text{O} \\
\text{ClO}_2 + 5 \text{e}^- + 4 \text{H}^+ & \rightarrow \text{Cl}^- + 2 \text{H}_2\text{O}
\end{align*}
\]
This analysis, however, neglects to account for the fact that in both systems (authentic ClO\(_2\) and NaClO\(_2\)/Mn) the ultimate oxidant of trichlorophenol is ClO\(_2\), and ClO\(_2\) is rarely fully reduced to chloride ion when oxidizing organic matter. The oxidation of phenols by ClO\(_2\) is believed to proceed by first a 1-electron oxidation, affording a phenolic radical and ClO\(_2^-\). A number of pathways have been suggested for the downstream oxidation of the phenolic radical to quinones and other products.\(^6\)

As we have shown (Chapter 2), manganese porphyrins can produce ClO\(_2\) from ClO\(_2^-\), and so we recognize the fact that the “by-product” of normal ClO\(_2\) oxidation is actually a reactant for the manganese porphyrin system! The trichlorophenol results suggest that the \textit{in situ} NaClO\(_2\)/Mn method activates ClO\(_2^-\) to ClO\(_2\) that can carry out productive chemical oxidation. Then, the inorganic by-product of those reactions (i.e. ClO\(_2^-\)) can be “re-fed” to the manganese porphyrin to keep the oxidation going, resulting in more oxidation on a molar basis of oxidant added (Scheme 2).
Scheme 2. Proposed mechanism of substrate (S) oxidation by a manganese porphyrin/NaClO₂ system, highlighting the possibility of chlorite ion “recycling”

It is interesting that essentially the same experiment using diclofenac or phenol as a substrate did not demonstrate this dramatically increased efficacy of the porphyrin system (Figure 6 and Table 2). The reason for this is unclear, but one can speculate that this is due to such phenomena as “unproductive” turnovers of the catalyst (e.g. those that ultimately consume ClO₂ and produce chlorate) and differing rates of reactivity of ClO₂ with each substrate. However, it should be noted that the partial consumption of phenol by authentic ClO₂ was made *quantitative* when the manganese porphyrin was present (Table 2). This latter result further corroborates the idea that the porphyrin “recycles” the ClO₂⁻ by-products of ClO₂ chemical oxidation.
Reduction in chlorination

One striking result of the oxidation of diclofenac by the NaClO₂/Mn system is that it did not generate the chlorinated by-product 3 that was observed using authentic ClO₂. Although ClO₂ is heralded as being “less chlorinating” than traditional chlorine (Cl₂/HOCl) treatments, it is known that ClO₂ can in fact produce a small amount of chlorinated species, especially upon reaction with some phenols.\(^6\) This is understood to be a result of HOCl production (Scheme 3):

![Scheme 3](image)

**Scheme 3.** Oxidation of phenols by ClO₂ can afford HOCl

We have shown (Chapter 2) that the manganese porphyrin catalysts and HOCl react rapidly (\(k_{\text{app}} > 10^4 \text{ M}^{-1} \text{ s}^{-1}\)). Therefore, we interpret these results to say that any HOCl generated by ClO₂ oxidation of diclofenac is quickly scavenged by the porphyrin, thus reducing any downstream chlorination reactions.

Another factor might also be the speed of the differing methods of ClO₂ treatment. In the case of authentic ClO₂, the ClO₂ reagent is added as a single bolus, allowing for both the generation of diclofenac radicals and subsequent capture of those
radicals by ClO₂ present in large relative amount (Scheme 3). With the NaClO₂/Mn system, however, the ClO₂ reagent is essentially being “metered in” to the reaction. Therefore, generated diclofenac radicals do not have as much opportunity to encounter an additional ClO₂ molecule (and generate HOCl). As seen in Figure 7, the NaClO₂/Mn treatment resulted in the formation of dimeric products not observed in the authentic ClO₂ treatment, consistent with relatively long-lived diclofenac radicals that have time to dimerize before they encounter more ClO₂.

**Efficacy of disinfection**

As ClO₂ can be used as a disinfectant in addition to chemical oxidation, we wanted to explore the potential benefits of using *in situ* ClO₂-generation by the NaClO₂/Mn system as a biocide. Figure 11 shows clearly that the porphyrin catalyst is not toxic to the *E. coli* cells. This is perhaps unsurprising, as iron and manganese porphyrins are actually potential therapeutics and antioxidants with beneficial pharmacological properties that are currently under study.¹⁶-¹⁷ NaClO₂ was also non-harmful to the cells but only at sub-mM concentrations. With low cell O.D. values and mM amounts of NaClO₂, cell death was observed (Figure 11). Together, however, the manganese porphyrin and NaClO₂ together indeed effective at killing *E. coli*.

However, unlike the chemical oxidation examples discussed above, here the *in situ* NaClO₂/Mn system was not as effective as authentic ClO₂. Complete *E. coli* disinfection required only 0.1 ppm authentic ClO₂, whereas the catalytic porphyrin system required at least an order of magnitude more NaClO₂. Perhaps this is another
manifestation of the differing rates of ClO₂ addition, as the authentic ClO₂ was added all at once, while the porphyrin system slowly generated ClO₂ over time.

The concentration of catalyst was also found to have an unusual effect, as 5 µM (but not 50 or 0.5 µM) was found to be the optimal catalyst concentration. We speculate that this could be due to slow ClO₂-forming kinetics (0.5 µM catalyst) or fast ClO₂-consuming kinetics (50 µM catalyst). Clearly, there is an optimal balance of NaClO₂ and catalyst concentration that would need to be carefully engineered in a practical device or water-treatment method.
CONCLUSIONS

The catalytic generation of chlorine dioxide (ClO$_2$) by manganese porphyrins has could be used as the basis for an alternative commercial method for ClO$_2$ production. Additionally, we have investigated the potential of using this catalytic system to generate ClO$_2$ \textit{in situ} in the context of water treatment. The \textit{in situ} generation of ClO$_2$ is shown to be as or more effective than exogenously-added ClO$_2$ in oxidizing chemical contaminants like phenols. Moreover, the \textit{in situ} generation of ClO$_2$ produces even fewer organohalogen by-products than traditional chlorine dioxide treatment. This method was also effective at killing \textit{E. coli} \textit{in situ}, but not as effective as authentic ClO$_2$. Regardless, the preliminary work reported here suggests that the manganese porphyrin-catalyzed \textit{in situ} ClO$_2$ generation could be effectively implemented in water treatment. Not only does this method of ClO$_2$ treatment avoid the need for reactor systems, it also prevents the need to handle ClO$_2$ gas or solutions and presents an opportunity to bring ClO$_2$ water treatment to remote geographic areas.
ACKNOWLEDGMENTS

The *E. coli* cell viability study was carried out in collaboration with Patrick Kates.
EXPERIMENTAL

Reagents

MnTDMImP, MnTM23PyPz, recrystallized NaClO₂, and authentic ClO₂ were prepared as described in Chapter 2. All other reagents were obtained from Sigma-Aldrich and used as received.

Trichlorophenol

To ca 0.8 mM trichlorophenol was added either: 0 – 1 equiv ClO₂; or 0 – 1 equiv NaClO₂ as a control; or first 5 µM MnTDMImP followed by 0 – 1 equiv NaClO₂. The reactions were allowed to proceed for ca. 1 hr, after which they were diluted 1:20 and analyzed by UV-Vis (Dichloroquinone, ε₂₇₂ = 14,000 M⁻¹ cm⁻¹).

Diclofenac

To 20 mL of 2mM diclofenac (stirring) was added either: 1 equiv ClO₂; or MnTDMImP (10 µM) and then 1 equiv NaClO₂. This reaction mixture was assayed directly by LC/MS after ca. 1 hr. For isolation of products, water was removed under vacuum and the remaining solids (buffer, catalyst, products) were redissolved in DMSO for separation by preparative LC. Collected fractions were evaporated under vacuum and then analyzed by NMR.

Phenol

To 1mM stirring phenol (pH 6.8 100 mM KPi) was added a combination of: 0 or 10 µM MnTDMImP; 1 equiv ClO₂ or NaClO₂; 0 or 10 mM NaNO₂ (8 total combinations).
Reactions were allowed to proceed for ca. 1 hr before being directly analyzed by LC/MS (0.1 mM 2,6-dichloropyridine internal standard).

_E. coli Cell Viability_

_E. coli_ (TOP10) were grown overnight in Miller’s LB Broth in 15 mL sterile culture tubes, inoculated from single colonies. For the cell viability assay, cultures were centrifuged, washed with 100 mM KPi (pH 6.8), and resuspended in 100 mM KPi (pH 6.8). Cell viability studies were carried out using the Promega CellTiter-Blue® Cell Viability Assay. In general, 50 µL of buffered cells were placed in a 96-well microplate. To each well was added more buffer, catalyst, NaClO₂, or ClO₂ (as appropriate) so that the final reaction concentration was 100 µL. The plate was shaken at room temperature for 20 minutes, after which 20 µL resazurin test solution (CelTiter-Blue® Reagent) was added to each well. The plate was then shaken and incubated for 1 h at 37 °C, and analyzed for fluorescence (560 ex, 590 em).

_Instrumentation_

UV-Vis spectroscopic measurements were taken using a Hewlett-Packard 8453 diode array spectrophotometer. LC-MS of the diclofenac and phenol oxidation studies was carried out using a Hewlett-Packard LC/MS system (HP 1100 MSD) equipped with a Phenomenex Luna C18 column (3 µm particle size, 100 Å pore size, 50 x 4.6 mm) running a solvent gradient from 97% water/3% acetonitrile/0.1% formic acid to 90% acetonitrile/10% water/0.1% formic acid. Preparative LC for the diclofenac studies was accomplished using a Varian PrepStar 218 semi-preparatory system (two Varian ProStar
solvent delivery pumps, ProStar 310 UV-Vis detector, Waters Fraction Collector II) equipped with a Sunfire Prep C18 column (5 µm particle size, 19 x 100 mm). The cell viability studies were monitored using a Varioskan fluorescence microplate reader (Thermo Electron Corporation). Further characterization of diclofenac oxidation products was done on an Agilent 6210 Time-of-Flight LC/MS and a Bruker 500 MHz NMR.
REFERENCES


CHAPTER IV

CHARACTERIZATION OF MANGANESE PORPHYRINS BY X-RAY ABSORPTION SPECTROSCOPY
ABSTRACT

Trans-dioxomanganese(V), oxomanganese(IV), and oxohydroxomanganese(IV) porphyrins were prepared and submitted for analysis by X-ray Absorption Spectroscopy (XAS) and Extended X-ray Absorption Fine Structure (EXAFS) analysis. The trans-dioxoMnV study revealed that the complex has two, short Mn-O bonds of 1.68 Å consistent with predicted orbital bonding arrangements and an analysis of the Mn-O vibration using Badger’s Rule. The manganese(IV) samples, however, behaved anomalously. XAS revealed that the oxomanganese(IV) sample submitted was contaminated by an apparent manganese(V) impurity, while the 6-coordinate oxohydroxoMnIV EXAFS suggested a Mn-O bond that is much shorter (R = 1.63 Å) than anticipated from its vibrational spectroscopy and compared to other oxomanganese(IV) complexes.
INTRODUCTION

Synthetic manganese porphyrin complexes have been chiefly developed and used as models of the iron porphyrin active site of cytochrome P450.\textsuperscript{1} Cytochrome P450 activates molecular oxygen (O\textsubscript{2}) in order to generate a high-valent oxoiron species that is understood to be the active oxidant in P450-catalyzed oxygen transfers.\textsuperscript{1} Analogous oxomanganese porphyrins have been implicated as reactive intermediates in manganese porphyrin-catalyzed oxidation reactions, including O-atom transfers, H-atom abstractions, and peroxidase-type reactivity.\textsuperscript{2-7} An understanding of the factors that govern the reactivity of oxomanganese(IV) and –(V) porphyrin complexes has not only provided a deeper understanding of metal-oxo complexes but also lead to the development of practical catalysts for hydrocarbon functionalization and the generation of the disinfectant chlorine dioxide (described in Chapter 2).\textsuperscript{2-3,5} Additionally, although manganese porphyrins do not exist in nature, high-valent oxomanganese species have been implicated as active intermediates in the water-splitting chemistry of Photosystem II;\textsuperscript{8-9} therefore, the study of oxomanganese porphyrin complexes, their structure, and their reactivity, are of even wider interest.

