A Functional Model for Vanadium Haloperoxidase

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A number of vanadium haloperoxidases have been isolated from marine algae and a lichen[1] and are thought to be involved in the production of a large number of halogenated organics *in vivo*.[2] The active site is shown to consist of an oxovanadium(V) with N/O donors.[3-5] The haloperoxidases catalyze the oxidation of halides by hydrogen peroxide, generating halogenated organic compounds in the presence of substrate or dioxygen by a halide-assisted disproportionation reaction of hydrogen peroxide.[6] The reactions catalyzed are shown in eqs 1 and 2:

$$RH + H_2O_2 + HX \rightarrow RX + 2H_2O \quad (1)$$

$$2H_2O_2 \rightarrow O_2 + 2H_2O \quad (2)$$

Although studies are consistent with an ordered mechanism employing hydrogen peroxide and then halide,[7-9] the exact compositions of the active oxidant and the active halogenating intermediate are unknown. However, the vanadium center does not appear to undergo redox cycling during turnover,[10] unlike the more widely distributed heme enzymes that appear to form ferryl intermediates during catalysis.[11]

Vanadate in acidic aqueous solution has been shown to catalyze the oxidation of bromide and the bromination of various organic substrates.[12] Dioxygen is generated by the disproportionation of hydrogen peroxide in the absence of organic substrate. Kinetic analysis of the oxidation of iodide in acidic aqueous solution showed that oxoperoxovanadate and oxodiperoxovanadate are the active catalysts.[13] Recent work by Clague and Butler has characterized the ability of some vanadium(V) phenolate complexes to catalyze bromination reactions using hydrogen peroxide in DMF solution.[14] We report here the structural characterization and reactivity of an oxoperoxovanadium(V) complex which is an efficient functional model for the vanadium haloperoxidase enzyme.

The reaction of potassium vanadate, hydrogen peroxide, and *N*-(2-hydroxyethyl)iminodiacetic acid (H_3heida) in water (pH = 4) yields the compound K[VO(O_2)Hheida].[15] This compound was characterized crystallographically and has a pentagonal-bipyramidal structure with the coordinated alcohol trans to the terminal oxo in the axial positions and a side-on bound peroxide

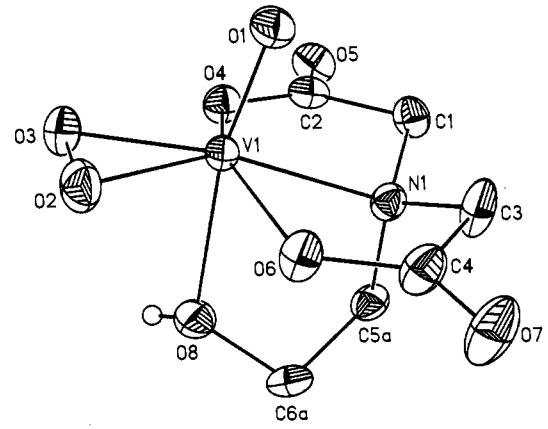

Figure 1. ORTEP diagram of [VO(O_2)Hheida]$^{1-}$ with thermal ellipsoids shown at 50% probability. Important distances (Å): V1–O1 1.601(1), V1–O2 1.865(1), V1–O3 1.864(1), O2–O3 1.432(2), V1–O4 2.051(1), V1–O6 2.038(1), V1–O8 2.236(2), V1–N1 2.194(2). Angles (deg): O1–V1–O2 105.19(7), O1–V1–O3 105.44(7), O2–V1–O3 45.17(7), O1–V1–O4 93.93(7), O1–V1–O6 93.93(7), O1–V1–N1 92.27(7), O1–V1–O8 167.72(7), O2–V1–O6 79.70(6), O3–V1–O4 80.64(6), O2–V1–O8 86.19(6), O3–V1–O8 85.79(6), O4–V1–N1 75.68(6), O4–V1–O6 150.28(6), O4–V1–O8 82.83(6), O6–V1–N1 75.41(6), O6–V1–O8 83.44(6), O8–V1–N1 75.46(6).

