Vanadium Bromoperoxidase and Functional Mimics

Alison Butler* and Anne H. Baldwin

Department of Chemistry, University of California, Santa Barbara, CA 93106-9510
E-mail: butler@sbmm1.ucsb.edu

Haloperoxidases are enzymes which catalyze the oxidation of halides (iodide, bromide and chloride) by hydrogen peroxide, resulting in the halogenation of appropriate organic substrates or the halide-assisted disproportionation of hydrogen peroxide forming dioxygen. Two classes of vanadium haloperoxidases are known: vanadium bromoperoxidase, isolated mainly from marine algae, and vanadium chloroperoxidase from terrestrial fungi. A review of the structure, reactivity and enzyme kinetics of these enzymes is presented. Kinetic and mechanistic studies of several functional mimics of the vanadium haloperoxidases are also presented, including peroxo-complexes of vanadium(V) and other transition metal ions. These catalysts have proved useful in testing hypotheses concerning the role of vanadium in the haloperoxidase enzymes.

Key words: Vanadium, haloperoxidase, bromoperoxidase, chloroperoxidase, biomimic.

* Corresponding Author.

Structure and Bonding, Vol. 89

List of Abbreviations

MCD	monochlorodimedone
V-BrPO	vanadium bromoperoxidase
V-HPO	vanadium haloperoxidase
V-ClPO	vanadium chloroperoxidase

1 Introduction

Haloperoxidases are enzymes which catalyze the oxidation of halides (iodide, bromide and chloride) by hydrogen peroxide, resulting in the halogenation of appropriate organic substrates [1] or the halide-assisted disproportionation of hydrogen peroxide forming dioxygen:

$$X^- + H_2O_2 + R\text{–}H + H^+ \rightarrow R\text{–}X + 2H_2O \quad (1)$$

$$X^- + 2H_2O_2 \rightarrow O_2 + 2H_2O + X^- \quad (2)$$

In the halogenation reaction, stoichiometric consumption of peroxide and protons occurs per equivalent of halogenated substrate produced. Fluoride is not a substrate because hydrogen peroxide does not have the potential to oxidize fluoride.

Two classes of haloperoxidase are known, the vanadium- and FeHeme-containing enzymes. Within the class of vanadium-haloperoxidase enzymes, both vanadium bromoperoxidases (V-BrPO), which are isolated mainly from marine algae, and vanadium chloroperoxidases (V-ClPO), which are isolated mainly from terrestrial fungi, have been identified. Many halogenated natural products have been isolated from marine organisms. These compounds range from volatile halogenated hydrocarbons, e.g., bromoform, chloroform, etc, which are produced in very large quantities [2–4], to chiral halogenated terpenes, acetogenins and indoles, among others, which are produced in smaller amounts, but which often have important biological activities (e.g., antifungal or antibacterial properties, feeding deterrents, etc) or pharmacological properties (e.g., antineoplastic activities) (Fig. 1). The reader is referred to a series of review articles on these natural products [5 and references therein]. Thus the function of the marine haloperoxidases is thought to be the biosynthesis of these natural products. Investigations of the role of the vanadium haloperoxidases and other marine haloperoxidases in the biosynthesis of such compounds are increasing.

The physiological role of vanadium chloroperoxidase (V-ClPO) remains to be determined. Halogenated natural products have not been identified in the

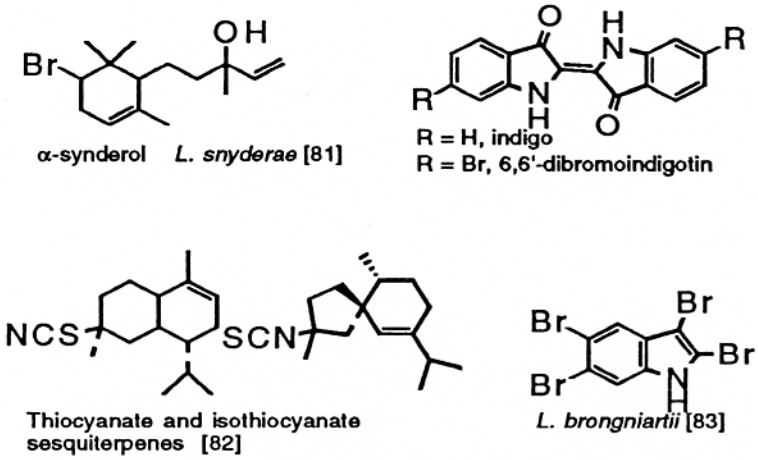


Fig. 1

fungi which produce V-ClPO, including V-ClPO from *Curvularia inaequalis* (the most studied of the V-ClPOs) and other dematiaceous hyphomycetes [6]. V-ClPO is reported to be secreted from these fungi and, as will be discussed below, produces hypochlorous acid (HOCl). HOCl is a strong bactericidal agent which may be produced as a defense mechanism, or as an attack mechanism in the invasion of the plant cell wall of the fungi's host.

The vanadium bromoperoxidases are all acidic glycoproteins [7, 8] with a very similar amino acid composition [9], molecular weight, charge (pI 4–5), and vanadium content. The subunit molecular weight of V-BrPO is ca. 65,000. The isolated form of V-BrPO contains a sub-stoichiometric ratio of vanadium per subunit. However, a content of one gram-atom of vanadium/subunit can be achieved by addition of excess vanadate and subsequent removal of adventitiously bound vanadium(V) by dialysis [10–12]. Isozymes of vanadium bromoperoxidase which differ in carbohydrate content have been isolated from *A. nodosum* [7, 13]. While the physical characteristics of V-BrPO isolated from marine algae are all very similar, some differences in reactivity have been observed, such as specific activity [14].

V-BrPO (*A. nodosum*) is particularly stable to strong oxidants, such as singlet oxygen ($^1O_2$, $^1\Delta_g$) and oxidized bromine and chlorine compounds (HOBr, HOCl, etc) [13, 15]. V-BrPO also has appreciable thermal stability [16] and stability in many organic solvents. For example V-BrPO does not lose activity when stored at room temperature for a month in 60% (v/v) acetone, methanol or ethanol or 40% (v/v) 1-propanol [15]. In addition V-BrPO retains activity after immobilization on solid support media, e.g., photo-crosslinked to DEAE cellulofine [17].

V-ClPO (*C. inaequalis*) is a 67,488 Da protein consisting of 609 amino acid residues, as determined from the DNA sequence analysis [18]. This protein lacks disulfide bonds, although two cysteine residues are present as free thiols. Two putative *N*-glycosylation sites were identified, however the protein is not glycosylated. V-ClPO is secreted from *C. inaequalis*. The isolated form may have a variable vanadium content, depending on the concentration of vanadate in the growth medium. However, one vanadium(V) per subunit can be achieved by

addition of excess vanadate to the growth medium or to the purified protein [19, 20] Like V-BrPO, V-ClPO is stable in the presence of organic substrates, at elevated temperatures and in the presence of high concentrations of strong oxidants (e.g., HOCl) [21].

Vanadium can be removed from V-HPO producing the inactive apo-enzyme derivative. The activity can be fully restored by addition of vanadate [19, 22]. Other metal ions have not been found to restore haloperoxidase activity [23]. Vanadium(V) is only fully incorporated in the absence of phosphate [6, 22, 24]. Like phosphate, molybdate, arsenate, tetrafluoroaluminate, and tetrafluoroberrylate inhibit coordination of vanadium(V) to apo-BrPO [25].

2
Structural Features of Vanadium Haloperoxidases

Recently, the crystal structure of vanadium chloroperoxidase isolated from *Curvularia inaequalis* was reported by Messerschmidt and Wever [26] (see the chapter by Messerschmidt in the succeeding volume). The X-ray structure (2.11 Å resolution) of the 67 kDa protein showed a cylindrical shape of approximately 80 Å by 55 Å. Analysis of the secondary structure revealed extended strands and loop regions and short β-strands arranged in pairs as anti-parallel β-ladders, but the main structural motif is α-helical, with two four- helix bundles. Vanadate is coordinated at the top of one of these bundles in a broad channel which is lined on one half with predominantly polar residues including an ion-pair between Arg-360 and Asp-292 and several main chain carbonyl oxygens (Fig. 2). The other half of the channel is hydrophobic, containing Pro-47, Pro-211, Try-350, Phe-393, Pro-395, Pro-396, and Phe-397.