Manganese porphyrins catalyze oxygen atom transfers from oxidants such as iodosylbenzene, acyl hydroperoxides, and hypochlorite to a variety of substrates including, most notably, unactivated hydrocarbons.\textsuperscript{4-6} Both oxomanganese(IV) and –(V) complexes have been implicated as reactive species in such catalysis,\textsuperscript{4} and oxomanganese(IV) porphyrins have been prepared, isolated, and thoroughly characterized.\textsuperscript{10-12} The more-reactive oxomanganese(V) porphyrins remained elusive for many years; however, they could be isolated at low temperatures\textsuperscript{4} and observed using
techniques such as rapid-mixing stopped flow spectroscopy.\textsuperscript{13} The observation that electron-withdrawing porphyrin ligands could stabilize these species by affecting their electronic structure\textsuperscript{14} laid the groundwork for their eventual isolation as stable trans-dioxomanganese(V) porphyrins.\textsuperscript{15}

A key to the characterization of both oxomanganese(IV) and (V) species was the use of vibrational spectroscopic techniques: infrared and resonance Raman.\textsuperscript{11,15} The latter technique is particularly useful for metalloporphyrins, as the excitation of the porphyrin chromophore normally results in an enhancement of the metal-ligand vibrational modes. Oxomanganese(IV) porphyrins were characterized with an unusually weak Mn-O stretch ($\nu_{\text{obs}} = 754 \text{ cm}^{-1}$) relative to neighboring oxovanadium(IV) ($\nu_{\text{obs}} = 1007 \text{ cm}^{-1}$), oxochromium(IV) ($\nu_{\text{obs}} = 1025 \text{ cm}^{-1}$) and oxoiron(IV) ($\nu_{\text{obs}} = 843 \text{ cm}^{-1}$) porphyrins.\textsuperscript{11} This trend has been attributed to a special stability of the high-spin $d^3$ configuration of manganese(IV) due to a half-filled $t_{2g}$ orbital set. Therefore, not one but two of the three $d$ electrons in oxoMn$^{IV}$ reside in $d_{\pi}^{*}$ orbitals, giving the manganese-oxo bond a formal bond order of 2 and not 2.5 (Figure 1).
Chapter IV

Figure 1. a) Schematic of \( \pi \)-interactions of \( O^{2-} \) p orbitals and metal (M) d orbitals; b) Qualitative splitting diagrams of metal \( t_{2g} \) d orbitals and calculated metal-oxo bond order

Oxomanganese(V) porphyrins, on the other hand, have been characterized as diamagnetic \( d^2 \) species,\(^4\),\(^14\)-\(^15\) suggesting that the \( d_{\pi^*} \) orbitals of the Mn ion are vacant and therefore predicting the manganese oxo bond is formally a triple bond (Bond Order = 3).\(^16\) Five-coordinate oxomanganese(V) corrolazines have been characterized with very strong and short Mn-O bonds (\( \nu_{\text{obs}} = 979 \text{ cm}^{-1} \) \( R = 1.56 \text{ Å} \)) that are consistent with this prediction.\(^17\) Oxomanganese(V) porphyrins, however, exist as 6-coordinate species, and the nature of this 6th ligand has profound effects on the reactivity of the oxo complex. Trans-dioxomanganese(V) porphyrins have been elegantly characterized by their IR-active, asymmetric (805 cm\(^{-1}\)) and Raman-active symmetric (743 cm\(^{-1}\)) vibrational modes, and the Mn-O vibrations were found to be insensitive to the electron-withdrawing or –donating properties of the porphyrin macrocycle.\(^15\) Deconvolution of the linear
triatomic O=Mn\textsuperscript{V}=O vibrational stretches observed, the hypothetical, unobservable O=Mn\textsuperscript{V} diatomic stretch can be calculated to be 788 cm\textsuperscript{-1}.

The \textit{trans}-dioxoMn\textsuperscript{V} arrangement has been experimentally observed and computationally predicted to be a ground state d\textsuperscript{2} singlet\textsuperscript{14-15, 18-19} The bonding arrangement is believed to consist of two, equivalent Mn-O double bonds resulting from \(\pi\)-donation from each oxygen atom into the vacant d\textsubscript{\pi} orbitals of manganese(V). Protonation of one of the oxo groups, transforming it formally into a hydroxo or aqua ligand, is predicted to shorten the remaining Mn-O bond, consistent with the reduced \(\pi\)-donating capabilities of these protonated ligands\textsuperscript{19} Most interestingly, perhaps, this predicted shortening of the Mn-O bond occurs concomitantly with a dramatic increase in reactivity\textsuperscript{18-19} This observation has been theoretically explored and attributed to a lowering in energy of triplet and quintet excited electronic states for the protonated species. The accessibility of these high-spin excited states has been further implicated in the reactivity of oxoMn\textsuperscript{V} porphyrins towards both O-atom transfer to bromide ion and hydrogen atom abstractions\textsuperscript{18-19}

In this chapter, we present the results of an X-ray Absorption Spectroscopy (XAS) and Extended X-ray Absorption Fine Structure (EXAFS) study for two, known oxomanganese(IV) porphyrin complexes and the \textit{trans}-dioxomanganese(V) porphyrin complex. These results are then discussed in the context of other characterizations of these complexes as well as that of other oxomanganese species in general.
RESULTS

A related family of known manganese porphyrins were prepared and analyzed by XAS and EXAFS spectroscopy (Scheme 1). The array of complexes include \textit{trans}-dioxoMn\textsuperscript{V},\textsuperscript{15} oxoMn\textsuperscript{IV} and oxohydroxoMn\textsuperscript{IV},\textsuperscript{10} and \textit{trans}-dihydroxoMn\textsuperscript{III}.\textsuperscript{11} The tetra-pentafluorophenyl porphyrin (TPFPP) ligand was chosen for all complexes, as this ligand was found to best stabilize the \textit{trans}-dioxoMn\textsuperscript{V} species. Attempts to similarly characterize the cationic manganese tetra-\textit{N},\textit{N}-dimethyl-imidazolium porphyrin (MnTDMImP, see Chapters 2 and 3) by XAS failed due to facile photoreduction by the X-ray source during data collection.

\[ \text{Scheme 1. Preparation of manganese porphyrins species studied in this chapter} \]

\textit{Trans}-hydroxomanganese(III) was prepared by dissolving manganese(III) TPFPP (chloride salt) in acetonitrile containing 40 mM tetrabutylammonium hydroxide. \textit{Trans}-dioxomanganese(V) was generated by the addition of 1 equiv \textit{m}-chloroperoxybenzoic acid (mCPBA) to the \textit{trans}-hydroxoMn\textsuperscript{III}. The negative-mode, electrospray ionization mass spectra of these two known complexes is shown in Figure 2 and Figure 3, below.
Figure 2. Negative mode, low-resolution ESI-MS of trans-dihydroxoMn$^{III}$ TPFPP

(Calculated m/z for C$_{44}$H$_{10}$F$_{20}$MnN$_{4}$O$_{2}^{-}$: 1061.0)

Figure 3. Negative mode, low-resolution ESI-MS of trans-dioxoMn$^{V}$ TPFPP (Calculated m/z for C$_{44}$H$_{8}$F$_{20}$MnN$_{4}$O$_{2}^{-}$: 1059.0)
Oxomanganese(IV) and oxohydroxomanganese(IV) were prepared analogously to the reported conditions\textsuperscript{10} but using acetonitrile in place of dichloromethane.\textsuperscript{*} The Mn\textsuperscript{IV} complexes were notably unstable at room temperature, decaying completely to a Mn\textsuperscript{III} species in under 10 minutes. Therefore, all manipulations of the Mn\textsuperscript{IV} complex, including the filling of the XAS sample cells, were done at -30 °C. The UV-Vis spectra of the Mn\textsuperscript{IV} complexes were checked and verified against the known spectra for these species (Figure 4). Additionally, these complexes were analyzed by EPR to confirm their previously determined d\textsuperscript{3}, Mn\textsuperscript{IV} oxidation state (Figure 5, Figure 6).

* XAS and EXAFS require that both a solvent capable of forming a glass and also that chlorinated solvents be avoided, as the chlorine atoms lead to scattering
Figure 5. EPR spectrum of oxoMn$^{IV}$ TPFPP
Figure 6. EPR spectrum of oxohydroxoMn$^{IV}$ TPFPP
All samples were ca. 1 mM porphyrin concentration and immediately frozen in liquid nitrogen after being prepared. The manganese(III) and -(V) samples were prepared on-site at the Stanford Linear Accelerator Center (SLAC) in Menlo Park, CA, immediately frozen in liquid nitrogen, and analyzed within an hour of preparation. The oxomanganese(IV) samples were prepared at Princeton University, immediately frozen in liquid nitrogen, and shipped overnight to SLAC using a liquid nitrogen dry shipping dewar. Data collection and analysis of all samples were carried out by Serena DeBeer.

The position of the so-called “rising edge” in XAS analysis is indicative of oxidation state, with more oxidizing metals blueshifting the edge to higher energy. The oxidation state assignments of the known trans-dihydroxoMn\textsuperscript{III}, oxoaquaMn\textsuperscript{IV}, and trans-dioxoMn\textsuperscript{V} complexes are in agreement with the XAS analysis. As seen in Figure 7, the energy of the rising edge increases with increasing oxidation state, corroborating the assignment of these three complexes as manganese(III), -(IV), and -(V).

Additionally, the pre-edge feature in an XAS spectrum is indicative of the complex’s local environment, as metal centers with an asymmetric local symmetry give rise to an intense pre-edge absorption. This pre-edge feature is quite apparent for the oxohydroxoMn\textsuperscript{IV} complex (Figure 8), consistent with its axially-distorted characterization, especially compared to the featureless (centrosymmetric) trans-hydroxoMn\textsuperscript{III} and trans-dioxoMn\textsuperscript{V}.

The sample prepared as the 5-coordinate oxoMn\textsuperscript{IV} did not fit these expected trends, having both a rising edge similar to that of the authentic Mn\textsuperscript{V} porphyrin and an insignificant pre-edge feature.
Figure 7. XAS spectra of samples of \textit{trans}-dihydroxoMn$^{\text{III}}$, oxo-Mn$^{\text{IV}}$, oxo-hydroxoMn$^{\text{IV}}$, and \textit{trans}-dioxoMn$^{\text{V}}$ TPFPP.
Figure 8. Pre-edge features in the XAS spectra of samples of trans-dihydroxoMn$^{\text{III}}$, oxo-Mn$^{\text{IV}}$, oxo-hydroxoMn$^{\text{IV}}$, and trans-dioxoMn$^{\text{V}}$ TPFPP
Analysis of the post-absorption fine structure provide further information on the local coordination sphere, specifically bond distances to ligated atoms. The results of an EXAFS analysis of the above data are collected in Table 1. The first coordination sphere of the trans-hydroxoMn$^{III}$ complex consists of six equal Mn-O/N bonds ($R = 1.99$ Å), while the oxohydroxoMn$^{IV}$ complex has one shorted Mn-O bond ($R = 1.63$ Å) relative to the other five ($R = 2.01$ Å). In the trans-dioxoMn$^{V}$ complex, four Mn-N/O bonds remain unchanged from the parent Mn$^{III}$ complex ($R = 2.01$ Å) and two bonds are equivalently shortened ($R = 1.68$ Å). Again, the oxoMn$^{IV}$ is an anomaly, with its EXAFS analysis suggesting six equivalent bonds ($R = 2.01$ Å).