in the equatorial plane, shown in Figure 1.[16] The structure of this complex is typical of structurally characterized oxoperoxovanadium(V) complexes, which all feature a pentagonal-bipyramidal coordination geometry around vanadium with a terminal oxo in an axial position and an equatorial side-on bound peroxide ligand.[17-23] The 1.432(1)-Å peroxo O–O bond length of the coordinated peroxide is similar to those of other vanadium complexes of this type, which average near 1.45 Å.[24]

Solution characterization by ^{13}C NMR in aqueous solution indicates that ligand exchange is slow, unlike that in similar dioxo species,[25] implying decreased lability for the peroxo complexes. The potassium salt can be dissolved in acetonitrile solution upon addition of 18-crown-6. The UV/visible spectrum of the compound shows a characteristic peroxo-to-vanadium charge-transfer band at 430 nm, observed in both aqueous and organic solutions.

The addition of 1 equiv of triflic acid to an acetonitrile solution of K(18-crown-6)[VO(O_2)Hheida] and excess tetra-*n*-butylammonium bromide or tetra-*n*-butylammonium iodide will rapidly and stoichiometrically form tribromide or triiodide (eq 3):

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(15) Characterization for K[VO(O_2)Hheida]·H_2O. IR (Nujol): ν(C=O, s) 1610, (C—O, s), 1461, (V=O, s) 961, (O—O, s) 920, (V—O_2, s, as) 570. ^{13}C{^{1}H} NMR (D_2O): δ 183.2, 68.8, 64.9, 59.9. ^{51}V NMR (D_2O): −565. Anal. Calcd (Found) for $C_6H_{11}NKO_9V$: C, 21.76 (21.61); H, 3.35 (3.11); N, 4.23 (4.32).
(16) X-ray parameters for K[VO(O_2)Hheida]·H_2O: monoclinic ($P2_1/n$, No. 14); a = 6.740(2), b = 11.510(3), and c = 14.841(4) Å; α = 90°, β = 93.12(2)°, γ = 90°; V = 1149.6(5) Å^3; Z = 4; d_{calc} = 1.902 g/cm^3; crystal dimensions (mm), 0.20 × 0.24 × 0.28; μ for Mo Kα = 13.12 cm^{-1}; T = 293(2) K; Syntex $P2_1$ m/v diffractometer; absorption coefficient, 1.235 mm^{-1}. Structure was solved and refined using the SHELXTL PLUS and SHELXL-93 programs on a VAXstation 3500. Hydrogen atoms were refined individually. Scan range $5 < 2\theta < 55$; unique reflections = 2655, refined reflections = 2655, no. of parameters = 243, R_{int} = 0.0279, R_w^2 = 0.0674.
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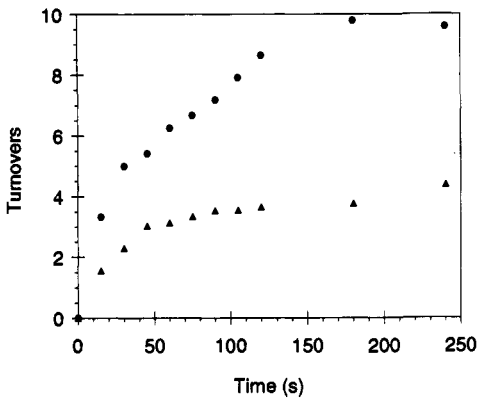