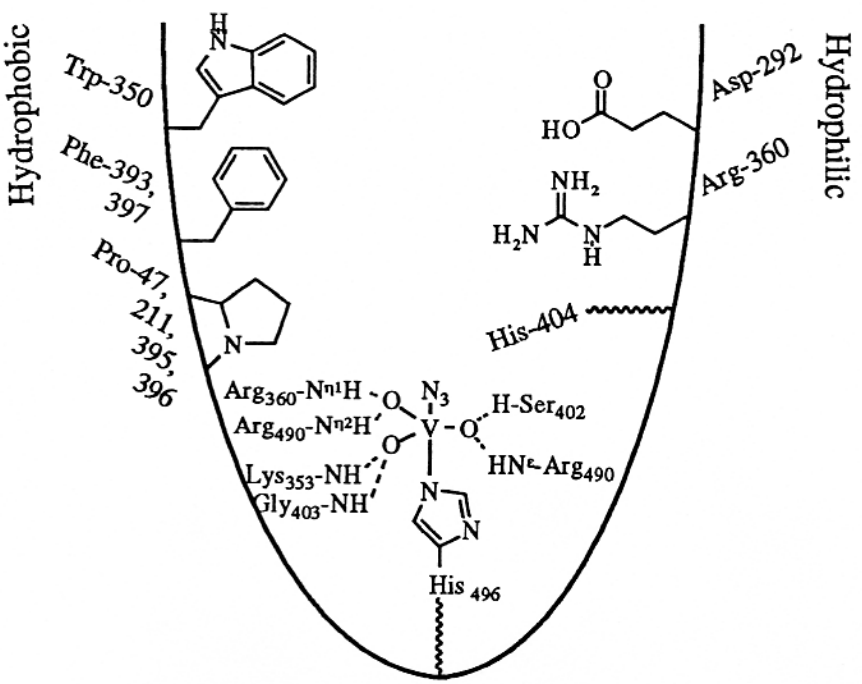


Fig. 2

Vanadate is bound with pentagonal bipyramidal geometry, ligated by azide (a result of the azide-containing crystallization buffer), three non-protein oxygen atoms, and histidine-496 (Fig. 3). The azide ligand is reportedly replaced by hydroxide in the native structure [26]. Vanadium coordination to the protein is stabilized by multiple hydrogen bonding between the vanadate oxygen atoms and the positively charged protein residues Lys-353, Arg-360, Arg-490, and Ser-402, as well as the amide nitrogen proton of Gly-403. Messerschmidt and Wever [26] propose that the hydrophobic residues Trp-350 and Phe-397 form a chloride binding site along with His-404; a hydrophobic binding site for halides is observed in other proteins such as haloalkane dehalogenase [27] and certain amylases [28]. His 404, which is present in the active site channel, must be deprotonated for H_2O_2 to bind to V-ClPO [21], and thus it is thought to function in acid-base catalysis.

Structural features of V-BrPO have not been published, although crystallization of V-BrPO from *A. nodosum* and *Corallina officinalis* has been reported at 2.4 Å and better than 2 Å resolution, respectively [12, 29]. The full sequence of V-BrPO (*A. nodosum*) has also not been reported, but from the partial sequence results [23] it is clear that there is sequence similarity between V-ClPO (*C. inaequalis*) and V-BrPO (*A. nodosum*), particularly in the active site region. Thus one can get an idea of the structure of V-BrPO. The similarities include regions containing four of the five amino acid residues which hydrogen bond to the vanadate oxygens (i.e. Arg-360, Ser-402, Gly-403, and Arg-490), the histidine ligand (His-496), and the acid-base histidine (His-404) [18, 23, 26]. In the proposed halide binding site [26], Trp-350 is present in both V-BrPO and V-ClPO, but Phe-397 is replaced by a histidine residue in V-BrPO, raising questions about the basis of halide specificity of each enzyme.

Extended X-ray absorption fine structure (exafs) analysis [30] and bond valence sum analysis [31, 32] are consistent with a trigonal bipyramidal vanadium site in V-BrPO (*A. nodosum*). These results along with the sequence similarities between V-BrPO and V-ClPO suggest that the vanadium site in V-BrPO has a trigonal bipyramidal structure, like that observed in V-ClPO, with coordination to one histidine ligand and multiple hydrogen bonds between the vanadate oxygens and positively charged residues.

The native vanadium(V) sites of V-BrPO and V-ClPO can be reduced to vanadium(IV) derivatives [19,30] which have nearly identical EPR signals, showing axially symmetrical vanadyl sites [19, 22]. While sequence similarities

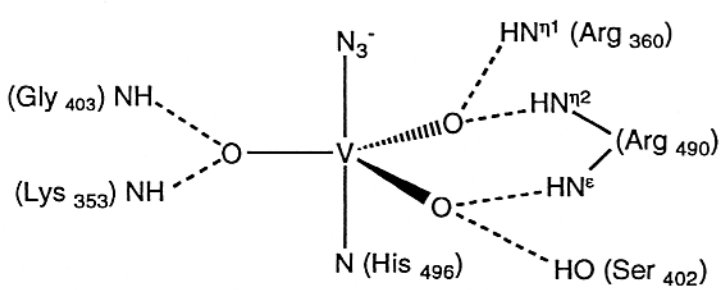


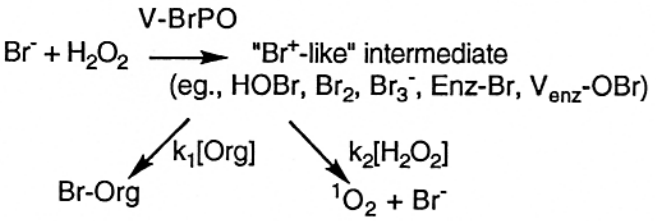
Fig. 3

between V-BrPO and V-ClPO suggest that the active sites of the two enzymes are similar, exafs analysis [30] and electron spin echo results [33] of reduced V-BrPO are consistent with a distorted octahedral geometry about the vanadium(IV) and an equatorial rather than an axial nitrogen ligand. These results imply either that the coordination environment of the vanadyl derivative of V-BrPO differs from the native structure, or that the coordination geometry of V-BrPO is different from that of V-ClPO.

3
Reactivity of the Vanadium Haloperoxidases

3.1
Halogenation and Halide-Assisted Disproportionation of Hydrogen Peroxide

Vanadium haloperoxidases (V-HPO) catalyze the halide-assisted disproportionation of hydrogen peroxide and the peroxidative halogenation of organic substrates. The enzymes catalyze the oxidation of the halide by hydrogen peroxide producing a two-electron oxidized halogen intermediate, such as hypobromous acid, bromine, tribromide, or an enzyme-bound bromonium ion equivalent, for bromide. This oxidation is followed by the halogenation of an appropriate organic substrate by the oxidized halogen intermediate, or a reaction with a second equivalent of hydrogen peroxide to form dioxygen, as shown in Scheme 1 for V-BrPO (*A. nodosum*) with bromide [34, 35].



Scheme 1

Several features of this scheme deserve further comment:

Firstly, when monochlorodimedone (MCD) is the organic substrate, the reactive intermediate in Scheme 1 (e.g. HOBr, Br_2, Br_3^-, Enz-Br) is shown to be common to both the substrate halogenation and the dioxygen formation pathways [34,35]. The supporting evidence is provided by the nearly identical rates of monochlorodimedone bromination (>75 mM MCD) and dioxygen formation in the absence of organic substrate [34], as well as the kinetic parameters resulting from the steady-state enzyme kinetics [35]. The kinetic parameters (K_m^{Br}, $K_m H_2O_2$, K_{ii}^{Br}, K_{is}^{Br}; see below for further discussion of the enzyme kinetics) obtained in the MCD bromination reaction and the dioxygen formation reaction agree to within a factor of two, providing evidence that the rate-limiting steps are the same for both the bromination of MCD and the bromide-assisted disproportionation of hydrogen peroxide. In addition, $k_2[H_2O_2]$ competes with k_1[MCD], because at variable concentrations of MCD or H_2O_2, the sum of k_1[MCD] and $k_2[H_2O_2]$ in the presence of MCD is equal to $k_2[H_2O_2]$ in the ab-

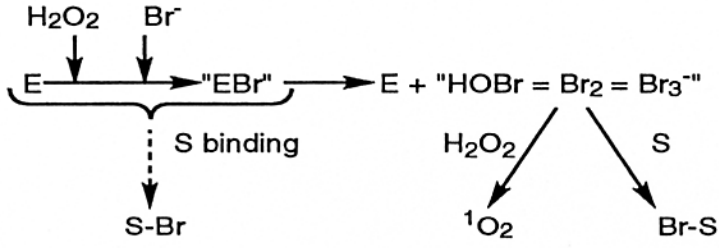
sence of an organic halogen acceptor [36]. Competitive dioxygen formation is strongly enhanced at higher pH.

Secondly, the dioxygen formed through the V-BrPO-catalyzed disproportionation of hydrogen peroxide assisted by bromide oxidation is in the singlet excited state ($^1O_2$; $^1\Delta_g$), which was identified spectroscopically using the near-IR emission characteristics [13]. It has been well established that singlet oxygen ($^1\Delta_g$) is a product of the oxidation of H_2O_2 by certain oxidized halogen species (e.g. HOBr, HOCl, and bromamines) [37, 38]. The finding that each atom of oxygen in dioxygen originates from the same molecule of hydrogen peroxide, as shown in recent $H_2{}^{18}O_2$ studies [36], is also consistent with singlet oxygen production.

Thirdly, the V-BrPO-catalyzed bromination reaction has been shown to be an electrophilic (Br^+) rather than a radical ($Br^\bullet$) process [36]. Product analysis of the bromination of 2,3-dimethoxytoluene (DMT) at pH 6.5 and 4 showed only a single product, ring-brominated 2,3-dimethoxytoluene. This product is indicative of electrophilic bromination. The product expected from a radical bromination process, 2,3-dimethoxybenzyl bromide, was not observed. In addition, two-electron oxidation of bromide is consistent with the formation of singlet oxygen.