<table>
<thead>
<tr>
<th></th>
<th># of Mn-O/N bonds (length / Å)</th>
</tr>
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<tbody>
<tr>
<td>trans-hydroxoMn$^{III}$</td>
<td>$6$ (1.99)</td>
</tr>
<tr>
<td>oxoMn$^{IV}$</td>
<td>$6$ (2.01)</td>
</tr>
<tr>
<td>oxohydroxoMn$^{IV}$</td>
<td>$1$ (1.63), $5$ (2.01)</td>
</tr>
<tr>
<td>trans-dioxoMn$^{V}$</td>
<td>$4$ (2.01), $2$ (1.68)</td>
</tr>
</tbody>
</table>

Table 1. Collected EXAFS Mn-O/N bond distances for the first coordination sphere of Mn TPFPP complexes
Figure 9. EXAFS data and fit for trans-dihydroxoMn$^{III}$ TPFPP
Figure 10. Fourier transform of EXAFS data and fit for \textit{trans}-dihydroxoMn$^{III}$ TPFPP
Figure 11. EXAFS data and fit for trans-dioxoMn$^V$ TPFPP
Figure 12. EXAFS data and fit for trans-dioxoMn$^V$ TPFPP
DISCUSSION

Four known manganese porphyrin complexes in different oxidation and protonation states were characterized by XAS and EXAFS spectroscopy: trans-dihydroxoMn$^{III}$, oxoMn$^{IV}$, oxohydroxoMn$^{IV}$, and trans-dioxoMn$^{V}$. The higher-valent compounds are of much interest for several reasons: their role in catalytic substrate oxidations,$^{2-6}$ as structural models of the oxoiron porphyrins that are generated by heme-containing monoxygenases,$^{1}$ and for the involvement of high-valent manganese species in oxygen evolution by Photosystem II.$^{8-9}$ In particular, the recent discovery of stable, isolable trans-dioxomanganese(V) porphyrins (compared to their quite reactive, protonated counterparts) has suggested the ability to control and tune this otherwise potent oxidizing species.$^{15}$ A summary of the data collected in this study is depicted graphically in Scheme 2.

\[
\text{Scheme 2. Bond lengths and oxidation states measured for the four manganese porphyrin complexes in this study.}
\]
The \textit{trans}-dihydroxoMn$^\text{III}$, characterized by mass spectroscopy and originally assigned by Spiro, \textit{et al.},$^{11}$ shows six equal Mn-O/N bonds. Presumably, four of these arise from ligation to the porphyrin and the other two to the axial hydroxide ligands, placing the central Mn$^\text{III}$ ion in a pseudo-octahedral environment.

Oxidation of the \textit{trans}-dihydroxoMn$^\text{III}$ porphyrin with mCPBA or H$_2$O$_2$ has been shown to afford a diamagnetic manganese complex, assigned as a \textit{trans}-dioxoMn$^\text{V}$ from the observation of both IR-active $\nu_{\text{assym}}$ and Raman-active $\nu_{\text{sym}}$ Mn-O stretches.$^{15}$ Mass spectroscopy (Figure 3) suggests the loss of two hydrogen atoms in this complex relative to the starting HO-Mn$^\text{III}$-OH (Figure 2). XAS data further corroborates the oxidation state assignment (Figure 7), and the EXAFS analysis reveals two shortened Mn-O bonds relative to the starting complex. The shortening of the two Mn-O bonds might be expected simply from the increased pi-donating ability of the oxo ligands, but is further supported by DFT predictions for the O=Mn bond lengths in singlet \textit{trans}-dioxoMn$^\text{V}$.\textsuperscript{19} Further, these bonds are longer than true manganese-oxygen triple bonds, consistent with the expected bond order of 2 for a \textit{trans}-dioxo species. A manganese porphyrin species with the same EXAFS-measured bond length (1.68 Å) was originally reported as a 5-coordinate, oxomanganese(V) species\textsuperscript{20}; however, a reanalysis of that data found that the original XAS and EXAFS results from that report are better fit by a \textit{trans}-dioxo bonding geometry.$^{21}$

Data collected for the \textit{trans}-dioxoMn$^\text{V}$ complex can be further subjected to an analysis using Badger’s Rule, which predicts an inverse correlation between the bond length and cubed vibrational force constant for a given diatomic oscillator.$^{22}$ A plot of terminal Mn-O bonds, and their stretching force constants, spanning five oxidation states
is given in Figure 13. The measured Mn-O bond length and calculated, hypothetical O-Mn vibration for \textit{trans}-dioxoMn$^V$ clearly fit the expected trend for terminal oxomanganese species. The data used to construct this plot are collected in Table 2.

\textbf{Figure 13.} Badger plot of Mn-O bond length versus $1/F^{1/3}$ for known terminal Mn-O bonds (black boxes) and the oxomanganese compounds studied herein (blue circles)
Table 2. Collected data for manganese-oxo bonds in a variety of oxidation states

<table>
<thead>
<tr>
<th>[O=Mn\textsuperscript{V}]\textsuperscript{3+}</th>
<th>ν (cm\textsuperscript{-1})</th>
<th>F (mdynes/Å)</th>
<th>R (Å)</th>
<th>Ref.</th>
</tr>
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<tbody>
<tr>
<td>[O=Mn\textsuperscript{V}]\textsuperscript{3+}</td>
<td>979</td>
<td>7.00</td>
<td>1.56</td>
<td>\textsuperscript{17}</td>
</tr>
<tr>
<td>Mn\textsuperscript{VII}O\textsubscript{4}\textsuperscript{-}</td>
<td>838</td>
<td>5.13</td>
<td>1.629</td>
<td>\textsuperscript{24}</td>
</tr>
<tr>
<td>[O=Mn\textsuperscript{IV}-OH]\textsuperscript{+}</td>
<td>712</td>
<td>3.70</td>
<td>1.630</td>
<td>\textit{a, 11}</td>
</tr>
<tr>
<td>Mn\textsuperscript{VI}O\textsubscript{4}\textsuperscript{2-}</td>
<td>814</td>
<td>4.84</td>
<td>1.659</td>
<td>\textsuperscript{25}</td>
</tr>
<tr>
<td>[O=Mn\textsuperscript{V}=O]\textsuperscript{+}</td>
<td>788\textsuperscript{c}</td>
<td>4.47\textsuperscript{c}</td>
<td>1.680</td>
<td>\textit{a, 15}</td>
</tr>
<tr>
<td>[O=Mn\textsuperscript{IV}]\textsuperscript{2+}</td>
<td>737</td>
<td>3.97</td>
<td>1.706</td>
<td>\textsuperscript{26}</td>
</tr>
<tr>
<td>[O-Mn\textsuperscript{III}]\textsuperscript{2+}</td>
<td>700</td>
<td>3.58</td>
<td>1.771</td>
<td>\textsuperscript{27}</td>
</tr>
<tr>
<td>[O=Mn\textsuperscript{IV}]\textsuperscript{2+}</td>
<td>754</td>
<td>4.15</td>
<td>2.01\textsuperscript{a}, 1.70\textsuperscript{b}</td>
<td>\textit{a, 11}</td>
</tr>
</tbody>
</table>

\(a\), this work; \(b\), reference \textsuperscript{12}  
\(c\), calculated from \(\nu_{\text{sym}} = \frac{1}{2\pi c} \sqrt{\frac{F + k}{m_0}}\) and \(\nu_{\text{asym}} = \frac{1}{2\pi c} \sqrt{(F - k) \frac{m_{Mn} + 2m_O}{m_0m_{Mn}}}\)

Although the \textit{trans}-dioxoMn\textsuperscript{V} XAS and EXAFS analysis is consistent with expected trends and previously-acquired data, both “Mn\textsuperscript{IV}” species studied in this chapter remain anomalous. The presumed, 5-coordinate oxoMn\textsuperscript{IV} species is most problematic, as its EXAFS-measured Mn-O bond length (2.01 Å) is much longer than would be predicted by the previously measured Mn-O stretch (\(\nu_{\text{obs}} = 754\) cm\textsuperscript{-1}, \(R_{\text{pred}} = 1.70\) Å). Correspondingly, a Mn-O bond length of 1.70 Å has been reported for oxoMn\textsuperscript{IV} tetra-(\(\alpha\), \(\alpha\), \(\alpha\), \(\alpha\)-pivalainidophenyl) porphyrin.\textsuperscript{12} In addition to the bond length discrepancy for the “oxoMn\textsuperscript{IV}” complex, the XAS oxidation state assignment does not agree with that determined by EPR. The EPR of a sample prepared analogously to that sent for XAS
analysis showed a characteristic $d^3$ Mn$^{IV}$ spectra, whereas he XAS analysis of “oxoMn$^{IV}$” assigns it more properly as a Mn$^V$ species! Clearly, the XAS/EXAFS, EPR, and resonance Raman data are not in agreement for this sample. Groves and Stern reported the generation of a Mn$^V$ complex by the same method used to prepare oxoMn$^{IV}$, although at much lower temperature, and perhaps this former species contaminated the XAS samples.

The oxohydroxoMn$^{IV}$ XAS is much more consistent with the oxidation state assignment of +4, as measured by EPR. However, the EXAFS analysis of the Mn-O bond ($R = 1.63 \, \text{Å}$) would predict a diatomic vibration of $\nu_{\text{pred}} = 844 \, \text{cm}^{-1}$ ($F = 5.21 \, \text{mdyn/Å}$), which does not agree with the Mn-O stretch observed by Stern for the same compound ($\nu_{\text{obs}} = 712 \, \text{cm}^{-1}$, $R_{\text{pred}} = 1.75 \, \text{Å}$) (Figure 13). Although the EXAFS-measured Mn-O bond length in oxohydroxoMn$^{IV}$ is uncharacteristically short for a terminal O=Mn$^{IV}$, it is possible that the EXAFS data does not fit the Badger Rule plot because the “wrong” Mn-O stretch is being plotted. The trans-dioxoMn$^V$ Mn-O vibrations, more properly described by a linear triatomic oscillator, had to be first deconvoluted to a hypothetical diatomic oscillator$^{15}$ in order to be analyzed by Bader’s Rule (see caption, Table 2). Similarly, the Mn-O stretch in oxohydroxoMn$^{IV}$ has been shown to be highly coupled to the trans-axial Mn-OH stretch,$^{11}$ and therefore it may be improper to use the directly-observed Mn-O stretch in this analysis.

Truly, however, the simplest explanation for the discrepancy observed for oxohydroxoMn$^{IV}$ may arise from the fact that these unstable compounds underwent some form of decomposition between being synthesized and analyzed. Unlike the Mn$^{III}$ and Mn$^V$ samples that were prepared immediately prior to analysis (and gave results
consistent with expectations), the fragile Mn$^{IV}$ species were subject to a much longer period between synthesis and analysis as they were prepared at Princeton and cryo-shipped to SLAC. Additionally, although these complexes were checked by EPR and UV-Vis prior to being submitted for XAS, EPR would not detect Mn$^V$ contamination and UV-Vis characterization (using diluted samples) may obscure subtle effects of concentration or dilution. Acetonitrile was found to either not ligate oxoMn$^{IV}$ or not have an appreciable effect upon binding oxoMn$^{IV}$, and therefore the slightly different synthetic procedure used for oxoMn$^{IV}$ should not be considered much of a concern. However, one is ultimately cautioned to not “over-interpret” these curious data until a further study is attempted.

Still, it remains a tantalizing curiosity that the so-called “oxohydroxoMn$^{IV}$” sample suggested the presence of a manganese(V) porphyrin species with six equivalent, long Mn-O/N bonds (2.01 Å). What is this highly oxidized species? Manganese(V) porphyrins are rare and normally exist only with strong pi-donating oxo or nitrido ligands that have much shorter bond lengths than those observed here.$^{15,28}$ Therefore, even should the anomalous EXAFS data reported here be a result of contamination, decomposition, or some other error, it may at least provide evidence that other isolable manganese(V) porphyrins exist and should be considered future research targets.
CONCLUSIONS

The XAS and EXAFS analysis of trans-dioxomanganese(V) porphyrins shows two equivalent, short (R = 1.68 Å) Mn-O bonds consistent with its proposed formulation, vibrational data, electronic structure, and predicted bonding arrangement. Indeed, these Mn-O bonds are longer than true manganese-oxygen triple bonds but reflect the increased pi-donor character of the oxo ligands. Similar attempts to study oxomanganese(IV) porphyrins by XAS and EXAFS provided data that conflicts with previous characterizations of these complexes. Notably, the EXAFS-measured Mn-O bond length is shorter than predicted. More strangely, XAS suggests that the sample intended to be analyzed as a “5-coordinate oxomanganese(IV)” was in actuality a hexacoordinate MnV with all long Mn-O/N bonds (R = 2.01 Å). Although this latter data suggests experimental issues with sample handling, the curious manganese(V) species that was accidentally analyzed could be the focus of a future study to isolate rare manganese(V) porphyrins.
ACKNOWLEDGMENTS

The XAS and EXAFS spectra were collected and computationally analyzed by Prof. Serena DeBeer at the Stanford Linear Accelerator Center. EPR measurements were collected under the guidance of Katharine A. Prokop and Prof. David P. Goldberg at Johns Hopkins University. We are also grateful to Prof. Frank Neese and Michael Röemelt for freely sharing Mr. Röemelt’s master’s thesis with us.
EXPERIMENTAL

Reagents

Mn$^{III}$TPFPP chloride was obtained from Mid Century. Tetra-butyl ammonium hydroxide was obtained from Sigma Aldrich. Recrystallized meta-chloroperoxybenzoic acid (mCPBA) was inherited from Dr. Ning Jin.

