A Functional Mimic of Vanadium Bromoperoxidase

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The unique marine metalloenzyme vanadium bromoperoxidase (V-BrPO) catalyzes the oxidation of chloride,[1] bromide,[2,3] and iodide[4] by hydrogen peroxide. The oxidized halogen species can halogenate selected organic compounds, which is the presumed biogenesis of the halogenated marine natural products, or oxidize a second equivalent of hydrogen peroxide, producing dioxygen, which in the case of bromide is known to be in the singlet excited state ($^1O_2$) (Scheme I).[5] Vanadium(V) is an essential cofactor for the catalytic activity of V-BrPO; however, because observation of the vanadium(V) site in V-BrPO is largely inaccessible by conventional spectroscopic techniques, we have turned to model compounds to address the role of vanadium. We report here the reactivity of the first functional mimic of V-BrPO, dioxovanadium(V) in acidic aqueous solution, which catalyzes the oxidation of bromide by hydrogen peroxide, resulting in bromination of organic substrates or the bromide-assisted disproportionation of hydrogen peroxide.

Dioxovanadium(V) catalyzes the bromination of trimethoxybenzene (TMB) using hydrogen peroxide as an oxidant of bromide (Figure 1). Bromotrimethoxybenzene (Br-TMB) formation was established by electron impact mass spectral analysis (i.e., for M^+, m/e 246 and 248 in the correct relative intensities) and by ^1H NMR.[6] Under conditions of excess TMB to H_2O_2 (as in Figure 1), the overall stoichiometry of the reaction is consumption of 1 equiv each of H_2O_2 and TMB per equivalent of Br-TMB produced.[7] In the absence of dioxovanadium(V), very little Br-TMB is formed, establishing that dioxovanadium(V) functions as a catalyst (Figure 1). The maximum turnover rate is 15 mol of Br-TMB/(mol of V) h^{-1}. Dioxovanadium(V) catalysis requires significantly greater acid concentration ($\geq$0.001 M H^+) than V-BrPO, which functions maximally at pH 5-7, with a turnover rate of 4.7×10^5 mol of Br product/(mol of enzyme) h^{-1} at pH 6.5.[1-5]

The dioxovanadium(V)-catalyzed bromination of TMB was also followed by ^{51}V NMR (Figure 2). The ^{51}V NMR chemical shift of 0.5 mM dioxovanadium(V) in the reaction medium is -540 ppm (Figure 2a). Addition of 5 mM H_2O_2 forms a mixture of monoperoxovanadium(V) [$VO(O_2)^+$, -529 ppm; Figure 2b] and diperoxovanadium(V) [$VO(O_2)_2^-$, -687 ppm; Figure 2b], consistent with previously reported chemical shifts for these species.[8] Upon initiation of the reaction by addition of bromide, the peroxovanadium(V) species are consumed with concomitant production of dioxovanadium(V) (Figures 2c-j). The final ^{51}V NMR spectrum (Figure 2j) is identical to the initial spectrum of dioxovanadium(V) in Figure 2a, showing that all of the active dioxovanadium(V) catalyst is retained after complete reaction of H_2O_2, the limiting reagent.

In the absence of an organic acceptor such as TMB, dioxovanadium(V) catalyzes the bromide-assisted disproportionation of hydrogen peroxide forming dioxygen (Figure 3a). Neither the reaction of dioxovanadium(V) and H_2O_2 (Figure 3b) nor the reaction of Br^- and H_2O_2 produces dioxygen (Figure 3c), showing that, under these catalytic conditions, both dioxovanadium(V) and Br^- are required for catalysis of the bromide-assisted disproportionation of H_2O_2. In the presence of TMB, dioxygen formation is completely inhibited (Figure 3d), due to the bromination of TMB. In the absence of TMB, tribromide (Br_3^-) is detected spectrophotometrically (λ_{max} 267 nm; data not shown); however, it reacts with excess hydrogen peroxide in a slower reaction forming dioxygen, which accounts for the initial lag phase in Figure 3a. Diperoxovanadium(V) oxidizes bromide more rapidly than does monoperoxovanadium(V) (Figure 2), leading to a biphasic rate of bromide-assisted dioxygen formation (Figure 3) and TMB bromination (Figure 1).

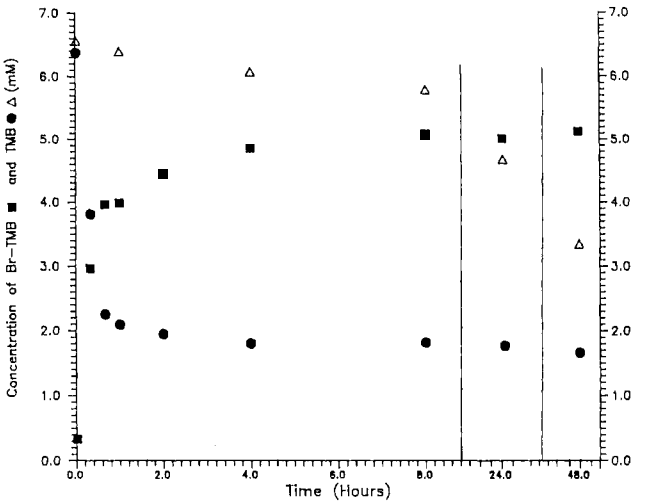


Figure 1. GC analysis of the dioxovanadium(V)-catalyzed bromination of TMB. Conditions: 0.5 mM NH_4VO_3, 6.66 mM TMB, 5.0 mM H_2O_2, 0.43 M KBr in 0.05 M $HClO_4$/25% MeOH. (●) TMB in catalytic reaction; (■) Br-TMB in catalytic reaction; (Δ) TMB in control reaction without VO_2^+.