3.2 Nature of the Oxidized Halogen Intermediate and the Question of Organic Substrate Binding to V-BrPO.

The nature of the oxidized intermediate, enzyme-bound or released, and the role of organic substrate binding to V-BrPO is not addressed in Scheme 1, nor in the experimental results described above. The oxidized halogen species cannot be detected because its reaction with organic substrates or with excess H_2O_2 to produce O_2 is too fast, preventing its build-up in solution. In competitive kinetic studies comparing the reactivity of the V-BrPO/H_2O_2/KBr system with HOBr, we have recently demonstrated that the nature of the halogenating species produced by V-BrPO depends on the nature of the organic substrate [38]. V-BrPO does not release an oxidized bromine species (e.g. HOBr, Br_2, Br_3^-) in the presence of certain indole derivatives, because these indoles bind to V-BrPO. (Halogenated indoles are marine natural products: see Fig. 1.) Indole binding was evaluated, in part, by fluorescence quenching experiments [38]. The Stern-Volmer analysis revealed a binding constant for 2-phenylindole to V-BrPO (*A. nodosum*) of $1.1 \times 10^5\ M^{-1}$ [39]. A mechanistic scheme involving substrate binding is shown in Scheme 2 [38]; V-BrPO binds H_2O_2 and Br^- leading to



Scheme 2

a putative enzyme-bound or active-site trapped brominating moiety, E-Br, which in the absence of an indole may release HOBr (or other bromine species, e.g. Br_2, Br_3^-). When indole is present, it binds to V-BrPO, preventing release of an oxidized bromine species and leading to indole bromination.

The overall reactivity of V-ClPO (*C. inaequalis*) has not been investigated as extensively as has V-BrPO (*A. nodosum*). However, one important difference is apparent. The nature of the oxidized halogen intermediate for V-ClPO (*C. inaequalis*) can be addressed because it accumulates in solution under turnover conditions (e.g. 0.2 mM H_2O_2, 1 mM Cl^-, 64 nM V-ClPO in 0.1 M phosphate buffer, pH 4.5) [21]. V-ClPO generates HOCl, which can be detected spectrophotometrically. In addition, HOCl could be separated from the reaction solution by ultrafiltration (30,000 MW membrane). Both of the experiments were carried out at pH 4.5, which means that the reduction of HOCl with excess H_2O_2 producing dioxygen is not efficient at this pH. Since only about 50 mM HOCl was detected starting with an initial concentration of 200 mM H_2O_2, it was inferred that some reduction of HOCl by H_2O_2 could have occurred. Thus the overall reactivity in Scheme 1 probably holds for V-ClPO with chloride.

V-BrPO (*A. nodosum*) also catalyzes the peroxidation of chloride, although a much larger concentration of chloride is required (> 1 M [40]) compared to V-ClPO. As with the reactivity of V-BrPO (*A. nodosum*) with bromide, the rate of peroxidative chlorination of MCD is equivalent to the rate of the chloride-assisted disproportionation of hydrogen peroxide forming dioxygen (i.e., Scheme 1). However, in the presence of primary, secondary or tertiary amines, the rate of dioxygen formation is much slower due to the formation of chloramines, NH_3, NH_2R, NHR_2, NR_3 (R is alkyl, etc.) which do not readily oxidize hydrogen peroxide [11].[1] The formation of chloramines can be observed spectrophotometrically using their characteristic UV absorption maxima.

The rate of chlorination of MCD catalyzed by V-BrPO (*A. nodosum*) is the same in the presence and absence of an amine [40]. However, because the rate of chlorination of the organic substrate by the chloramine is very rapid, we were unable to determine whether MCD chlorination proceeds through a chloramine intermediate when an amine is present. Chloride oxidation seems to be most favorable at about pH 5 under conditions of 1 mM H_2O_2, 1.5 M KCl, 50 M MCD in 0.1 M citrate buffer, pH 5.0 [40].

3.3
The Steady-State Kinetics of V-BrPO and V-ClPO

Steady-state kinetic analyses of the rates of MCD bromination and dioxygen formation catalyzed by V-BrPO from *A. nodosum* [35, 41], *M. pyrifera* [11], and *F. distichus* [11] fit a substrate-inhibited bi bi ping-pong mechanism. At pH 6.5, the K_m^{Br} and $K_m^{H_2O_2}$ are ca. 22 mM and ca. 100 mM, respectively, for V-BrPO (*A.*

[1] Bromamine formation is not observed with V-BrPO using hydrogen peroxide [11] since bromamines are rapidly reduced by H_2O_2 to singlet oxygen and bromide [37]. Using acyl peracids as the oxidant instead of hydrogen peroxide, bromamines were observed [11]; see the section on peroxide reactivity.

nodosum).[2] Although bromide and hydrogen peroxide are substrates for V-BrPO, they are also both non-competitive inhibitors of V-BrPO in certain pH ranges. Bromide inhibition is strongest at pH 5–5.5 for V-BrPO from the three sources examined; $K_i^{Br^-}$ is 200–400 mM [11, 35, 41]. Hydrogen peroxide inhibition increases with increasing pH, ranging from $K_{ii}^{H_2O_2}$ of 312 mM at pH 5.5 to 64 mM at pH 8 [36].[3] Fluoride is a competitive inhibitor of H_2O_2 binding in both the MCD bromination reaction and the bromide-assisted disproportionation of hydrogen peroxide in V-BrPO: K_i^F is 1.75 mM at pH 6.5 [35]. Fluoride inhibition (K_i^F) is uncompetitive with respect to bromide [35].

The MCD chlorination kinetics for V-ClPO (*C. inaequalis*) also fit a substrate-inhibited bi bi ping-pong mechanism. The kinetic constant for chloride, K_m^{Cl}, is reported to be 0.25 mM at pH 4.5 [20]. The kinetic constant for hydrogen peroxide, $K_m^{H_2O_2}$, varies as a function of pH: 0.5 mM at pH 3.2 to 0.01 mM at pH 5 [21]. As with V-BrPO (*A. nodosum*), chloride is both a substrate for and an inhibitor of V-ClPO [20, 21]. At pH 3.1, chloride inhibition is competitive: K_i^{Cl} is 6 mM. At pH 4.1 chloride inhibition becomes non-competitive. Nitrate also inhibits V-ClPO competitively with respect to chloride and uncompetitively with respect to hydrogen peroxide at pH 5.5. The $K_i^{nitrate}$ is 2 mM at pH 5.5.

The kinetic studies of both V-BrPO and V-ClPO enzymes suggest that a residue with pK_a of 5.7–6.5 is important for reactivity [21, 35, 36, 41]. Under peroxide inhibition conditions of V-BrPO (*A. nodosum*), the steady-state kinetic constants indicate that an ionizable group with a pK_a between 6.5–7 is involved in the inhibition [36]. This residue must be deprotonated prior to binding of hydrogen peroxide. It seems reasonable to assume, given these pK_a values, that this residue is histidine, i.e. specifically the histidine in V-BrPO analogous to His 404 in V-ClPO.

3.4
Reversible and Irreversible Inactivation of V-BrPO under Turnover Conditions

H_2O_2 is a fully reversible, non-competitive inhibitor of V-BrPO. This is shown by the steady-state kinetics observed during the initial portion of the reaction (e.g. first ten minutes at the highest concentrations of H_2O_2) [36]. If the reaction proceeds longer, the specific activity of V-BrPO decreases. The inactivation that occurs on prolonged turnover between pH 6 and 8 can be fully reversed by the addition of vanadate, suggesting that protein-bound vanadium is released under turnover at high concentrations of hydrogen peroxide, forming the apo-enzyme derivative. Vanadium release was confirmed by atomic absorption analysis. At pH 4 and 5, inactivation also occurs under turnover, accompanied by the release of vanadium, but this turnover is not reversed on addition of vanadate.

---

[2] The K_m values reported are the average of the K_m values for the MCD bromination reaction and the dioxygen formation reaction.

[3] Inhibition by hydrogen peroxide could be investigated only using the bromide-assisted dioxygen formation reaction because the kinetics of the MCD bromination reaction were complicated by competing dioxygen formation at the high concentrations of hydrogen peroxide required for inhibition [36].

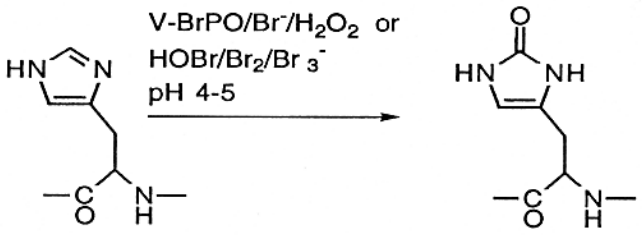


Fig. 4

The irreversible inactivation of V-BrPO which occurs at low pH was found to produce 2-oxohistidine as identified by HPLC using electrochemical detection [42]. Inactivation of V-BrPO and formation of 2-oxohistidine require all the components of turnover (i.e. bromide, hydrogen peroxide and V-BrPO) as well as low pH (Fig. 4). Neither inactivation nor 2-oxohistidine formation is observed in the presence of hydrogen peroxide alone. The inactivation and 2-oxohistidine formation are not the result of oxidation by singlet oxygen produced by V-BrPO, since they do not occur under conditions in which V-BrPO produces singlet oxygen quantitatively [42].

The oxidation of histidine to 2-oxohistidine is mediated by aqueous bromine species, as confirmed by the formation of N^{α}-benzoyl-2-oxohistidine upon addition of aqueous bromine to N^{α}-benzoylhistidine at low pH (Fig. 4). When hypobromite is added to N^{α}-benzoylhistidine in the presence of hydrogen peroxide at neutral pH, conditions under which HOBr would react with H_2O_2 to form $^1O_2$, the formation of N^{α}-benzoyl-2-oxohistidine is not observed. In addition, several functional mimics of V-BrPO (*cis*-VO_2^+ in strong acid, $MoO(O_2)_2(oxalato)_2$- at pH 5; see below) catalyze the bromide and hydrogen peroxide-mediated oxidation of N^{α}-benzoylhistidine to N^{α}-benzoyl-2-oxohistidine [42].