Preparation of manganese porphyrin samples

Trans-dihydroxomanganese(III) was prepared by dissolving the MnTPFPP complex (1 mM) in 40 mM TBA(OH) in acetonitrile. Trans-dioxomanganese(V) was prepared by adding 1 equiv mCPBA to the dihydroxomanganese(III) complex. The manganese(IV) species were prepared in a manner similar to that reported by Stern. Oxomanganese(IV) was prepared by adding 1.2 equiv mCPBA to a cold (-30 °C), stirring solution of Mn$^{III}$TPFPP and 1 equiv TBA(OH) in acetonitrile. Oxohydroxomanganese(IV) was prepared analogously to oxomanganese(IV), above, but here 10 equiv TBA(OH) were added after the oxidation was complete. The oxidation reactions in all cases were monitored by UV-Vis.

XAS Collection and Analysis

XAS data were recorded at the Stanford Synchrotron Radiation Lightsource (SSRL) on focused beam line 9-3. All XAS samples (~1 mM) were measured as solutions in acetonitrile. Samples were loaded into 2-mm Delrin XAS cells with Kapton windows and then frozen immediately in liquid nitrogen prior to XAS measurements. During XAS measurements, samples were maintained at a constant temperature of 10 K by an Oxford
Instruments CF1208 continuous-flow liquid-helium cryostat. Data were measured in fluorescence mode using a Canberra Ge 30-element array detector. XAS data were measured to $k = 11 \text{ Å}^{-1}$ due to contributions from both Fe contamination and diffraction (resulting from a poor glass). Internal energy calibration was performed by simultaneous measurement of the absorption of a Mn foil placed between a second and third ionization chamber. The first inflection point of the Mn foil was assigned to 6539.0 eV. Samples were monitored for photoreduction throughout the course of data collection. Only those scans, which showed no evidence of photoreduction, were used in the final average. The averaged data were processed as described previously\textsuperscript{29} by fitting a second-order polynomial to the post-edge region and subtracting this background from the entire spectrum. A three-region cubic spline was used to model the smooth background above the edge. Normalization of the data was achieved by subtracting the spline and normalizing the post-edge region to 1. The resultant EXAFS was $k^3$-weighted to enhance the impact of high-$k$ data. Theoretical EXAFS signals $\chi(k)$ were calculated using FEFF (version 7.0)\textsuperscript{30-31} and fit to the data using EXAFSPAK.\textsuperscript{32} The non-structural parameter $E_0$ was also allowed to vary but was restricted to a common value for every component in a given fit. The structural parameters varied during the refinements were the bond distance ($R$) and the bond variance ($\sigma^2$). The $\sigma^2$ is related to the Debye-Waller factor, which is a measure of thermal vibration and to static disorder of the absorbers/scatterers. Coordination numbers were systematically varied in the course of the analysis, but they were not allowed to vary within a given fit. Single scattering paths and the corresponding multiple scattering paths were linked during the course of refinements.
REFERENCES


CHAPTER V

SYNTHESIS AND CHARACTERIZATION OF NOVEL N-METHYL PYRIDYL AND N,N-DIMETHYLMIDAZOLYL VANADYL PORPHYRINS
ABSTRACT

A family of three cationic, \( N \)-alkylated, and water-soluble vanadyl (oxovanadium(IV)) porphyrins (tetra-\( N,N \)-dimethyl-imidazolyl, tetra-\( N \)-methyl-2-pyridyl, and tetra-\( N \)-methyl-4-pyridyl) has been prepared and characterized by UV-Vis, mass spectrometry, infrared spectroscopy, electron paramagnetic resonance, and two electrochemical techniques. The complexes display two pH-dependent spectral changes in the UV-Vis. The oxygen atom in the vanadyl complex readily exchanges slowly with \( \text{H}_2^{18}\text{O} \) \((t_{1/2} = \text{ca. 1h})\) in a pH-independent manner up to pH 10, but in more concentrated base, the exchange occurs faster than can be measured. Based on the pH-dependent spectral features, a reanalysis of published resonance Raman data (Su, Y. O.; Czernuszewicz, R. S.; Miller, L. A.; Spiro, T. G., *J Am Chem Soc* 1988, *110* (13), 4150-4157.), and the kinetics of O-atom exchange, we propose a new interpretation of the two spectrally observed \( \text{pK}_a \) transitions as sequential deprotonations of an axially-bound water, ultimately generating a *trans*-dioxovanadium(IV) species at high pH. Although an oxidized form of the three complexes could not be chemically produced or isolated, the electrochemical analysis of the three compounds coupled with the \( \text{pK}_a \) behavior can be used to estimate the O-H bond dissociation energy of \( \text{O} = \text{V}^{\text{IV}} \cdot \text{OH} \) to be ca. 99 kcal mol\(^{-1}\), and therefore a *trans*-dioxovanadium(V) should be a potent H-atom abstractor. The dramatic effect of subtle porphyrin ligand modifications, as has been seen for iron and manganese, is reflected by a strengthening of the \( \text{O} = \text{V}^{\text{IV}} \) bond, increased acidity of axial water, and stronger O-H BDE for the more electron-withdrawing imidazolyl and 2-pyridyl porphyrins.
INTRODUCTION

Vanadium porphyrins are an interesting class of metalloporphyrin that have been studied for many years. Under ambient conditions, vanadium porphyrins exist as monomeric, stable oxovanadium(IV) (‘vanadyl’) complexes.¹ Thus, vanadyl porphyrins have been the focus of many fundamental investigations owing to their structural similarity to highly reactive, and therefore difficult to study, oxoiron porphyrins (Chapter 1).²⁻³ Additionally, interest in vanadyl porphyrins has been generated as a result of the recent discovery that such complexes have pharmacological activity as insulin mimetics and anti-HIV therapeutics.⁴ Intriguingly, although vanadyl porphyrins are completely unknown in biology, they can be found as dominant and problematic impurities in fossil fuels such as crude oil, and the majority of research surrounding vanadyl porphyrins has actually been focused on overcoming their presence during petroleum refining.⁵

Synthetic vanadium porphyrin complexes have been prepared with metal oxidation states of V^{II} and V^{III}.⁶⁻⁹ As noted above, however, the stable form of these complexes under ambient conditions are oxovanadium(IV) complexes.¹ Indeed, successful synthetic preparations of vanadyl porphyrins can either directly insert a vanadyl O\text{V}^{2+} ion into the ligand or alternatively ligate a V^{III} ion under an inert atmosphere and expose the resulting complex to air.¹ This is perhaps unsurprising given that the higher oxidation states of free vanadium are in general the predominant, thermodynamically stable species (Figure 1).¹⁰
Figure 1. Pourbaix diagram (E vs. pH) of vanadium (reproduced directly from Crans, et al.\textsuperscript{10})

Remarkably, the vanadyl d\textsuperscript{1} species resist metal-centered oxidations and instead produce vanadyl porphyrin cation-radicals upon electrochemical oxidation (Scheme 1).\textsuperscript{2, 11-12} The accessibility and relative stability of oxovanadium(IV) porphyrin cation radicals made them the focus of spectroscopic research as models of the elusive oxoiron(IV) porphyrin cation-radical (so-called “Compound I”) that is the active oxidant in Cytochrome P450.\textsuperscript{2} Perhaps more astoundingly, further oxidation at even higher potentials results in a second porphyrin ring oxidation to produce a porphyrin dication!\textsuperscript{12-13} Similarly, electrochemical, 1-electron reduction of vanadyl porphyrins places the new electron in a porphyrin LUMO rather than the metal center.\textsuperscript{12}
Scheme 1. Oxidation and reduction of vanadyl porphyrins occurs on the porphyrin ligand

This “redox silence” of oxovanadium species is similarly reflected in nature. The active site of vanadium-containing chloroperoxidase (VCPO) consists of a histidine-ligated vanadate ($V^{V}$). It is believed that VCPO catalyzes the generation of HOCl from $H_2O_2$ and $Cl^-$ without undergoing metal-centered redox chemistry. Rather, the vanadium(V) center ligates a molecule of $H_2O_2$ to generate a $\eta^2$-peroxovanadium(V) species that directly oxidizes a halide ion. The mechanism of the only other known vanadium enzyme, a vanadium-containing nitrogenase, is not yet known.$^{10}$

This is not to suggest that vanadium is an unreactive transition metal. Vanadium has a rich chemistry surrounding its ability to function as a catalyst in a variety of oxidation reactions. Industrially, vanadium is used in the conversion of hydrocarbon precursors into feedstocks for polymer manufacture and fine chemical production. For example, vanadium pyrophosphate (VPO) is currently used for driving the aerobic production of maleic anhydride from $n$-butane,$^{14}$ and heterogeneous vanadium catalysts are employed to carry out the selective oxidative dehydrodgenation (ODH) of propane.$^{15-17}$ Although the mechanism of these solid phase, heterogeneous catalysts remain a mystery experimentally, DFT calculations suggest that the rate-determining step of the catalysis is hydrogen atom abstraction by a single $O=V^{IV}$ group (Figure 2).$^{18}$ This observation, coupled with recent advances in similar hydrogen atom abstractions by high-valent oxometalloporphyrins, is a driving factor behind the present study into vanadyl porphyrins.
The theoretical prediction by Goddard and coworkers that a single vanadyl site in VPO catalysts can abstract a hydrogen atom from propane suggests that oxovanadium porphyrins may have utility as more than mere models of more reactive oxometalloporphyrins. Moreover, the discovery that trans-dioxo metalloporphyrins can indeed exist for first-row transition metals\(^1\) (c.f. Chapter 4) calls for a reinvestigation of vanadyl porphyrins. All previous reports of trans-axial binding to vanadyl porphyrins ignored the possibility of this dioxo binding motif.\(^3\) Notably, the original prediction by Ballhausen and Gray that a trans-dioxo species could not exist for a first-row transition metal was largely based on their study of the vanadyl ion.\(^2\)

The remainder of this chapter describes the synthesis and characterization of a family of water-soluble, cationic vanadyl porphyrins. These ligands were chosen so that
their electron-deficient properties might raise the oxidation potential of porphyrin ring and perhaps force a metal-centered (rather than ligand-centered) oxidation. Moreover, the sterically-encumbered TM2PyP and TDMImP ligands were selected in order to facilitate \textit{trans}-axial ligation and the generation of \textit{trans}-dioxo species. These same ligands have been used previously in studies of oxoiron and oxomanganese chemistry and have revealed how subtle ligand modifications dramatically affect the electronics of metalloporphyrins as well as the thermodynamics and kinetics of high-valent, metal oxo species.\textsuperscript{21-24}
RESULTS

Three cationic vanadyl (oxovanadium(IV)) porphyrin complexes were prepared: tetra-\(N, N\)-dimethyl-imidazolyl (TDMImP), tetra-\(N\)-methyl-2-pyridyl (TM2PyP), and tetra-\(N\)-methyl-4-pyridyl (TM4PyP) (Figure 3). This section describes the synthesis and characterization of these compounds.

![Structures of vanadylTDMImP, TM2PyP, and TM4PyP](image)

**Figure 3.** Structures of vanadylTDMImP, TM2PyP, and TM4PyP

Synthesis

\(N\)-methylated pyridyl and \(N, N\)-dimethylated imidazolyl vanadyl porphyrins could be prepared by a number of methods which are summarized below. However, many of the synthetic procedures and protocols suffered from variations in reproducibility and/or difficulties in isolation. In all cases, preparation of the vanadyl complexes from unalkylated freebase porphyrin requires two steps: insertion of the vanadyl (\(\text{VO}^{2+}\)) ion ("metallation") and \(N\)-alkylation. In practice, the order of these two steps is interchangeable, although optimal conditions for the preparation of purified complex are discussed below (Scheme 2).

Based on previous reports for the synthesis of vanadyl porphyrins, the metallation step was investigated using 10 equiv \(\text{OV(SO}_4\text{)}\) in a refluxing, high-boiling solvent (e.g. DMF or NMP) for many hours.\(^{25-26}\) Indeed, metallation of both the neutrally-charged,
unalkylated and cationic, \( N \)-alkylated freebases could be carried out in this manner, as determined by UV-Vis spectroscopy. In practice, however, this method was fraught by inconsistencies in reaction times (5 – 12 hours), often proceeded incompletely (e.g. necessitating the addition of many additional equivalents of OV(SO\(_4\)) to drive the reaction to completion), and often partially dealkylated the \( N \)-methylated porphyrins (as observed by ESI-MS). An alternative method using instead a 2:1 (v/v) mixture of acetic acid and pyridine as solvent was found that reproducibly metallated the unalkylated porphyrin freebases in quantitative yield.\(^{27}\)

Perhaps interestingly, the unalkylated vanadyl pyridyl and imidazolyl porphyrins were discovered to be soluble in water. Their isolation was accomplished by the addition of the acetic acid/pyridine reaction mixture to cold, stirring ether (to precipitate the metalloporphyrin) followed by washing of the precipitate with saturated sodium bicarbonate. The vanadyl pyridyl porphyrins are not soluble in alkaline aqueous solution, and this washing afforded solid product. The vanadyl imidazolyl porphyrin in contrast was readily soluble in even saturated bicarbonate solution, but could easily be extracted with chloroform.