Figure 2. $[VO(O_2)Hheida]^-$ catalyzed bromination of phenol red as followed by UV/visible spectroscopy at 595 nm. Reaction conditions are 0.5 mM K(18-crown-6)$[VO(O_2)Hheida]$, 15.0 mM tetra-*n*-butylammonium bromide, 5.0 mM H_2O_2, 5.0 mM triflic acid, and 1.25 mM Phenol Red in acetonitrile at 0 °C. Spectral data taken of aliquots in pH = 7.1 aqueous phosphate buffer. Vanadium-catalyzed reaction data indicated by ●, control reaction (minus vanadium complex) indicated by ▲.

$$VO(O_2)L + X^- + H^+ \rightarrow VO_2L + X^+ + OH^- \quad (3)$$

This can be observed by UV/visible spectroscopy and requires 1 equiv of peroxide and 1 equiv of acid for each oxidation. This reaction corresponds to the effective oxidations of Br^- to Br^+ and I^- to I^+. Chloride is not oxidized under the same conditions.

Halogenation of an organic substrate is shown by the conversion of Phenol Red to Bromophenol Blue.[26] This reaction is used to assay activity of haloperoxidase enzymes by UV/visible spectroscopy.[27] The reaction is rapid and stoichiometric, producing the halogenated product by reaction of the oxidized halogen species with the organic substrate as shown in eq 4:

$$RH + X^+ + OH^- \rightarrow RX + H_2O \quad (4)$$

The combination of eqs 3 and 4 then corresponds to the halogenation of organic substrate using hydrogen peroxide as oxidant (eq 1).

The halogenation reaction is also catalytic in the vanadium complex, performing multiple turnovers in the presence of added hydrogen peroxide and acid. The reaction as followed by UV/visible spectroscopy went to completion doing approximately 10 turnovers in under 3 min at 0 °C under the conditions indicated in Figure 2. A control reaction performed without the vanadium complex present showed significant initial reactivity;[28] however, the reaction slowed under conditions of lowered acid concentration, and halogenation of the substrate was not completed for several hours.

If an organic substrate is not present and 1 equiv of hydrogen peroxide in acetonitrile and tetraethylammonium hydroxide in methanol is added to the solution obtained upon oxidation of halide, dioxygen is generated as shown in eq 5:

$$H_2O_2 + X^+ + 2OH^- \rightarrow O_2 + X^- + 2H_2O \quad (5)$$

The combination of eqs 3 and 5 then corresponds to the halide-assisted disproportionation of hydrogen peroxide to produce water and dioxygen (eq 2).[29]

The peroxo-to-vanadium charge-transfer band is observed at 430 nm (ϵ = 300) in both aqueous and organic solvents. It is retained with a small shift in energy upon addition of 1 equiv of triflic acid to an acetonitrile solution of the complex, indicating the stability of the peroxide coordination to the vanadium. This may indicate an oxohydroperoxovanadium(V) complex as the active oxidizing agent. This species could be considered electronically analogous to an (alkylperoxo)oxovanadium(V) complex utilizing a similar ligand which is crystallographically characterized and shown to be an oxidizing agent.[30] In addition, a 5-fold excess of bromide shows no effect on the UV/visible spectrum of the peroxo complex.

We have also explored the chemistry of other oxoperoxovanadium(V) complexes utilizing aminocarboxylic ligands derived from iminodiacetic acid, such as nitrilotriacetic acid and ((2-pyridyl)methyl)iminodiacetic acid, and observed similar reactivity. The coordination spheres of the compounds employed here closely resemble the proposed coordination sphere of the vanadium site as determined from the known spectroscopic details of the vanadium haloperoxidase enzymes. The reactivity obtained here models the vanadium haloperoxidases enzyme as known at this time. The conditions under which the halogenation reaction is observed may indicate the presence of a hydrophobic active site with a nearby acid/base catalyst in the vanadium haloperoxidase enzymes.

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Supplementary Material Available: Details of the crystal structure determination, including tables of atomic coordinates, equivalent isotropic displacement parameters, bond distances and angles, anisotropic displacement parameters, hydrogen coordinates, and isotropic displacement parameters (8 pages); observed and calculated structure factors (6 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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