3.5
Reactivity of V-BrPO with Pseudohalides

In addition to iodide, bromide and chloride, V-BrPO catalyzes the oxidation of pseudohalides such as thiocyanate, azide, and cyanide [43, 44]. The reduction potentials for the oxidation of the pseudohalides fall within or below the range of those for the oxidation of I^-, Br^-, or Cl^- (i.e, ε^o 0.53 V, 1.08 V, 1.36 V (vs. NHE) respectively). This suggests that vanadium haloperoxidases may also catalyze the oxidation of these anions. Indeed, in the presence of cyanide ($\varepsilon^o = 0.375$ V) or thiocyanate ($\varepsilon^o = 0.77$ V) the V-BrPO-catalyzed oxidation of bromide by hydrogen peroxide is inhibited [43, 45]. Once these pseudohalides are consumed, the V-BrPO-catalyzed peroxidation of bromide occurs at its normal rate. V-BrPO catalyzes the thiocyanation of 1,2-dimethylindole and 1,3,5-trimethoxybenzene to 1,2-dimethyl-3-thiocyanato-indole and 1-thiocyanato-2,4,6-trimethoxybenzene, respectively. ^{13}C NMR of the oxidation of $KS^{13}CN$ (133.4 ppm vs. TMS) by H_2O_2 catalyzed by V-BrPO showed the production of several oxidized thiocyanate species, including hypothiocyanate (OSCN-; 128.6 ppm), thio-

oxime (SCNO- or SCNOH; 156.5 ppm), and the putative dithiocyanate ether anion (NCS-O-SCN; 127.6 ppm) which is unstable, as well as bicarbonate (HCO_3^-; 160.2 ppm). Azide, however, is a mechanism-based inactivator of V-BrPO [44, 46].

3.6
Peroxide Reactivity of V-BrPO

In place of hydrogen peroxide as the oxidant, V-BrPO can use acyl peracids (i.e. peracetic acid, phenyl peracetic acid, *p*-nitroperoxybenzoic acid, *m*-chloroperoxybenzoic acid), but not alkylhydroperoxides (i.e. *tert*-butyl hydroperoxide, ethyl hydroperoxide, cuminyl hydroperoxide) [11]. The peracid reactivity results from direct use of the peracid and not from hydrogen peroxide which is a result of peracid hydrolysis. Bromamines (BrNHR) have been proposed as possible intermediates in other haloperoxidase reactions, and the formation of bromamines is observed when the reaction of V-BrPO with acyl peroxides is carried out in the absence of organic substrates and in the presence of amines (including buffers or amino acids). Since acyl peroxides are not efficient two-electron reductants, BrNHR species can accumulate in solution. The peracids do not readily reduce the oxidized bromine species (e.g. HOBr, Br_2, Br_3^-, BrNHR) in the time-frame of the enzymatic reactions (i.e. several minutes), thus dioxygen is not formed [11].

4
Functional Mimics of the Vanadium Haloperoxidases

4.1
cis-Dioxovanadium(V) in Acidic Aqueous Solution and Other Vanadium(V) Species.

The first reported functional mimic of vanadium bromoperoxidase was *cis*-dioxovanadium(V) (VO_2^+) in acidic aqueous solution [47, 48]. *cis*-Dioxovanadium(V) functions by coordination of hydrogen peroxide forming the corresponding oxomonoperoxo- and oxodiperoxo-vanadium(V) species (i.e. $VO(O_2)^+$ and $VO(O_2)_2^-$, respectively). The relative amounts of $VO(O_2)^+$ and $VO(O_2)_2^-$ produced depend on the acidity of the medium and the concentration of hydrogen peroxide according to the following equilibria:

$$VO_2^{2+} + H_2O_2 = VO(O_2)^+ + H_2O \qquad K_1 \quad (3)$$

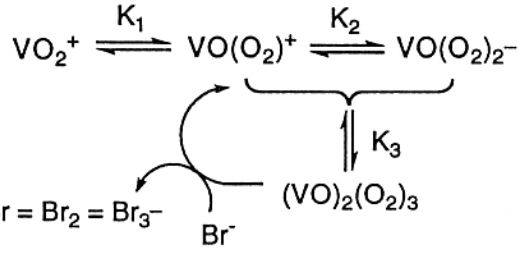
$$VO(O_2)^+ + H_2O_2 = VO(O_2)_2^- + 2H^+ \qquad K_2 \quad (4)$$

where K_1 and K_2 are $(3.7 \pm 0.4) \times 10^4$ M^{-1} and 0.6 ± 0.1 M at 25 °C, respectively [47]. In general, as the pH is raised, the relative amount of $VO(O_2)^+$ decreases. In fact at neutral pH, only $VO(O_2)_2^-$ is present when at least two equivalents of hydrogen peroxide are added to a solution of vanadate. In acidic medium (e.g. 0.05 M H^+) containing ca. 5 mM H_2O_2 and 0.5 mM vanadium(V), both $VO(O_2)^+$ and $VO(O_2)_2^-$ are present [48]. Neither of these species oxidize bromide [49] (see below for oxidation of iodide), but rather they associate forming a binuclear

oxovanadium(V), triperoxo- species, $(VO)_2(O_2)_3$, as supported by a new ^{51}V NMR signal, at 670 ppm (vs $VOCl_3$) [49, 50] (when carried out at a much higher vanadium concentration: 40 mM) and kinetic evidence [49]:

$$VO(O_2)^- + VO(O_2)_2^- = (VO)_2(O_2)_3 \qquad K_3 \qquad (5)$$

Despite the small equilibrium constant for formation of this triperoxo-, bis-oxovanadium(V) species (i.e. $K_3 = 9\ M^{-1}$ at pH 0–2), we have recently shown that it is the sole oxidant of bromide as depicted in Scheme 3 [49].



Scheme 3

The oxidation of bromide by $(VO)_2(O_2)_3$ produces HOBr, which is in a rapidly established equilibrium with bromine (Br_2) and tribromide (Br_3^-). In the absence of an organic substrate and in an acidic medium, the predominant species in solution is Br_3^-, which can be observed spectrophotometrically.[4] In the presence of an organic substrate, including MCD and trimethoxybenzene, the brominated product is formed quantitatively [48, 49].

The bimolecular rate constant for the oxidation of bromide by $(VO)_2(O_2)_3$ is $4.1 \pm 0.1\ M^{-1}s^{-1}$ [49]. Our kinetic analysis reveals that this bimolecular reaction is independent of acid, although it is required for the reaction to proceed: the acid participates in the overall catalytic cycle by maintaining the speciation of both vanadium(V) (i.e. *cis*-dioxovanadium(V) vs vanadate: $H_2VO_4^-$, HVO_4^{2-}, VO_4^{3-}) and oxoperoxo-vanadium(V) (i.e. the presence of both $VO(O_2)^+$ and $VO(O_2)_2^-$).

Wever estimated a lower limit for the bimolecular rate constant of the peroxo-V-BrPO complex with bromide [41]. This value, $k_{cat}/K_m^{H_2O_2}$, is strongly pH-dependent, primarily because $K_m^{H_2O_2}$ is sensitive to pH. The calculated bimolecular rate constant varies from $2.78 \times 10^3\ M^{-1}s^{-1}$ at pH 7.9 to $1.75 \times 10^5\ M^{-1}s^{-1}$ at pH 4.0, which is 10^3–10^5 times larger than the rate constant ($4.1 \pm 0.1\ M^{-1}s^{-1}$) for the reaction of $(VO)_2(O_2)_3$ and bromide [41, 49]. The speciation of the peroxo-vanadium(V) oxidant may control some of the reactivity difference. However, clearly the protein ligand mediates enzyme catalysis via a monomeric vanadium(V) center at neutral pH and a much faster rate (see below for further discussion).

In contrast to the mechanism of oxidation of bromide by the $VO_2^+/H_2O_2/Br^-$ system in acid, which is mediated by $(VO)_2(O_2)_3$ [48], Secco has found that iodide is oxidized by $VO(O_2)^+$, $VO(O_2)_2^-$ and VO_2^+ or their protonated forms [52, 53].

[4] The high concentrations of bromide and acid stabilize tribromide with respect to HOBr and Br_2 [51].

The diperoxo- species are the most reactive. The protonated form (i.e., $HVO(O_2)_2$) has a rate constant which is 200 times greater than the unprotonated species, which, Secco rationalizes, is a result of the more positive charge of the protonated form. Secco has used this result to argue for nucleophilic attack of iodide on the vanadium(V) center as opposed to a vanadium-bound peroxo-oxygen atom, although no direct experimental results address this point.

Other groups have also examined the vanadium(V)-catalyzed oxidation of bromide by hydrogen peroxide in aqueous or aqueous/organic mixtures, although without examining the detailed speciation of the vanadium peroxo-species [54–60]. These reports have focussed more on the nature of the substrate brominated and the product distribution under different conditions. DiFuria and co-workers have suggested that the oxidation of bromide by peroxo-V-BrPO may occur in a hydrophilic portion of the enzyme, followed by migration of the oxidized bromine intermediate to a more hydrophobic portion of the protein where bromination of organic substrates occurs and where decomposition by hydrogen peroxide is limited [54, 55]. In support of this suggestion, this group has used a two-phase 0.1 M $HClO_4$-CH_2Cl_2 (or 0.1 M $HClO_4$-$CHCl_3$) system to show that bromine can be transferred and accumulate in the organic phase [54]. If the reaction is carried out with an organic substrate, quantitative bromination is observed.[5]

The two-phase system [54, 55] has low selectivity; dibromo-derivatives and bromohydrins are observed along with the mono-brominated products. However, the ratio of dibromo-derivatives and bromohydrins to mono-brominated product is different in the two-phase system with the vanadium catalyst than with bromination with Br_2 or HOBr in a two phase system [55]. These authors conclude that because the vanadium-oxidized bromine-equivalent intermediate behaves differently than bromine, a vanadium(V)-hypobromite-like species is formed and is an active brominating moiety. However, it is not clear whether the acid and bromide concentration of the two-phase system is the same as in the vanadium catalytic system [55]. Clearly the results of further investigations will be interesting.