Alkylation of the vanadyl porphyrin complexes to produce the cationic, \( N \)-methyl species was quantitatively carried out using methyl iodide in chloroform and microwave heating in sealed reaction tubes.\(^{28}\) The iodide salt of the isolated, cationic metalloporphyrin could be purified and converted to the corresponding chloride salt using the well-established “double precipitation” procedure.\(^{29}\) Briefly, the cationic metalloporphyrin is dissolved in a minimal amount of water to which is added \( \text{NH}_4\text{PF}_6 \) to precipitate the metalloporphyrin hexafluorophosphate salt. This precipitate is isolated and
washed with a 1:1 mixture of ethyl ether/isopropanol before being dissolved in a minimal amount of acetone. To the metalloproprhyin hexafluorophosphate salt in acetone is added a minimal amount of saturated tetrabutylammonium chloride (TBACl) in acetone, which precipitates the purified metalloporphyrin chloride salt.

It is important to note that the presence of other anions, especially sulfates, was found to complicate or completely impede this method. Therefore, when metallation of the N-methylated, cationic porphyrin freebases was carried out with OV(SO$_4$)$_2$, sulfate anion impurities carried through to the end made purification by double precipitation difficult or impossible. To avoid the complications of double precipitation (and because the double precipitation procedure is known to introduce tetrabutyl ammonium impurities that are difficult to completely remove), another procedure for purifying the cationic vanadyl porphyrins was sometimes employed. Batch anion exchange using a chloride exchange resin followed by size exclusion chromatography has been used to purify cationic, alkylated iron porphyrins,$^{30}$ and this method was successfully used here as an alternative to double precipitation.
UV-Vis Spectra

In water, the cationic, vanadyl porphyrins $\text{OVTM2yP}$, $\text{OVTM4PyP}$, and $\text{OVTDMImP}$ give characteristic metalloporphyrin UV-Vis spectra, consisting of an intense Soret absorbance between 400 and 500 nm and two so-called Q-bands between 500 and 600 nm (Figure 4). Wavelengths of Soret and Q-band absorbances as well as Soret extinction coefficients are collected in Table 1. Notably, the extinction coefficient of the TM2PyP complex was experimentally nearly twice as large as those of the other two complexes.
Figure 4. UV-Vis spectra of cationic, vanadyl porphyrins at pH 6.8

<table>
<thead>
<tr>
<th></th>
<th>Soret / nm (ε /M⁻¹ cm⁻¹)</th>
<th>Q-bands / nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>(OV)TDM1mP</td>
<td>425 (10⁵.11)</td>
<td>552, 589</td>
</tr>
<tr>
<td>(OV)TM2PyP</td>
<td>431 (10⁵.08)</td>
<td>558, 593</td>
</tr>
<tr>
<td>(OV)TM4PyP</td>
<td>440 (10⁵.32)</td>
<td>564, 605</td>
</tr>
</tbody>
</table>

Table 1. Collected UV-Vis spectral data for cationic, vanadyl porphyrins at pH 6.8

The UV-Vis spectra of these compounds were found to be pH-dependent. Between pH 6 and 9, all of the complexes underwent a decrease in extinction coefficient as the Soret flattened and slightly broadened but there was no shift in wavelength of the Soret or Q-bands. At more alkaline pH, however, each complex underwent a dramatic redshift with a single isosbestic point. The pKₐ for each of these two transitions was
determined by titrating an unbuffered aqueous sample of each metalloporphyrin with a small amount (<< 0.1% v/v) of concentrated NaOH. Between each addition of NaOH, the spectrum of the complex was taken and the pH of the medium measured using a pH meter. For the TM2PyP and TDMImP complexes, the total dilution was < 3%. For the TM4PyP complex the dilution was much more, and so the absorbances of the raw spectra were corrected for dilution. The absorbance of the Soret chromophores was plotted against pH and fit to a two-pK_a titration curve (Figure 5 - Figure 10).
Figure 5. Titration of (OV)TDMImP with NaOH from pH 6.60 to 12.81

Figure 6. Absorbance at 425 nm (diamonds) and 435 nm (circles) of (OV)TDMImP as a function of pH. The red line is a fit of the data to a titration curve. (pK$_{a1}$ = 6.31 ± 0.09, pK$_{a2}$ = 11.89 ± 0.03)
Figure 7. Titration of (OV)TM2PyP with NaOH from pH 8.59 to 12.96

Figure 8. Absorbance at 431 nm (diamonds) and 443 nm (circles) of (OV)TM2PyP as a function of pH. The red line is a fit of the data to a titration curve. ($pK_{a1} = 6.99 \pm 0.10$, $pK_{a2} = 12.20 \pm 0.02$)
Figure 9. Titration of (OV)TM4PyP with NaOH from pH 10.14 to 13.29

Figure 10. Absorbance at 440 nm (diamonds) and 450 nm (circles) of (OV)TM4PyP as a function of pH. The red line is a fit of the data to a two $pK_a$ titration curve ($pK_{a1} = 8.67 \pm 0.05$, $pK_{a2} = 12.75 \pm 0.02$)
High-resolution Electrospray MS

The cationic, vanadyl porphyrins all produce ions by electrospray mass spectrometry with charges of +4, consistent with the expected charge state of the vanadyl complexes. The masses of the thus observed ions are consistent with those predicted for each complex (Table 2). Additionally, smaller peaks corresponding to the tri-cationic, tri-alkylated complexes can be observed.

$^{18}$O-labeled vanadyl complexes were obtained by incubating the $^{16}$OV complexes in H$_2^{18}$O for approximately 3 hours. The corresponding mass spectra accordingly showed this incorporation as a change of +0.5 m/z (+2 mass units for the $^{18}$O atom and +4 charge corresponding to the tetra-N-alkylation).

<table>
<thead>
<tr>
<th></th>
<th>$^{16}$OV complexes</th>
<th></th>
<th>$^{18}$OV complexes</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Obs.</td>
<td>Exp.</td>
<td>Δ</td>
<td>Obs.</td>
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<tr>
<td>(OV)TDMImP</td>
<td>755.2916</td>
<td>755.2866</td>
<td>0.0050</td>
<td>757.2968</td>
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<tr>
<td>(OV)TM2PyP</td>
<td>743.2484</td>
<td>743.2452</td>
<td>0.0032</td>
<td>745.2520</td>
</tr>
<tr>
<td>(OV)TM4PyP</td>
<td>743.2484</td>
<td>743.2452</td>
<td>0.0032</td>
<td>745.2520</td>
</tr>
</tbody>
</table>

Table 2. Observed and expected masses for $^{16}$O- and $^{18}$O-labeled vanadyl porphyrins
**Figure 11.** High-resolution mass spectrum (TOF-ESI) of ($^{16}$OV)TDMImP

**Figure 12.** High-resolution mass spectrum (TOF-ESI) of ($^{18}$OV)TDMImP
Figure 13. High-resolution mass spectrum (TOF-ESI) of $^{16}$OV)TM2PyP

Figure 14. High-resolution mass spectrum (TOF-ESI) of $^{18}$OV)TM2PyP
**Figure 15.** High-resolution mass spectrum (TOF-ESI) of \((^{16}\text{OV})\text{TM}4\text{PyP}\)

**Figure 16.** High-resolution mass spectrum (TOF-ESI) of \((^{18}\text{OV})\text{TM}4\text{PyP}\)
ATR-IR Spectra

The cationic, vanadyl porphyrin complexes were analyzed as solid samples using Attenuated Total Reflectance – Fourier Transform Infrared spectrometry (ATR-FTIR), as were the corresponding $^{18}$O-labeled vanadyl complexes. (Figure 17-Figure 19). The $^{16}$O-labeled vanadyl stretches of (OV)TDMImP and (OV)TM2PyP can both be clearly seen to disappear upon $^{18}$O-labeling, concomitant with the appearance of a strong, redshifted $^{18}$O=V. The oxygen isotope-sensitive vanadyl stretches in the case of (OV)TM4PyP overlapped with porphyrin bands (at 920 and 888 cm$^{-1}$) (Figure 19), and therefore these spectra were deconvoluted using the IGOR Pro software package. The results of this analysis are summarized in Table 3.
Figure 17. ATR-IR of $^{16}$OV)TDMImP (red) and $^{18}$OV)TDMImP (blue).
Figure 18. ATR-IR of (\(^{16}\text{O})\text{TM2PyP}\) (red) and (\(^{18}\text{O})\text{TM2PyP}\) (blue).
Figure 19. ATR-IR of $^{16}$OV)TM4PyP (red) and $^{18}$OV)TM4PyP (blue). The $^{18}$O=V stretch occurs near a porphyrin band at 885 cm$^{-1}$ (seen clearly in the red trace). The exact position of the $^{18}$O=V stretch (897 cm$^{-1}$) was determined by a computer-assisted, multiple-peak fitting analysis.
\[ \nu_{\text{obs}} \left( \text{cm}^{-1} \right)^{16}\text{O}=\text{V} = \nu_{\text{obs}} \left( \text{cm}^{-1} \right)^{18}\text{O}=\text{V} = \nu_{\text{exp}} \left( \text{cm}^{-1} \right)^{18}\text{O}=\text{V} \]

<table>
<thead>
<tr>
<th>Complex</th>
<th>(\nu_{\text{obs}}) (cm(^{-1})) (^{16}\text{O}=\text{V})</th>
<th>(\nu_{\text{obs}}) (cm(^{-1})) (^{18}\text{O}=\text{V})</th>
<th>(\nu_{\text{exp}}) (cm(^{-1})) (^{18}\text{O}=\text{V})</th>
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<tr>
<td>(OV)TDM1mP</td>
<td>960</td>
<td>919</td>
<td>919</td>
</tr>
<tr>
<td>(OV)TM2PyP</td>
<td>956</td>
<td>916</td>
<td>915</td>
</tr>
<tr>
<td>(OV)TM4PyP</td>
<td>938</td>
<td>897</td>
<td>897</td>
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</table>

**Table 3.** Observed vibrational data of the V=O stretch for \(^{16}\text{O}-\) and \(^{18}\text{O}-\)labeled complexes, and expected \(^{18}\text{O}=\text{V}\) stretch predicted by a harmonic oscillator analysis of the observed \(^{16}\text{O}=\text{V}\) data.

**EPR**

The EPR spectrum of (OV)TM2PyP at 77 K consists of two overlapping 8-line signals resulting from \(^{51}\text{V}\) hyperfine coupling (Figure 20), with \(g_{\parallel} = g_{\perp} = 2.01\), \(A_{\parallel} = 167\) and \(A_{\perp} = 62\).* This arrangement is extremely typical of known, axially-distorted d\(^{1}\) vanadyl porphyrins.\(^{13,31}\) Although the complex is known to undergo a dramatic UV-Vis change at high pH hydroxide (*vide supra*), there is only a slight shift in the EPR spectrum with the addition of up to 0.63 M NaOH (Figure 20).

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* Note: Due to instrumental limitations at the time of this analysis, the value of \(g\) could not be calculated more precisely than to two decimal places.
Figure 20. X-band EPR spectra of (OV)TM2PyP in 1:1 H₂O:ethylene glycol (black) and 1:1 H₂O:ethylene glycol containing 0.63 M NaOH (red).

**Kinetics of O-atom exchange in water**

As is known for vanadyl porphyrins, the oxygen atom of the ligated vanadyl ion (OV²⁺) readily exchanges with that of water. Incubation of the vanadyl complexes in ¹⁸O-labeled water (H₂¹⁸O) afforded the correspondingly labeled vanadyl complex (¹⁸OV porphyrin) as observed by high-resolution ESI MS and ATR-FTIR (*vide supra*).

The kinetics of oxygen-atom exchange with water was followed using the labeled complex (¹⁸OV porphyrin) and high-resolution ESI-MS. The labeled compound was incubated in buffered H₂¹⁶O, and the disappearance of the ¹⁸O complex (and appearance
of the $^{16}\text{O}$ complex) was monitored. Complete conversion of the labeled complexes required approximately 2-3 hours, independent of initial $[^{18}\text{OV}]$. The ratio $^{16}\text{OV}/^{18}\text{OV}$ could be directly measured by relative peak heights, and was used to estimate $[^{18}\text{OV}]$ as a function of time (Figure 21). Plots of the 1st-order decay of labeled vanadyl complex provided first-order rate constants, which are collected in Table 4.

**Figure 21.** First-order decay of [$[^{18}\text{OV}]\text{TDMImP}$] in H$_2^{16}\text{O}$ (pH = 8.5)
<table>
<thead>
<tr>
<th></th>
<th>pH 5.9</th>
<th>pH 6.8</th>
<th>pH 8.5</th>
<th>pH 9.4</th>
<th>pH 10.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>(OV)TDMImP</td>
<td>(2.63 ± 0.14) x 10^{-4}</td>
<td>(3.14 ± 0.21) x 10^{-4}</td>
<td>(4.48 ± 0.27) x 10^{-4}</td>
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<td>n.d.</td>
</tr>
<tr>
<td>(OV)TM2PyP</td>
<td>(1.75 ± 0.06) x 10^{-4}</td>
<td>(1.78 ± 0.07) x 10^{-4}</td>
<td>(1.78 ± 0.04) x 10^{-4}</td>
<td>(2.46 ± 0.11) x 10^{-4}</td>
<td>(3.37 ± 0.17) x 10^{-4}</td>
</tr>
<tr>
<td>(OV)TM4PyP</td>
<td>(1.64 ± 0.14) x 10^{-4}</td>
<td>(1.61 ± 0.14) x 10^{-4}</td>
<td>(1.69 ± 0.13) x 10^{-4}</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

*n.d. – not determined*

**Table 4.** First-order rate constants for O-atom exchange

**Electrochemical Oxidation**

All three vanadyl complexes were analyzed by square-wave voltammetry (SWV) and cyclic voltammetry (CV) (1 mM complex, 100 mM NaNO₃ supporting electrolyte). In unbuffered water (pH 7), CV showed no oxidation of the complexes. At pH 13 (0.1 M NaOH), however, a single, irreversible oxidation can be weakly observed by CV. The peak voltage for this oxidation wave was +895 mV, +872 mV, and +736 mV (vs Ag/AgCl) for the vanadyl TDMImP (Figure 22), TM2PyP (Figure 23), and TM4PyP (Figure 24), respectively.

A similar analysis of these complexes in 0.1 M NaOH using the square-wave technique, which is known for its increased sensitivity, showed two oxidation features for each complex. The more pronounced peak can be observed at potentials consistent with the single oxidation wave reported in the CV traces. Additionally, the SWV analysis of each complex showed a less-positive peak at +652 mV, +545 mV, and +414 mV (vs Ag/AgCl) for the vanadyl TDMImP, TM2PyP, and TM4PyP, respectively.
Figure 22. Overlaid square-wave (black) and cyclic (blue) voltammetry traces for (OV)TDMLmp
Figure 23. Overlaid square-wave (black) and cyclic (blue) voltammetry traces for (OV)TM2PyP
Figure 24. Overlaid square-wave (black) and cyclic (blue) voltammetry traces for (OV)TM4PyP
Table 5. Measured oxidation peak potentials for vanadyl porphyrins by CV and SWV

<table>
<thead>
<tr>
<th></th>
<th>CV</th>
<th>SWV</th>
</tr>
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<tbody>
<tr>
<td>(OV)TDMImP</td>
<td>+895</td>
<td>+652</td>
</tr>
<tr>
<td>(OV)TM2PyP</td>
<td>+872</td>
<td>+545</td>
</tr>
<tr>
<td>(OV)TM4PyP</td>
<td>+736</td>
<td>+414</td>
</tr>
</tbody>
</table>

**Chemical Oxidation**

The addition of common iron and manganese porphyrin oxidants including hydrogen peroxide, tert-butyl hydroperoxide, oxone, peroxynitrite, ceric ammonium nitrate, and sodium chlorite to aqueous solutions of the cationic, vanadyl porphyrins did not elicit any UV-Vis spectral changes at pH 7 or 12 indicative of porphyrin oxidation (as has been observed for analogous iron and manganese porphyrins).\(^{21,32}\) In the case of stable oxidants that are UV-active, such as sodium hypochlorite and hydrogen peroxide, the oxidant chromophore persisted in solution with the vanadyl porphyrin for long periods (ca. 60 minutes) with no indication of reaction.

Notably, however, the addition of excess amounts of sodium hypochlorite to solutions of the cationic, vanadyl complexes resulted in a reversible, spectral redshift in the UV-Vis (Figure 25) similar to but different from that observed in the NaOH titration, above. This change is indicative of binding of the hypochlorite ion to the vanadyl complex. At pH 7, the $K_d$ for this binding is ca. 7 mM. This same complex could also be
generated using the acetonitrile-soluble PF$_6^-$ salts of the vanadyl porphyrins. In acetonitrile, the binding of hypochlorite is more favorable, with a $K_d$ of ca. 100 µM.

**Figure 25.** Titration of (OV)TM2PyP with HOCl. Each scan is ca. +1.3 mM HOCl. The inset shows the slight decrease and redshift of the Soret region.
DISCUSSION

Effect of porphyrin ligand on the vanadyl bond strength

The vibrational data observed in this work is collected in Table 6 along with that for some other selected vanadyl porphyrins.\textsuperscript{3, 12} The cationic prophyrrins consistently result in the most redshifted V=O stretches (938 – 960 cm\textsuperscript{-1}), compared to non-cationic ligands such as octaethyl porphyrin (OEP) or tetraphenyl porphyrin (TPP). However, care should be taken to note that the vanadyl stretch is quite sensitive to axial ligation as well as the solvation of the complex.\textsuperscript{3} Therefore, any direct comparison between the data collected in this study on solid samples and the solution-phase Raman data ubiquitous in the literature must consider the different environments. As a direct example, (OV)TM4PyP in the solid phase has a vanadyl stretch at 938 cm\textsuperscript{-1}, which is blueshifted by as much as 20 – 40 cm\textsuperscript{-1} when dissolved in water or methanol.
### Table 6. Collected vibrational data for the V=O stretch of selected vanadyl porphyrins.

Nevertheless, the wavenumber of the vanadyl stretch (V=O) can be considered an indicator of the V-O bond strength and length. Strictly speaking, the TM4PyP ligand (938 cm\(^{-1}\)) must therefore weaken the V=O bond relative to the TM2PyP and TDMImP ligands (956 and 960 cm\(^{-1}\), respectively). This suggests that, unlike previous assertions that the vanadyl bond strength is independent of porphyrin ligation, the porphyrin ligand indeed does perturb the O=V bond or at least does so in the solid state. However, when the complex is solvated, the effects of solvation and axial ligand binding are larger influences.
On the pKₐ of axially-ligated water molecules

In water, vanadyl porphyrins exist as water-ligated oxoaqua species. Spiro observed that the resonance Raman stretch of cationic (OV)TM4PyP undergoes a redshift at pH ca. 13.5.³ Similarly, the anionic vanadyl tetra-sulfanatophenyl porphyrin (TSPP) showed a single pH-sensitive UV-Vis redshift at ca. pH 13.³³ This pKₐ was attributed to the deprotonation of an axially-ligated water molecule (e.g. O=V^{IV}-OH₂ → O=V^{IV}-OH + H⁺). This is an interesting assignment, as it suggests that the oxovanadium(IV) species does not dramatically perturb the H-OH pKa. Oxochromium(IV)³⁴, oxomanganese(IV)²⁴, and oxomanganese(V)³⁵ porphyrins, on the other hand, have been shown to reduce the pKₐ of an axially bound water to as low as 9.0, 9.4, and 4.4, respectively. Even oxoMn^{V}TM4PyP shifts the pKₐ of an axially-ligated water to 7.7.²³

We have similarly observed that the UV-Vis spectra of the vanadyl porphyrins studied in this chapter are sensitive to pH. However, in this report we note two pKₐ transitions: one near pH 11-13 and one near pH 7-8 (Table 7). The trend for pKₐ values follows that established for oxoMn^{IV} and oxoMn^{V} complexes in the same ligand: the most electron-withdrawing TDMImP ligand yields the most acidic pKₐ values, while the less-electron deficient TM4PyP complex has the most basic transitions. The question then immediately arises, “If the more alkaline pKₐ is deprotonation of an axially-ligated water molecule as suggested by Spiro, then what is the more acidic pKₐ?”
Table 7. Spectrally-observed pK$_a$ values for oxo-metal, cationic porphyrins.

<table>
<thead>
<tr>
<th>Porphyrin</th>
<th>O=V$^{IV}$-OH$_2$</th>
<th>O=Mn$^{IV}$-OH$_2$</th>
<th>O=Mn$^V$-OH$_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pK$_{a1}$</td>
<td>pK$_{a2}$</td>
<td>pK$_a$</td>
</tr>
<tr>
<td>TDMImP</td>
<td>6.3</td>
<td>11.9</td>
<td>9.4</td>
</tr>
<tr>
<td>TM2PyP</td>
<td>7.0</td>
<td>12.2</td>
<td>10.5</td>
</tr>
<tr>
<td>TM4PyP</td>
<td>8.67</td>
<td>12.8</td>
<td>n.o.</td>
</tr>
</tbody>
</table>

*a, this work; b, refs. 36 and 35; c, ref. 23; n.o., not observed*

There are only a few options for this first pK$_a$ transition. It is unlikely that the first pK$_a$ represents a simple axial ligand-binding event. Similar, subtle spectral changes have been reported, for instance, for PO$_4^{3-}$ displacing a water molecule in Mn$^{III}$ porphyrins; however, the only possible such ligands we could expect are solvent water and the chloride counterions that are part of the complex. There is no reasonable explanation why a change from pH 6 to 9 would result in binding of water over chloride ion (or *vice versa*). Regardless of pH, the concentration of water is ca. 55 M, orders of magnitude more than the chloride counterions, and therefore water should outcompete the well-solvated chloride ion at all pH values, or at least compete in a manner unaffected by pH.

Another possibility for the first pK$_a$ transition might be a change from 5- to 6-coordinate vanadyl complex. Spiro observed by resonance Raman that vanadyl octaethylporphyrin existed as a mixture of 5- and 6-coordinate species in solvents such as pyridine and piperidine (although this was not reported for (OV)TM4PyP in water). Such a combination of 5- and 6-coordinate species could exist similarly in water, although it is not clear why pH would perturb such speciation.

A more satisfying explanation for the first pK$_a$ is that it indeed describes a proton transfer from the complex to the medium. An interpretation consistent with Spiro’s...
assignment would be that at low pH the vanadyl complexes exist instead as hydroxoaquavanadium(IV) species (Scheme 3). A similar protonation of the manganyl O=MnIV has been suggested for oxoMnIV-TDMImP.\textsuperscript{35} Protonation of the vanadyl bond would be unprecedented, however; electron-rich (OV)TSPP resisted protonation even at pH 0.\textsuperscript{33}

\begin{equation*}
\begin{array}{c}
\text{OH} \\
\text{V}^{IV} \\
\text{OH}_2 \leftrightarrow \text{pK}_{a1} \\
\text{O} \\
\text{V}^{IV} \\
\text{OH}_2 \leftrightarrow \text{pK}_{a2} \\
\text{O} \\
\text{V}^{IV} \\
\end{array}
\end{equation*}

\textbf{Scheme 3.} Protonation of the vanadyl ion as an interpretation of the observed pH-behavior of vanadyl porphyrins

It is worth being concerned that this subtle spectral pK\textsubscript{a} arises due to an impurity in solution with the vanadyl porphyrin complex of interest. One likely candidate for such an impurity is an iron metalloporphyrin, an inadvertent by-product of metallation with iron-contaminated vanadyl sulfate. However, the reported pK\textsubscript{a} values\textsuperscript{37} for diaquairon(III)TM2PyP and TM4PyP are 5.1 and 5.5, respectively; well below those observed here.