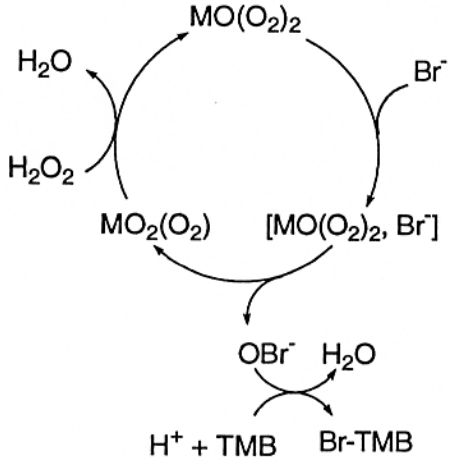
An oxoperoxo-vanadium(V) complex of glycine $[V_2O_2(O_2)_3(GlyH)_2(H_2O)_2]$ which oxidizes bromide in water is interesting because it may be related to the $(VO)_2(O_2)_3$ complex described above. It was proposed that one of the peroxide ligands is present as a μ-peroxo-moiety, but X-ray structural analysis has not been reported [58].

[5] It should be noted in reference [55] that the aqueous phase is acidic which stabilizes Br_3^- over HOBr. Thus oxidation of excess H_2O_2 by the oxidized bromine species is not favored [54]. The point is made that quantitative bromination indicates that the side-reaction of oxidation of excess H_2O_2 by the bromine species is minimized, because bromine transfers easily to the organic phase but the hydrogen peroxide or the peroxo vanadium species remain in the aqueous phase. However, even in a purely aqueous medium, quantitative bromination of trimethoxybenzene was observed [48, 49]. Thus a suitable organic substrate competes favorably against hydrogen peroxide for the oxidized bromine species in acidic solution.

4.2
Molybdenum(VI), Tungsten(VI) and Rhenium(VII)

That peroxo-vanadium(V) species oxidize halides raises questions about the reactivity of other peroxo-metal complexes. Indeed, peroxidative halogenation has been shown to occur with molybdenum(VI) [61–64], tungsten(VI) [61], and methyl-rheniumtrioxide [65, 66]. However, aqueous titanium(IV) has been found to actually stabilize peroxide against oxidation of even iodide [67].

Molybdenum and Tungsten. The catalytic cycle for the aqueous species of molybdenum(VI) and tungsten(VI) is shown in Scheme 4 [61]. Oxodiperoxo-Mo(VI) and W(VI) are the only detectable species present in acidic solution (3 mM–0.2 M $HClO_4$) in the presence of two or more equivalents of H_2O_2 per equivalent of Mo(VI) or W(IV). The rate of bromination of TMB is proportional to $[Br^-]$ and [Mo] (or [W]) and zero-order in $[H_2O_2]$. The independence of the rate on $[H_2O_2]$ is consistent with rapid recoordination of H_2O_2 to Mo(VI) and a rate-limiting step of bromide oxidation. The fate of the monoperoxide species is not known: it could coordinate H_2O_2, oxidize another equivalent of bromide, or disproportionate, forming MoO_3 and $MoO(O_2)_2$. The pH dependence shows an inflection at pH 2.1 which is the pK_a of $(H_2O)_2MoO(O_2)_2$.[6]



Scheme 4

The general reactivity for bromide oxidation is $WO(O_2)_2 > MoO(O_2)_2 > VO(O_2)_2^-$.[7] It has been suggested that the increase in activity of the group VI metals compared to vanadium(V) mimics may be due to their higher oxidation state, which would increase the oxidation potential of the bound peroxide [61]. In addition, a correlation has been drawn between the acidity of a complex and

[6] The inflection point in the acid dependence reaction with W(VI) could not be determined due to an increase in the rate of the uncatalyzed oxidation of bromide by hydrogen peroxide at H^+ concentrations higher than 0.1 M. The W(VI)-catalyzed reaction is faster at lower pH, consistent with the lower pK_a of 0.12 [61].

[7] In fact one must remember, as described above, that $VO(O_2)_2^-$ does not oxidize bromide directly [49].

its reactivity: under similar conditions, V(V), Mo(VI), and W(VI) show relative reactivities of $10:10^4:10^5$ [68]. As mentioned above, Ti(IV), which has less Lewis acid character, stabilizes peroxide [67]. In the case of V(V), the slow rate of bromide oxidation when compared to $MoO(O_2)_2(H_2O)_2$ and $WO(O_2)_2(H_2O)_2$ under identical conditions (see Fig. 7 in reference [61]) is due to $(VO)_2(O_2)_3$ and not $VO(O_2)_2^-$. In fact $VO(O_2)_2^-$ does not oxidize bromide. $(VO)_2(O_2)_3$ is present in very low concentrations which is a result of its small formation constant (i.e., 9 M^{-1}) [49]. Nevertheless, the second-order rate constant for bromide oxidation by $(VO)_2(O_2)_3$ is faster than for $MoO(O_2)_2(H_2O)_2$ or $WO(O_2)_2(H_2O)_2$ (see Table 1 below).

The oxalato complex of oxodiperoxo-molybdenum(VI), $MoO(O_2)_2(C_2O_4)^{2-}$, also catalyzes the oxidation of bromide [61, 62]. The proposed mechanism involves cycling between the diperoxo-$MoO(O_2)_2(C_2O_4)^{2-}$ species and a monoperoxo-intermediate, $MoO_2(O_2)(C_2O_4)^{2-}$, which combines with hydrogen peroxide to regenerate the initial diperoxo-complex [62]. In the case of $MoO_2(O_2)(C_2O_4)^{2-}$, which was studied at pH 5, dioxygen formation was observed in the absence of an organic substrate acceptor, whereas in the case of $MoO(O_2)_2(H_2O)_2$ in strong acid, dioxygen formation was not observed. This apparent difference in reactivity is a result of the speciation of HOBr, Br_2 and Br_3^- as a function of acid; thus Br_3^- which is favored in acid, does not oxidize H_2O_2, while at pH 5 more HOBr is present which does oxidize H_2O_2, forming dioxygen [69].

Chloride was found to inhibit the oxidation of bromide by the molybdenum(VI)-oxodiperoxo-complex [61]. The nature of the inhibition is not

Table 1. Bimolecular rate constants for the oxidation of bromide by peroxo-metal species

Oxidant	Conditions	Rate Constant $M^{-1}s^{-1}$	Reference
V-BrPO-(O_2)	pH 7.9[a]	2.78×10^3	41
	pH 4.0[a]	1.75×10^5	41
$MeReO_2(O_2)$	pH 0	350	65
$MeReO(O_2)_2$	pH 0	190	65
$VO(O_2)$(Hheida)	CH_3CN[b]	280	73
$VO(O_2)$(bpg)	CH_3CN[b]	21	73
$(VO)_2(O_2)_3$	pH 0.7–2.0	4.1	49
$MoO(O_2)_2(H_2O)_2$	pH 1.0–5.1	1.5×10^{-2}	61
$MoO(O_2)_2(H_2O)(OH)^-$	pH 1.0–5.1	2.4×10^{-3}	61
$MoO(O_2)_2(C_2O_4)^{2-}$	pH 5.1 with 20% MeOH	9.2×10^{-3}	61
	pH 5.0	4.9×10^{-3}	62
$VO(O_2)^+$	pH 0.7–2.0	nd	49
$VO(O_2)_2^-$	pH 0.7–2.0	nd	49

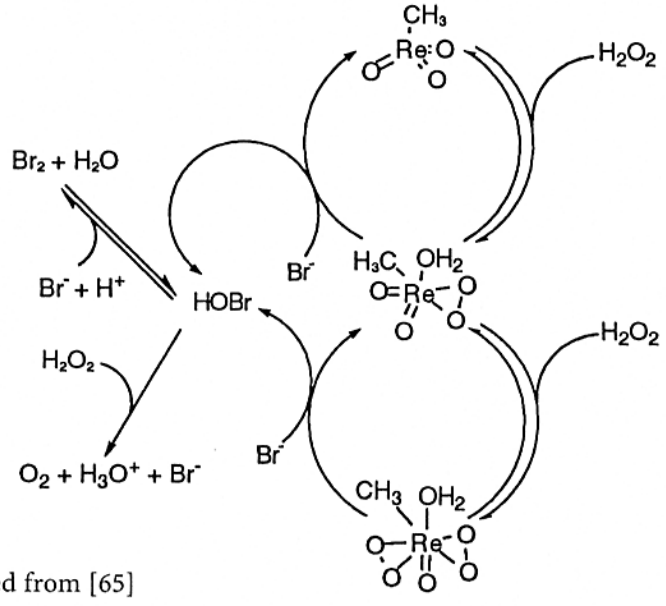
nd = not detected.

[a] lower limit as calculated by $k_{cat}/K_m^{H_2O_2}$.

[b] bromide oxidation did not occur in water.

clear but may be due to a change in the oxidation potential and the charge of the complex when it is bound to chloride. Chloride inhibition with $MoO(O_2)_2(H_2O)_2$ is accompanied by a shift in λ_{max} from 324 nM to 329 nM upon chloride addition, suggesting that chloride may coordinate to Mo(VI). A similar shift was not observed when only bromide was present, but this may be because the rate of bromide oxidation is too fast for a bromo-oxoperoxo-molybdenum(VI) intermediate to be observed. When chloride is added to the oxalato complex of $Mo(O_2)_2$, i.e. $MoO(O_2)_2(C_2O_4)^{2-}$, which does not have a dissociable ligand, no shift in the UV-vis spectrum of the complex was observed and there was no decrease in the rate of bromide oxidation [61]. Thus, halide coordination to the metal can occur, but it is not necessary for bromide oxidation.

Rhenium. Methylrhenium trioxide, CH_3ReO_3, has also been found to catalytically oxidize halides in acidic solution (Scheme 5) [65, 66]. The monoperoxo-complex, $CH_3ReO_2(\eta^2\text{-}O_2)$, and the diperoxo- complex, $CH_3ReO_2(\eta^2\text{-}O_2)_2(H_2O)$ both oxidize bromide and chloride in 1.0 M $HClO_4$. The reaction of the catalytic species (i.e, $CH_3ReO_2(\eta^2\text{-}O_2)$ + $CH_3ReO_2(\eta^2\text{–}O_2)_2(H_2O)$) with chloride is 10^5 times faster than the uncatalyzed reaction [66]. The rate of oxidation of bromide is at least 10^3 times faster than for chloride [65]. Espenson and Hansen [66] argue that the activity of monoperoxo-complexes in other systems should not be disregarded since the monoperoxo- to diperoxo-equilibria for these complexes are in excess of 10^6 M^{-1} [78,79] and thus the monoperoxo-complexes are in such low concentrations that their reactivity cannot be observed.

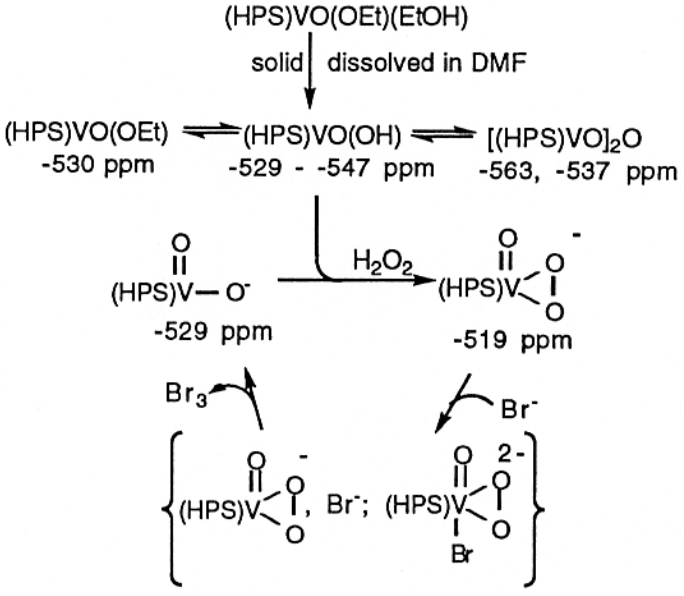


Scheme 5. Adapted from [65]

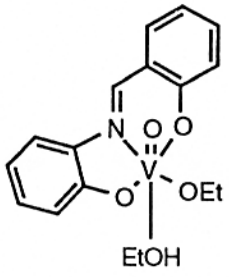
4.3
The Hydroxyphenyl-Salicylideneamine Complex of VO(OH)

Mononuclear vanadium(V) complexes also catalyze the oxidation of halides by hydrogen peroxide. One example is the (hydroxo)oxovanadium(V) complex of hydroxyphenyl-salicylideneamine (H_2HPS) [70]. (HPS)VO(OH) is one of sev-

eral species formed when the starting material, (HPS)VO(OEt)(EtOH) (Fig. 5), is dissolved in DMF (Scheme 6). In addition to (HPS)VO(OH), four other complexes are formed: (HPS)VO(OEt), and three stereochemically distinct dimers, $[(HPS)VO]_2O$, as identified by ^{51}V NMR (Scheme 6) [71,72]. A single oxoperoxovanadium(V) species, $(HPS)VO(O_2)^-$, is formed on addition of H_2O_2 to the mixture. When bromide is added to this solution, one turnover stoichiometric with respect to the concentration of the vanadium complex is observed in the absence of added acid, stopping at $(HPS)VO_2^-$. If acid is added, so that it is at least stoichiometric with H_2O_2, bromination becomes catalytic in the vanadium complex. Bromination is also stoichiometric with respect to H_2O_2 consumption. Like V-BrPO and the functional mimics discussed above (*cis*-VO_2^+, Mo(VI), W(VI)), bromination of organic substrates was shown to occur by an electrophilic process (Br^+) and not a radical process (Br•) [71]. Thus with the HPS^{2-} complex, unlike *cis*-VO_2^+, the monomeric monoperoxo-vanadium(V) complex oxidizes bromide.



Scheme 6



VO(HPS)(OEt)(EtOH)

Fig. 5

4.4 Other Complexes

Bromide oxidation by the peroxide complexes of several other V(V)-L complexes is similar to (HPS)$VO(O_2)$ described above. Such complexes include the salicylidene-amino acid Schiff base ligands (H_2Sal:Phe, H_2Sal:Gly), iminodiacetic acid (H_2IDA), nitrilotriphosphoric acid (H_3NTP) and other tripodal amine chelates (see below) and citric acid (Fig. 6) [31, 73]. On the other hand, the vanadium(V) complex of pyridine-2,6-dicarboxylic acid (H_2dipic) stabilizes coordinated H_2O_2 against bromide oxidation. Finally, some ligands cannot compete with simultaneous coordination of H_2O_2; for example carboxyphenylsalicylideneamine (H_2CPS) and pyridine-2-carboxylic acid (H_2pic) dissociate in the presence of H_2O_2 [31,71].

The oxoperoxo-vanadium(V) complexes of *N*-(2-hydroxyethyl)iminodiacetic acid (Hheida), *N*-(2-amidomethyl)iminodiacetic acid (ada), and *N,N*-bis(2-pyridylmethyl)-glycine (bpg) were shown to have a distorted pentagonal bipyramidal geometry [73, 74], consistent with the structures of all other oxoperoxo-vanadium(V) complexes reported to date [72]. The reactivity of these complexes in acetonitrile is as described above for the other monomeric vanadium(V) complexes [70]. The bimolecular rate constants for bromide oxidation by the peroxo-complexes varied from 21 $M^{-1}s^{-1}$ for the Hbpg complex to 280 $M^{-1}s^{-1}$ for the Hheida complex (see below for further discussion of these rate constants) [73]. The rates for iodide oxidation were approximately 5–6 times faster than for bromide.

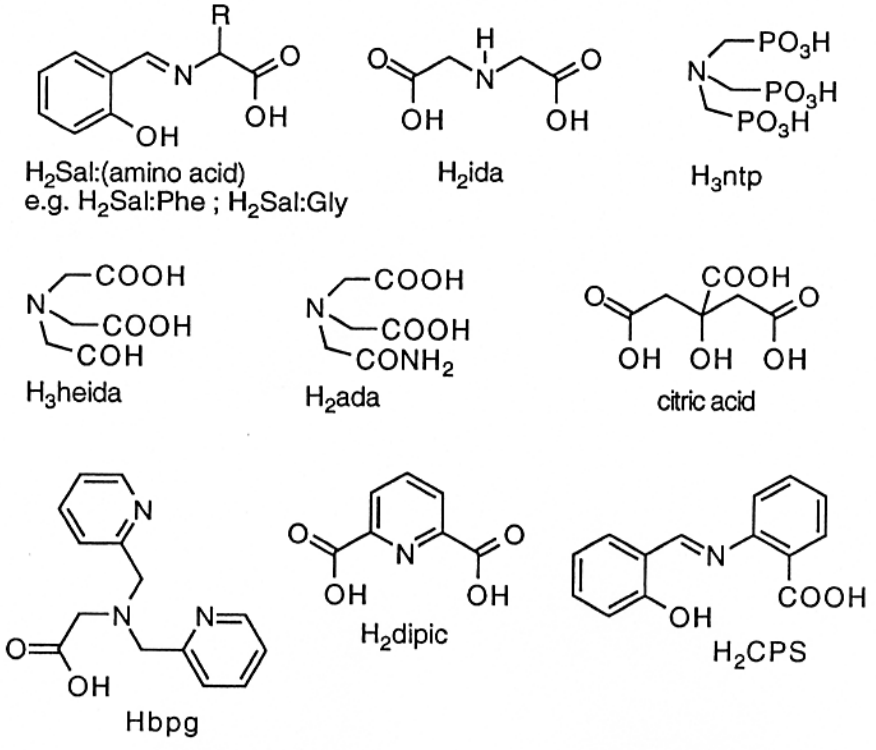
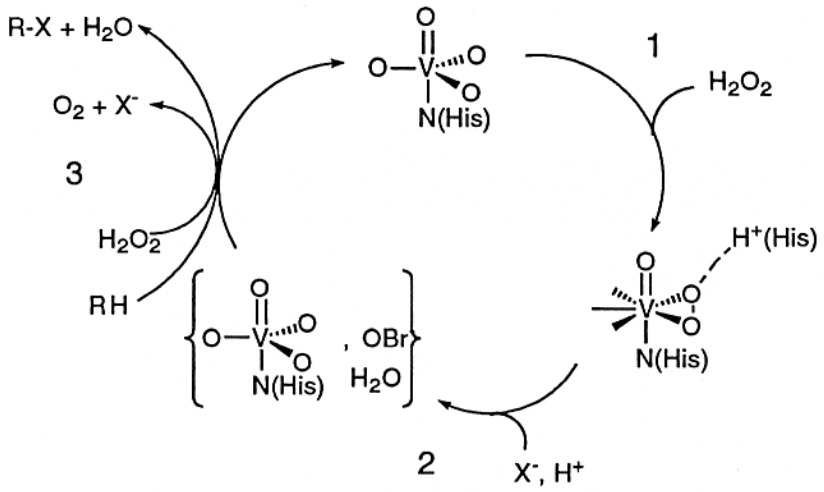


Fig. 6

5
On the Mechanism of V-HPO: Consideration of the Reaction Sequence, Role of Vanadium and Role of the Protein

Spectrophotometric analysis indicates that the first step in the catalytic cycle of V-HPO is coordination of hydrogen peroxide to vanadium(V) (Step 1 in Scheme 7). Upon addition of H_2O_2 to V-BrPO, a small absorbance decrease occurs between 300–340 nM [75]. Subsequent addition of bromide yields the original UV spectrum, a result consistent with bromide oxidation by an enzymatic peroxo-vanadium(V) species. In a separate experiment, it was found that oxidation of bromide by V(V)-BrPO, prior to reaction with hydrogen peroxide, could be ruled out: when excess bromide was added to V-BrPO (20 mM) and MCD (50 mM), MCD bromination was not observed, indicating that V-BrPO does not oxidize bromide in the absence of a peroxide source (hydrogen peroxide or an acyl peroxide) [36, 44].