Another possible impurity would be an underalkylated vanadyl porphyrin complex (e.g. a tri-methylated pyridyl porphyrin or a hepta-methylated imidazolyl porphyrin). High-resolution ESI-MS indeed shows peaks consistent with undermethylated species, although these could also be attributed to ionic fragments from the electrospray procedure. Regardless, if such species were present as an impurity, they
might be acid-base active \textit{on the meso substituent} (i.e. protonated pyridinium vs. pyridyl). This might give rise to the subtle spectral changes observed at lower pH. However, the pK\textsubscript{a} of free pyridine is 5.23; again, lower than we observe for the electron-poor, vanadyl pyridyl porphyrin complexes (TM2PyP and TM4PyP) here.\textsuperscript{38}

Another interpretation of this data is suggested by the chemistry of related manganese porphyrins. Oxomanganese(V) porphyrins are known to exist as a prototropically-related set of intermediates, from \textit{trans}-dioxoMn\textsuperscript{V} to oxoaquaMn\textsuperscript{V}.\textsuperscript{19, 23} The existence of the \textit{trans}-dioxo binding mode was unknown (and indeed unexpected) until 2006, well after the initial vanadyl porphyrin pK\textsubscript{a} assignments by Spiro \textit{et al.} Therefore, it is possible that the two pK\textsubscript{a} transitions observed for the cationic porphyrin complexes in this report represent transitions analogous to deprotonation of oxoaquaMn\textsuperscript{V}, forming a \textit{trans}-dioxoV\textsuperscript{IV} at high pH (Scheme 3).

\textbf{Scheme 4.} Two sequential deprotonations of oxoaquavanadium(IV) to form a \textit{trans}-dioxo species as an interpretation of the observed pH-behavior of vanadyl porphyrins

Spiro, \textit{et al.} reported that (OV)TM4PyP had a resonance-enhanced O=V Raman stretch at 955 cm\textsuperscript{-1} in both pH 7 and pD 7 aqueous solution. Under more alkaline conditions (pH or pD 14), this 955 cm\textsuperscript{-1} stretch was significantly decreased, with a concomitant appearance of an intense Raman band at 895 cm\textsuperscript{-1}. A plot of 5-coordinate (OV)TM4PyP Raman stretches as a function of solvent acceptor number (AN) predicted
that an unobserved 5-coordinate vanadyl species in pure water would have a V=O stretch of 970 cm\(^{-1}\). These predictions and data were taken together to suggest that the 5-coordinate oxovanadium(IV)TM4PyP in pure water has a V=O stretch at 970 cm\(^{-1}\) which is redshifted to 955 cm\(^{-1}\) (\(\Delta \nu_{5c-6c(H2O)} = 15 \text{ cm}^{-1}\)) upon coordination of an axial water ligand. Deprotonation of this axial water was suggested to further redshift the Raman stretch to 895 cm\(^{-1}\) (\(\Delta \nu_{5c-6c(OH)} = 75 \text{ cm}^{-1}\)). This successive weakening of the \textit{trans}-axial vanadyl bond is consistent with the \(\pi\)-donor properties of aqua and hydroxo ligands.

The six-coordinate vanadyl stretching bands were reportedly sensitive to \(^{18}\text{O}\) incorporation (but not D\(_2\)O), shifting to 916 and 858 cm\(^{-1}\), respectively, and consistent for a single, terminal vanadyl (\(\Delta \nu_{\text{pred}} = 922\) and 864 cm\(^{-1}\), respectively). Were the latter band due to a \textit{trans}-dioxovanadium O=V stretch, one would instead predict a shift to 843 cm\(^{-1}\) from a linear triatomic oscillator analysis\(^\dagger\). \textit{However}, a half-exchanged \(^{16}\text{O}=\text{V}^{18}\text{O}\) triatomic oscillator would be predicted to have a stretching band at 868 cm\(^{-1}\), which is closer to the value experimentally observed.

It is interesting also to compare the resonance Raman behavior of oxovanadium(IV) porphyrins with their oxomanganese(IV) analogues. The manganyl (O=Mn\(^{IV}\)) stretch in 5-coordinate oxoMn\(^{IV}\) tetra-mesityl porphyrin (TMP) has been reported at 754 cm\(^{-1}\), while oxoMn\(^{IV}\) TM4PyP in 1M NaOH (assigned as a 6-coordinate O=Mn-OH species) is only shifted to either 712 cm\(^{-1}\) in H\(_2\)O or 734 cm\(^{-1}\) in D\(_2\)O. The blueshift observed from deutertium exchange has been attributed to a relaxation of a vibrational resonance interaction between Mn=O and Mn-OH oscillators, and so the 734

\[^\dagger\] Symmetric O=M=O vibration calculated by \( \nu_{\text{sym}} = \frac{1}{2\pi c} \sqrt{\frac{F+k}{m_0}} \); half-labeled vibration calculated assuming \(m_0 = 17\) amu
cm\(^{-1}\) is suggested as the “true” manganyl stretch. Therefore, the observed shift of hydroxide binding to 5-coordinate oxoMn\(^{IV}\) (\(\Delta \nu_{5c-6c(OH)} = 20\) cm\(^{-1}\)) is much less than that for the purported hydroxide binding to 5-coordinate oxoV\(^{IV}\) (\(\Delta \nu_{5c-6c(OH)} = 75\) cm\(^{-1}\)). Perhaps the d\(^1\) vanadyl is more sensitive to the \(\pi\)-donor properties of trans-axial ligands than the high-spin d\(^3\) manganyl, but without further evidence one cannot completely rule out that the original assignments were in error.

To summarize, a single, Raman-observed pK\(_a\) transition occurs at ca. pH 13.5 whereby the O=V stretch in (OV)TM4PyP is weakened (\(\Delta \nu = 60\) cm\(^{-1}\)). Originally, this transition was assigned as a single deprotonation of an axially-bound aqua ligand. However, in the UV-Vis, two such pK\(_a\) transitions can be clearly observed (at ca. pH 9 and 13). Therefore, a discrepancy exists in the proton inventory of the assigned species as we traverse the pH from 7 to 14. Attempts to directly measure this proton inventory by a combination of titrimetry and UV-Vis spectroscopy were inconclusive. If there is indeed a deprotonation event at pH 9, the unshifting Raman band at that pH would require that both oxoaqua- and oxohydroxovanadium(IV) have the same or similar O=V stretch, which is unlikely given the increased \(\pi\)-donor properties of hydroxide. However, if there is only one deprotonation event, and (OV)TM4PyP exists as an oxoaquavanadium(IV) at all pH below 13, what is the transition that gives rise to a UV-Vis spectral change near pH 9?

Neither the original Raman data nor the UV-Vis titrations reported herein can alone or together resolve this discrepancy. However, given the inconsistency, it isn’t improper to suggest that the original assignments be at least reconsidered. Trans-dioxomanganese(V) porphyrins are now known, and the possibility of this bonding
arrangement was most certainly not considered fully in the original assignments by Spiro. Furthermore, it is curious that hydroxide binding to the vanadyl (but not the manganyl) would give rise to such a dramatic Raman shift. If there is indeed only a single pK$_a$ transition attributable to axially-ligated water at pH 13.5, then is it even more curious that binding of a water molecule to a Lewis-acidic vanadium center does not perturb the water molecule’s pK$_a$ more. Clearly, then, a reinvestigation of the pH-effects on the vanadyl stretch by resonance Raman is needed.

**O-atom exchange with water**

Exchange of the vanadyl oxygen of vanadyl porphyrins with water is known. Spiro and colleagues reported that O-atom exchange of ($^{16}$OV)TM4PyP with H$_2^{18}$O monitored by resonance Raman required ca. 3 hours at neutral pH but was nearly instantaneous at pH 13. From these observations, it was suggested that the mechanism of O-atom exchange is base catalyzed.$^3$

Oxygen-atom exchange of oxo-metal complexes with water has been explained by Meunier to proceed by so-called “oxo-hydroxo tautomerism.”$^{39-40}$ Coordination of an axially-ligated water molecule that can undergo rapid and reversible prototropy results in a dioxo compound that undergoes scrambling of the original oxo with the oxygen atom of bulk water. This mechanism has been corroborated by experiments showing the incorporation of $^{18}$O from solvent into the products of oxoMn and oxoFe substrate oxidations,$^{41}$ the slow O-atom exchange of oxoMn$^{IV}$ relative to oxoMn$^{V},$$^{42}$ and the inhibition of such exchange in the presence of strongly-coordinating axial ligands such as
5-chloro-1-methylimidazole.\textsuperscript{43} Oxo-hydroxo tautomerism has been similarly proposed even for systems that cannot adopt a \textit{trans} oxo-hydroxo geometry.\textsuperscript{44}

The kinetics of O-atom exchange in high-valent oxo metal species can be challenging to quantify, owing to the inherent instability of most such compounds. Most information on the phenomenon has relied on indirect measurements such as the incorporation of \textsuperscript{18}O from solvent into oxidized products\textsuperscript{41,45} or the measurement of the solvent H\textsubscript{2}\textsuperscript{16}O/ H\textsubscript{2}\textsuperscript{18}O composition post-oxometal exchange.\textsuperscript{46} The stable, water-soluble, and easily characterizeable oxovanadium(IV) porphyrins synthesized in this report therefore provided an opportunity to directly measure the incorporation of isotope label into and out of the vanadyl species.

As described above, the rate of O-atom exchange with vanadyl porphyrins was followed by incubating \textsuperscript{18}O-labeled vanadyl porphyrin in buffered H\textsubscript{2}\textsuperscript{16}O and directly analyzing the incubated sample at regular intervals by HRMS. In this way, the \textsuperscript{18}O=V\textsuperscript{IV} complex disappearance was directly observed alongside the appearance of the \textsuperscript{16}O=V\textsuperscript{IV} complex. The rate of isotope exchange is independent of initial complex concentration and occurs with slow, first-order kinetics.

Surprisingly, the rate constant of O-atom exchange is relatively invariant with pH in the range 5.9 – 10.5 (Figure 26). However, when we attempted to investigate this exchange at alkaline pH (13-14), it was suddenly too fast to monitor by our method, consistent with previous reports from Spiro.\textsuperscript{3} That the rate of exchange does not dramatically correlate with pH in the neutral range suggests that the mechanism of O-atom exchange is not dependent on base (e.g. a rate-limiting deprotonation step). Still, there appears to be a role for pH, given that both Spiro\textsuperscript{3} and the current investigation have
observed that the rate of exchange is nearly instantaneous in concentrated sodium hydroxide (> 0.1 M), as well as the very slight increases observed at higher pH for the vanadyl TDMIImP and TM2PyP complexes. It is worth noting that these two complexes are also the one with the most acidic pKₐ values measured (Table 7).

![Figure 26](image.jpg)

**Figure 26.** First-order rate constants for O-atom exchange between vanadyl porphyrins and water as a function of pH.

Briefly, we can speculate on the mechanism of this exchange phenomenon in spite of the pH-effects observed and the pKₐ values discussed above. It is important to remember that the electrospray ionization technique used here detects only the 5-coordinate, unligated vanadyl complexes (*vide supra*), although these species are presumably 6-coordinate in aqueous solution.

The oxoaquavanadium(IV) porphyrin would be anticipated to exchange its axial water molecule with bulk solution rapidly, as has been observed for both cationic and
anionic manganese porphyrins.\textsuperscript{47} A pH-independent incorporation of the oxygen atom of the axial water into the vanadyl would require an intramolecular proton shift to generate a HO-V\textsuperscript{IV}-OH species, which would be thermodynamically unfavorable owing to the strong acidity of the vanadyl moiety (Scheme 5a). The oxohydroxovandium(IV) species could incorporate the labeled oxygen atom into the vanadyl either by a pH-insensitive, concerted, intramolecular proton transfer or subsequent deprotonation/protonation via a \textit{trans}-dioxo intermediate (Scheme 5b). The former path would be entropically demanding, while the latter would only become available in the vicinity of the oxohydroxo pK\textsubscript{a}. Finally, a \textit{trans}-dioxovanadium(IV) might be initially expected to be the least likely to exchange its oxygen atoms; however, if it is in rapid equilibrium with even a small amount of oxohydroxovanadium(IV), the latter species could quickly pick up labeled oxygen from the medium and therefore the dominant \textit{trans}-dioxo species would become labeled as quickly as the ligand exchange occurred (Scheme 5c).
Scheme 5. Possible mechanisms for O-atom exchange into vanadyl porphyrins by oxoaqua (a), oxohydroxo (b), and trans-dioxo (c) complexes

Together, the observed pKa values, kinetics of O-atom exchange, and the above interpretation are all consistent with the suggested predominance of oxohydroxo- and oxoaquavanadium(IV) complexes below pH 11 and the formation of trans-dioxovianadium(IV) in concentrated NaOH.
Implications on the bond dissociation energy of oxovanadium(V) porphyrins

Although we have not in the present study been able to employ the novel vanadyl porphyrins as oxidation catalysts or characterize a higher-valent vanadium(V) species, the data presented in this report can be used to perhaps make the elusive oxovanadium(V) porphyrin even more alluring.