Scheme 7

The coordination mode of the peroxo-V-HPO complex is not known. From small molecule studies, it is known that all peroxo- vanadium(V) complexes, whether productive catalysts of halide oxidation reactions or not, contain η^2-coordinated peroxide (for a review see [72, 76]). Oxomonoperoxo-vanadium(V) complexes are characterized by symmetrical η^2-coordination of the peroxide ligand. Oxodiperoxo-vanadium(V) complexes contain a longer peroxo-O-O bond than the oxomonoperoxo-compounds and they are characterized by asymmetrical η^2-coordination of the peroxide: one M-O bond is longer in the diperoxo- complexes (i.e., the V-O_{trans} bond) than the other (Fig. 7). Thus, one might expect the oxodiperoxo-complexes would be more reactive to oxidation. Indeed, $VO(O_2)_2^-$ oxidizes iodide faster than $VO(O_2)^+$ [47] and in general aqueous oxodiperoxo-vanadate species are more reactive in organic oxidation reactions than their monoperoxo-counterparts [51]. However, $VO(O_2)_2^-$ does not oxidize bromide. Thus the question is raised as to whether the reactivity of V-HPO arises from a different mode of coordination in the enzyme. For example is an η^1 or hydroperoxide species the active oxidant of the halide? Or does

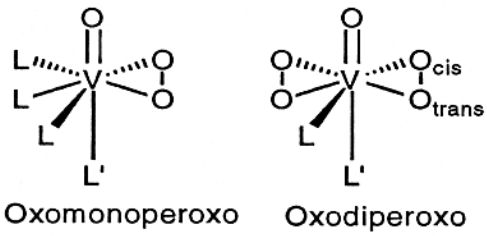


Fig. 7

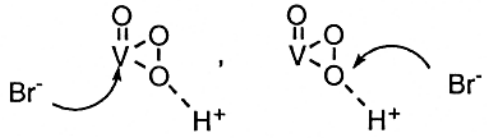


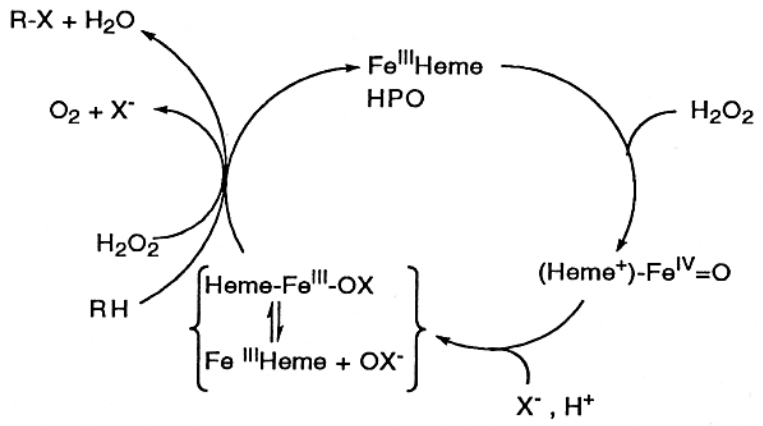
Fig. 8

the enzyme cycle between the diperoxo- and monoperoxo-species? The answers to these questions may be addressed in part by crystallographic analysis of the peroxo-V-HPO complexes.

After the first step of peroxide coordination to vanadium(V) in V-HPO, the halide is oxidized (Step 2 in Scheme 7). It has not been established conclusively whether the halide attacks the coordinated peroxide directly or whether it coordinates to vanadium(V) prior to oxidation (Fig. 8). From mechanistic studies with the molybdenum(VI) functional mimics, comparing $MO(O_2)_2(H_2O)$ with $MO(O_2)_2(C_2O_4)^{2-}$, it appears that a vacant coordination site on the oxoperoxo- metal species is not a prerequisite for halide oxidation. Thus, the oxidation probably occurs via nucleophilic attack by the halide on the coordinated peroxide.

Enzyme kinetics with V-BrPO and V-ClPO show saturation in bromide and chloride, establishing that a halide binding site exists, but the nature of this site remains unclear. Messerschmidt and Wever propose a chloride binding site of Trp-350, Phe-397 and His 404 in V-ClPO (*C. inaequalis*) based on similarity with other halide binding proteins [26]. All of these residues, however, are not conserved in V-BrPO (*A. nodosum*) (i.e., Phe is replaced by His). Thus, the basis of the preferred halide reactivity in V-HPO may be related to the nature of these amino acid residues. Of course the halide binding site could be vanadium(V), however, no difference in the exafs of V-BrPO (*A. nodosum*) was observed in the presence of bromide [80].

No direct evidence of an electron transfer role for vanadium has been detected in V-BrPO, which is surprising given the number of stable oxidation states available to vanadium in aqueous solution (see discussion in previous reviews: [14, 31, 45, 72, 76]). The reaction of V-BrPO is consistent with a Lewis acid role of vanadium(V), in which hydrogen peroxide coordination to vanadium(V) activates peroxide for halide oxidation. No epr signal has been observed under turnover conditions [41], and VO^{2+}-BrPO does not have bromoperoxidase activity, so the vanadyl-BrPO state is probably not an important component in the



Scheme 8

catalytic cycle. Also, a long-lived reduced oxidation state is not observed in ^{51}V NMR studies of functional model complexes [48, 49].

The FeHeme haloperoxidases function as two-electron redox catalysts (Scheme 8). Hydrogen peroxide oxidizes the Fe(III)-heme moiety by two electrons, forming Compound 1 ($Heme^{+}$-Fe^{IV} = O). Compound 1 then oxidizes the halide with two electrons, reforming the Fe(III)-heme moiety and a hypohalite species.

This sequence cannot be the mechanism in V-BrPO since vanadium is already in the highest oxidation state. Native V-BrPO (protein-V^{V} = O), which is analogous to Compound 1 ($Heme^{+}$-Fe^{IV} = O), does not oxidize bromide directly, as demonstrated by the lack of stoichiometric bromination when bromide was added to V-BrPO in the absence of hydrogen peroxide [36, 44]. An interesting inorganic system pertains to the discussion of oxidation state changes of the vanadium center in V-HPO as well as the models. The vanadium(V) complex of tetraethyleneglycol (H_2teg) oxidizes HBr in 1,2-dichloroethane forming a vanadium(III) complex, [V(teg)Br_2]Br, which was crystallographically characterized [77]. In the presence of air, V(III) is oxidized to vanadium(V). This reaction sequence differs from V-BrPO and the vanadium(V) catalysts discussed above where neither the reduction of vanadium(V) nor the oxidation of bromide is observed in the absence of hydrogen peroxide.

The second-order rate constants for the oxidation of bromide by various peroxo-metal species are summarized in Table 1. The peroxo-V-BrPO complex has the largest rate constant for the oxidation of bromide; thus the protein active site must provide a favorable environment. One feature of V-ClPO, which is probably present in V-BrPO, is the acid/base histidine (i.e., His 404 in V-ClPO from *C. inaequalis*). His 404 lies in hydrogen bonding distance of vanadium(V). Thus it probably assists in the reductive cleavage of coordinated peroxide upon nucleophilic attack by bromide. None of the model complexes has this feature. The relatively large rate constants for the oxidation of bromide by $VO(O_2)$(Hheida) and $VO(O_2)$(bpg) which were observed in acetonitrile, but not water, may be a result of the protonation of the η^2-coordinated peroxide which occurs in acetonitrile, but not water [73].

The state of the oxidized halogen intermediate, enzyme-bound or released, is affected by the nature of the organic substrate [38]. As discussed above, substrates which bind to V-BrPO (*A. nodosum*) block the release of a diffusible oxidized bromine intermediate. Thus one may anticipate that regioselective halogenation can be achieved with this enzyme. Indeed, this is an active area of research. Another active area, and one which when achieved will certainly advance our understanding of the mechanism of halogenation by the marine haloperoxidases, is that of stereoselective halogenation.