Hydrocarbon functionalization by oxoiron, -manganese, and some -ruthenium porphyrin complexes proceeds by hydrogen atom abstraction. In the simplest sense, the strength of the strongest C-H bond that can be broken is equal to the energy of the O-H bond formed by a hydrogen-atom transfer. This argument has been used to explain the differing hydrogen-atom abstraction reactivities of oxometal species in both heme-containing oxygenases and model compounds.\textsuperscript{48-49} The strength of an O-H bond (and indeed any X-H bond) can be estimated by a thermodynamic cycle that takes into account the acidity of the proton and the redox potential of the thus-formed conjugate base (Scheme 6). Each of these steps can be characterized by a free energy change (\(\Delta G\)) that allows one to calculate the bond dissociation energy of the bond of interest (Scheme 7), assuming a negligible entropic contribution.
Scheme 6. O-H bond strength can be deconvoluted as separate redox and acid/base reactions

\[
\begin{align*}
\text{O\textsuperscript{\cdot-}\text{H}} & \quad \text{M}^{+n} \quad \text{pK}_a \quad \text{O\textsuperscript{-}\text{H}} \\
\Delta E & \quad \text{H}^\cdot \quad \Delta E \\
\text{O} \quad \text{M}^{+(n+1)} \quad \text{pK}_a & \quad \text{O} \quad \text{M}^{+(n+1)} \\
\end{align*}
\]

\[\text{BDE}_{\text{O-H}} = -nF\Delta E_{\text{ox}} + 2.3 \text{ RT log}(K_a) + 57 \]
\[= 23.06 \Delta E_{\text{red}} + 1.37 \text{ pK}_a + 57\]

Scheme 7. Calculation of O-H BDE using pK\textsubscript{a} and redox potential (\Delta E).

We can use this analysis to estimate the reactivity of a hypothetical oxovanadium(V) porphyrin. As described above, the pH-induced spectral changes
observed for the three vanadyl complexes represent the subsequent deprotonations of an oxoaquavanadium(IV), and therefore the pKa values describe the basicity of a trans-dioxovanadium(IV). Although we have not definitely proven that the second pK\textsubscript{a} transition leads to a trans-dioxovanadium(IV), we can still use this pK\textsubscript{a} in our analysis. Should this pK\textsubscript{a} not describe a trans-dioxovanadium(IV), then it still provides a hypothetical lower bound for the true value. To complete the analysis, then, we require the 1-electron oxidation potential of this dioxovanadium(IV).

Electrochemical analysis of the vanadyl complexes at pH 13 (where the proposed dioxo species are predominant for all complexes) showed a single, irreversible oxidation by CV. Although more conventional vanadyl porphyrins are known to generate porphyrin cation radicals upon 1-electron oxidation, the oxidation potentials measured in this report (at pH 13) are all much lower than what is expected for these tetra-cationic ligands. Therefore, a preliminary assumption is that this CV-active oxidation wave indicates a metal-centered oxidation reaction.

The SWV results are more troubling because they in fact reveal two oxidation features, and a d\textsuperscript{1} metalloporphyrin should not be capable of undergoing two oxidations at such low potentials (given the inertness of the ligand towards oxidation). The more oxidizing feature observed by SWV occurs at the same potential as the single oxidation seen by CV, suggesting that the “first,” less-oxidizing feature observed by SWV is not a requirement for the “second” oxidation. In other words, the two oxidations seen by SWV are not consecutive events. This interpretation would therefore suggest that the two potentials observed by SWV are for different complexes. Multiple electrochemical oxidation waves such as these have been observed before for vanadyl porphyrins in cases
where the complexes can dimerize either before or after oxidation. Another possibility is that an undetectable amount of tri-N-alkylated porphyrin impurity exists in solution, or perhaps a mixture of cis and trans-ligated isomers. SWV is a more sensitive technique and might allow us to observe the presence of such impurities. Although either of these explanations could account for the odd electrochemical behavior seen by SWV, the matter deserves further attention. For the present purposes, however, we will assign the CV-active oxidation to a metal-centered V\textsuperscript{IV/V} oxidation.

The electrochemical and pH analyses described herein can now be used to predict the strength of the O-H bond formed by HAT by a hypothetical dioxovanadium(V) porphyrin (Table 8). The strength of the O-H bond calculated for O=V\textsuperscript{IV}-OH is 99 kcal mol\textsuperscript{-1} for the TDMImP and TM2PyP complexes and 95 kcal mol\textsuperscript{-1} for the TM4PyP complex. For comparison, the O-H bond in O=Mn\textsuperscript{IV}-OH has a BDE of 93 kcal mol\textsuperscript{-1} and 92 kcal mol\textsuperscript{-1} in TM2PyP and TM4PyP, respectively. The O-H BDE for Mn\textsuperscript{V} porphyrins is understood to increase with protonation state (up to a BDFE of 105 kcal mol\textsuperscript{-1}).\textsuperscript{50} Should this trend also hold for the vanadium porphyrins, an oxidant capable of cleaving C-H bonds stronger than that of methane remains a tantalizingly reachable possibility.

<table>
<thead>
<tr>
<th>Ligand</th>
<th>pK\textsubscript{a}</th>
<th>E (V vs. NHE)</th>
<th>BDFE (kcal mol\textsuperscript{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>TDMImP</td>
<td>11.9</td>
<td>+1.10</td>
<td>99</td>
</tr>
<tr>
<td>TM2PyP</td>
<td>12.2</td>
<td>+1.01</td>
<td>99</td>
</tr>
<tr>
<td>TM4PyP</td>
<td>12.8</td>
<td>+0.90</td>
<td>95</td>
</tr>
</tbody>
</table>

*Table 8. Calculated O-H BDE values for O=V\textsuperscript{IV}-OH porphyrins*
Independently, the Goddard group at the California Institute of Technology provided a similar estimate based on a DFT analysis of hypothetical, dioxovanadium(V) porphyrins.\textsuperscript{51} Their data suggests that a dioxovanadium(V) porphyrin will thermodynamically prefer the \textit{cis}-dioxo geometry over the \textit{trans} geometry. However, both \textit{cis} and \textit{trans} forms were studied computationally, and their work revealed that a metastable \textit{trans}-dioxovanadium(V) should be able to cleave a C-H bond of 102 kcal mol\textsuperscript{-1}. The more stable \textit{cis}-dioxo species is calculated to be able to break a C-H bond of 62.7 kcal mol\textsuperscript{-1}. Experimentally, a bipyridine-ligated \textit{cis}-oxohydroxovanadium(IV) species has been reported to have an O-H bond of 70.5 ± 1.2 kcal mol\textsuperscript{-1}.\textsuperscript{52} The higher BDE values calculated for the vanadyl porphyrins studied herein are in much closer agreement with the Goddard group’s prediction of a \textit{trans}-oxohydroxo binding arrangement, as might have been expected based on the \textit{trans}-dioxoMn\textsuperscript{V} precedent.

As an aside, the prediction that a \textit{trans}-dioxovanadium(V) might be capable of breaking a strong C-H bond makes the preparation of a hypochlorito-vanadyl porphyrin (Figure 25) even more relevant. If the hypochlorito adduct could be activated (e.g. chlorine atom abstraction by some radical initiator), a mechanism for chlorination analogous to that observed for oxomanganese(V) porphyrins\textsuperscript{53} might be initiated (Figure 27).
Figure 27. Hypothetical chlorination mechanism for a vanadyl porphyrin-hypochlorite adduct (c.f. reference 53)
CONCLUSIONS

A family of water-soluble, cationic vanadyl porphyrins was synthesized and characterized by a number of methods. Previously, it was suggested using resonance Raman spectroscopy that water-soluble vanadyl porphyrins exist as an acid-base pair of oxoaqua- and oxohydroxovanadium(IV) complexes. However, we show here that there are clearly two spectrally-observed pK\textsubscript{a} events that need to be assigned. Given the precedent of trans-dioxomanganese(V) porphyrins, we suggest a re-assignment of the the observed pK\textsubscript{a} events and propose that trans-dioxovanadium(IV) porphyrins are accessible, pending a reinvestigation by Raman spectroscopy. The vanadyl porphyrin species prepared here are remarkably inert towards chemical oxidation; however, the observation of an irreversible oxidation feature by cyclic voltammetry in concentrated NaOH allows us to estimate that a hypothetical trans-dioxovanadium(V) porphyrin is reactive enough to cleave a C-H bond of ca. 99 kcal mol\textsuperscript{-1}. This result has been corroborated by DFT calculations for a trans (but not cis) dioxovanadium(V) porphyrin and suggests that vanadium porphyrins might be capable of being developed as oxidation catalysts (as has been accomplished with manganese).
ACKNOWLEDGMENTS

The electrochemical analyses reported in this chapter were carried out with the kind assistance of Dr. Dong Wang. Prof. William Goddard, III and Dr. Robert “Smith” Nielsen (California Institute of Technology) are thanked for sharing their unpublished theoretical work on vanadyl porphyrins. Also, special thanks to Prof. Thomas G. Spiro for reading this chapter and providing useful feedback.
EXPERIMENTAL

Reagents

Freebase tetra-2-pyridyl porphyrin (H$_2$2PyP) and tetra-$N$-methyl-imidazolyl porphyrin (H$_2$TMImP) were prepared as previously reported.$^{54-55}$ Freebase tetra-4-pyridyl porphyrin (H$_2$4PyP) was obtained from Mid Century and purified by double precipitation. The AG 1-X8 anion exchange resin was obtained from BioRad.

Synthesis of Vanadyl Porphyrins

All three vanadyl porphyrins were prepared by a similar procedure. Typically: 0.1 – 0.2 mmol unalkylated freebase (i.e. H$_2$2PyP, H$_2$4PyP, H$_2$TMImP) was dissolved in 5 mL acetic acid/pyridine (2:1 v/v), to which was added 20 equiv (VO)SO$_4$. The reaction mixture was refluxed (ca. 130 °C) for 20 h and monitored by UV-Vis (e.g. taking an aliquot of the reaction mixture and dissolving it in methanol). Once metallation was complete, the reaction mixture was allowed to cool and then poured into a 100x volume of cold, stirring diethyl ether. Immediately, the porphyrin precipitated. The precipitate was collected by vacuum filtration, washed with saturated, aqueous sodium bicarbonate, and redissolved in chloroform ($n.b.$ vanadylTMImP is soluble in saturated sodium bicarbonate and had to be extracted with chloroform). The chloroform was removed under reduced pressure, affording unalkylated vanadyl porphyrin in > 80% yield. The unalkylated vanadyl porphyrin was then dissolved in chloroform (ca. 1 mg/mL) to which was added 200 equiv methyl iodide. This mixture was placed in a sealed, 5mL, microwave reaction vial and subjected to microwave irradiation (see Instrumentation) at 120 °C for 20 minutes. The tetra-alkylated porphyrin precipitated from the chloroform
and was collected by vacuum filtration. The porphyrin iodide salt was converted to the chloride salt by dissolving the iodide salt in water (ca. 100 mL) and slurrying this solution with a calculated amount of AG 1-X8 20-50 mesh chloride anion exchange resin for 2 days. The chloride salt of the porphyrin was isolated by filtration (to remove the resin) and evaporation of water under vacuum. Typical overall yields were > 50%.

**O-atom Exchange**

The synthesized vanadyl complexes were dissolved in $\text{H}_2\text{O}^{\text{18}}$ (10 mM porphyrin concentrations) and allowed to sit for 3 hours or longer. Immediately before analysis, an aliquot of this stock was diluted 1:100 in 10 mM buffer ($\text{KP}_i$) in $\text{H}_2\text{O}^{\text{16}}$. The exchange process was monitored by high-resolution mass spectrometry (see below).

**Instrumentation**

Most UV-Vis spectra were collected on Hewlett-Packard 8453 diode array spectrophotometer. The titrations of vanadyl TM2PyP and TDMImP were monitored using a Cary 300 Bio double-beam spectrophotometer to limit instrumental noise. Infrared spectra of solid samples were acquired using a Thermo Nicolet 6700 FTIR equipped with a SmartOrbit Attenuated Total Reflectance (diamond stage) accessory. High-resolution mass spectrometry was done on an Agilent 6210 Time-of-Flight LC/MS with a running solvent system of 50% A (90% acetonitrile, 10% water, 0.1% formic acid) and 50% B (97% water, 3% acetonitrile, 0.1 % formic acid). EPR experiments were recorded on a Bruker Elexys 580 X-band CW-EPR system using a liquid nitrogen-cooled sample chamber. EPR samples were prepared by dissolving the porphyrin complex (1
mM) in 1:1 water:ethylene glycol or 0.63 M NaOH:ethylene glycol. Microwave heating was done using a CEM Discover microwave reactor.
REFERENCES