Vanadium has been used as a catalyst in many industrial processes. Here we have illustrated the catalytic role of a vanadium enzyme in a biological process. The rate of halogenation catalyzed by V-HPO at its physiological pH (pH 5–7) is faster than the functional mimics reported to date, despite the very simple vanadium site in the enzyme (i.e., vanadate coordination to the protein by one histidine ligand). The protein clearly plays a very important role in the activation of vanadium-bound peroxide towards halide oxidation. We eagerly await the results of future mechanistic studies as well as novel applications of this robust and active class of enzymes.

Acknowledgements: Alison Butler gratefully acknowledges support from the National Science Foundation (CHE96-29374; MCB90-18025. A.B. is an Alfred P. Sloan Research Fellow. Partial support for this work is also sponsored by NOAA, U.S. Department of Commerce under grant numbers NA89-D-SG138, project number R/MP-53 and NA66RGO477 project number R/MP-76 through the California Sea Grant College System, and in part by the California State Resources Aqency. The views expressed herein are those of the authors and do not necessarily reflect the views of NOAA. The U.S. Government is authorized to reproduce and distribute for governmental purposes.

6 References

1. Hager LP, Morris DR, Brown FS, Eberwein H (1966) J Biol Chem 241:1769
2. Gschwend PM, MacFarlane JK, Newman A (1985) Science, 227:1033
3. Walter B, Ballschmiter K (1991) Chemosphere 22:557
4. Manley SL, Goodwin K, North WJ (1992) Limnology And Oceanography 37:1652
5. Faulkner DJ (1993) Natural Product Reports, 10:497 and references therein
6. Vollenbroek EGM, Simons LH, van Schijndel JWPN, Barnett P, Balzer M, Dekker H, van der Linden C, Wever R (1995) Biochem Soc Trans 23:267
7. Krenn BE, Tromp MGM, Wever R (1989) J Biol Chem 264:19287
8. de Boer E. Tromp MGM, Plat H, Krenn BE, Wever R (1986) Biochim Biophys Acta 872:104
9. Wever R, Krenn BE, de Boer E, Offenberg H, Plat H (1988) Prog Clin Biol Res (Oxidases Relat Redox Syst) 274:477
10. de Boer E, van Kooyk Y, Tromp MGM, Plat H, Wever, R (1986) Biochim Biophys Acta 869:48
11. Soedjak HS, Butler A (1990) Biochemistry 29:7974
12. Muller-Fahrnow A, Hinrichs W, Saenger W, Vilter H (1988) Febs Lett 239:292
13. Everett RR, Kanofsky JR, Butler A (1990) J Biol Chem 265:4908
14. Butler A, Walker JV (1993) Chem Rev 93:1937
15. de Boer E, Plat H, Tromp MGM, Wever , Franssen MC, Van der Plas HC, Meijer EM, Schoemaker HE (1987) Biotechnol Bioeng 30:607
16. Sheffield, DJ, Smith AJ, Harry TR, Rogers LJ (1993) Biochem Soc Trans 21:445S
17. Itoh N, Cheng LY, Izumi Y, Yamada H (1987) J Biotech 5:29
18. Simons LH, Barnett P, Vollenbroek EGM, Dekker HL, Muijsers AO, Messerschmidt A, Wever R (1995) Eur J Biochem 229:566

19. van Schijndel JWP, Vollenbroek, EGM, Wever R (1993) Biochim Biophys Acta 1161:249
20. van Schijndel JWPM, Simons LH, Vollenbroek EGM, Wever R (1993) FEBS Lett 336:239
21. van Schijndel JWPM, Barnett P, Roelse J, Vollenbroek EGM, Wever R (1994) Eur J Biochem 225:151
22. de Boer E, Boon, K, Wever R (1987) Biochemistry 27:1629
23. Vilter, H (1995) Metal Ions in Biological Systems 31:325
24. Soedjak HS, Butler A (1991) Biochim Biophys Acta 1079:1
25. Tromp M, Tran TV, Wever R (1991) Biochim Biophys Acta 1079:53
26. Messerschmidt A, Wever R (1996) Proc Natl Acad Sci, USA, 93:392
27. Verschueren KHG, Kingma J, Rozeboon HJ, Kalk KH, Janssen DB, Dijkstra BW (1993) Biochemistry 32:9031
28. Machius M, Wiegand G, Huber R (1995) J Mol Biol 246:545
29. Rush C, Willetts A, Davies G, Dauter Z, Watson H, Littlechild J (1995) FEBS Letters, 359:244
30. Arber JM, de Boer E, Garner CD, Hasnain SS, Wever R (1989) Biochemistry 28:7968
31. Butler A, Clague MJ (1995) in Mechanistic Bioinorganic Chemistry, (ACS Symposium Volume, eds, HH Thorp and VL Pecoraro) 329
32. Carrano CJ, Mohan M, Holmes SM, de la Rosa RI, Butler A, Charnock JM, Garner CD (1994) Inorg Chem 33:646
33. de Boer E, Keijzers CP, Klassen AAK, Reijerse EJ, Collison D, Garner CD, Wever, R (1988) FEBS Lett 235:93
34. Everett RR, Butler A (1989) Inorg Chem 28, 393
35. Everett RR, Soedjak HS, Butler A (1990) J Biol Chem 265:15671
36. Soedjak HS, Walker JV, Butler A (1995) Biochemistry 34:12689
37. Kanofsky JR (1989) Arch Biochem Biophys 274:229
38. Tschirret-Guth RA, Butler A (1994) J Am Chem Soc 116:411
39. Tschirret-Guth RA (1996) PhD Dissertation, UC Santa Barbara, p 223
40. Soedjak HS, Butler A (1990) Inorg Chem 29:5015
41. de Boer E, Wever R (1988) J Biol Chem 263:12326
42. Winter GEM, Butler A. (1996) Biochemistry 35:11805
43. Walker JV, Butler A (1996) Inorg Chim Acta 243:201
44. Soedjak HS (1991) PhD Dissertation, UC Santa Barbara, p 283
45. Wever R, Kustin K (1990) Adv Inorg Chem 35:81
46. Everett RR (1990) PhD Dissertation. UC Santa Barbara, p 211
47. Secco F (1980) Inorg Chem 19:2722
48. de la Rosa RI, Clague MJ, Butler A (1992) J Am Chem Soc, 114:760
49. Clague MJ, Butler A (1995) J Am Chem Soc 117:3475
50. Harrison AT, Howarth OW (1985) J Chem Soc Dalton Trans 1985:1173
51. Thompson RC (1986) Adv Inorg Bioinorg Mech 4:65
52. Secco F, Celsi S, Grati C (1972) JCS Dalton 1972:1675
53. Celsi S, Secco F, Venturini M (1974) JCS Dalton 1974:793
54. Conte V, DiFuria F, Moro S (1994) Tet Lett, 35:7429
55. Andersson M, Conte V, DiFuria F, Moro S (1995) Tet Lett, 36:2675
56. Bhattacharjee M (1992) Polyhedron 11:2817
57. Bhattacharjee M, Ganguly S, Mukherjee J (1995) J Chem Res, 1995:80
58. Bhattacharjee M, Chaudhuri MK, Islam NS, Paul PC (1990) Inorg Chim Acta 169:97
59. Dinesh C, Kumar R, Pandey B, Kumar P (1995) Chem Commun 1995:611
60. Hegde VR, Pais GCG, Kumar R, Kumar P, Pandey, B (1996) J Chem Res-S, 1996:62
61. Meister GE, Butler A (1994) Inorg Chem 33:3269
62. Reynolds MS, Morandi SJ, Raebiger JW, Melican SP, Smith SPE (1994) Inorg Chem 33: 4977
63. Smith RH, Kilford J (1976) International Journal of Chemical Kinetics 8:1
64. Arias C, Mata F, Perez-Benito JF (1990) Can J Chem 68:1499
65. Espenson JH, Pestovsky O, Hansen P, Staudt S (1994) J Am Chem Soc 116:2869
66. Hansen P, Espenson JH (1995) Inorg Chem 34:5839

67. Lydon J.D, Thompson RC (1986) Inorg Chem 25:3694.
68. Ghiron A F, Thompson RC (1990) Inorg Chem 29:4457
69. Bray WC, Livingston RS (1923) J Am Chem Soc 45:1251
70. Clague MJ, Keder NL, Butler A (1993) Inorg Chem 32:4754
71. Clague MJ (1994) PhD dissertation, UC Santa Barbara
72. Butler A, Clague MJ, Meister G (1994) Chem Rev 94:625
73. Colpas GJ, Hamstra BJ, Kampf JW, Pecoraro VL (1996) J Am Chem Soc 118:3469
74. Colpas GJ, Hamstra BJ, Kampf JW, Pecoraro VL (1994) J Am Chem Soc 116:3627
75. Tromp MGM, Olafsson G, Krenn BE, Wever R (1990) Biochim Biophys Acta 1040:192
76. Butler A, Carrano CJ (1991) Coord Chem Rev 109:61
77. Neumann R, Assael I (1989) J Am Chem Soc 111:8410
78. Lydon JD, Schwane LM, Thompson RC (1987) Inorg Chem 26:2606
79. Ghiron A F, Thompson RC (1989) Inorg Chem 28:3647
80. Kusthardt U, Hedman, B, Hodgson KO, Hahn R, Vilter H (1993) Febs 329:5
81. Kato T, Ichinose I, Kamoshida A, Hirata Y (1976) Chem Commun 518
82. He H-Y, Faulkner DJ, Shumsky JS, Hong K, Clardy J (1985) J Org Chem 54:2511
83. Carter GT, Rinehart KL, Li LH, Kuentzel SL, Conner JL (1978) Tetra Lett 46:4479
84. Foote CS (1979) In: Wasserman HH and Murray RW (eds) Singlet Oxygen Academic Press, Orlando, FL, p 139