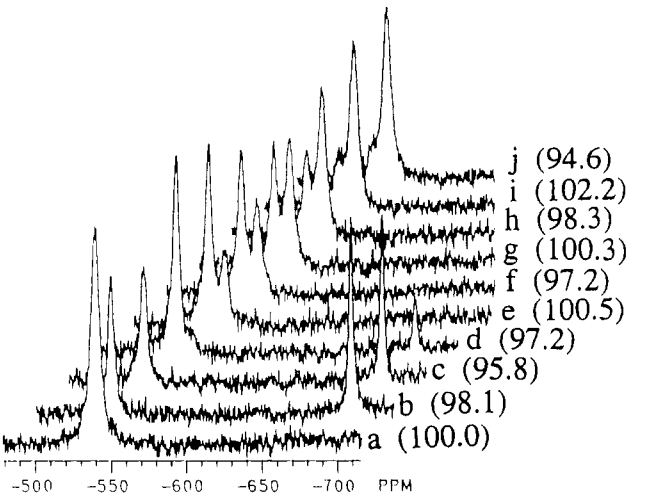
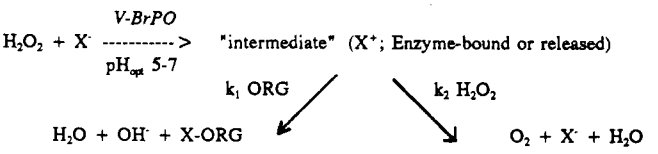


Figure 2. ^{51}V NMR[10] stack plot of the dioxovanadium(V)-catalyzed bromination of TMB. Conditions are the same as in Figure 1. (a) 0.5 mM NH_4VO_3 in 0.05 M $HClO_4$/25% MeOH; (b) a + 5 mM H_2O_2 + 6.66 mM TMB; (c) b + 0.5 M Br^- at the start of the reaction; (d) 20 min from the start of the reaction; (e) 40 min; (f) 60 min; (g) 2 h; (h) 4 h; (i) 24.5 h; (j) 48 h. The total ^{51}V signal area is shown in parentheses.

Scheme I



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(6) ^1H NMR for Br-TMB (300 MHz) in CD_3OD: δ = 6.177 (2 H, s), 3.878 (6 H, s), 3.321 (3 H, s). For further characterization, see: Goyne, T. E. Dissertation, UCLA, 1983.
(7) Br_2-TMB (M^+ m/e 324, 326, 328; correct relative intensities) is produced if [H_2O_2] is greater than [TMB].
(8) Campbell, N. J.; Dengel, A. C.; Griffith, W. P. *Polyhedron* **1989**, *8*, 1379-1386. Our ^{51}V chemical shifts of $VO(O_2)^+$ and $VO(O_2)_2^-$ differ slightly (15 ppm) from those reported due to the presence of MeOH, although in the absence of MeOH the same chemical shifts as those reported in this reference are observed.

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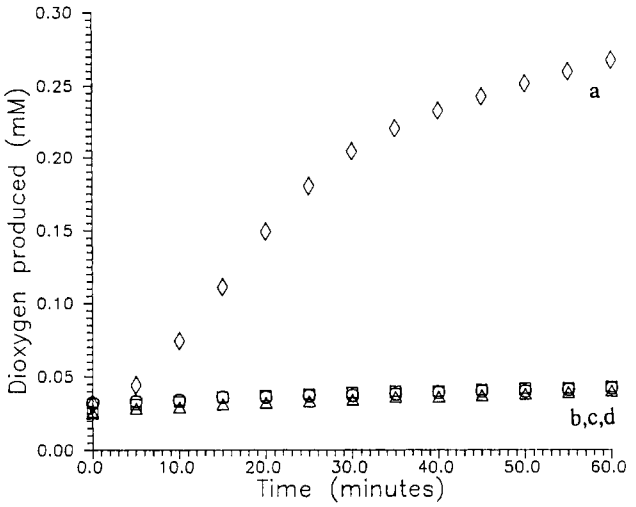
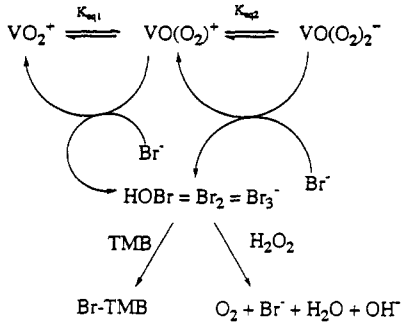


Figure 3. Dioxygen formation in the bromide-assisted disproportionation of hydrogen peroxide. Conditions are the same as in Figure 1. O_2 was measured with a YSI Clark-type electrode. (a) $VO_2^+ + Br^- + H_2O_2$; (b) $VO_2^+ + H_2O_2$; (c) $Br^- + H_2O_2$; (d) $VO_2^+ + Br^- + H_2O_2$ + TMB. Note: The solubility of O_2 in this reaction solution is ca. 0.345 mM; the limited solubility prevents determination of the stoichiometry under these conditions.

Scheme II



Dioxovanadium(V) also catalyzes the oxidation of iodide and chloride by H_2O_2 in acid. Peroxovanadium(V) species oxidize iodide; however, dioxovanadium(V) is reduced by iodide in acid,[9] consistent with the observed decrease in the ^{51}V NMR signal over time of the reaction solution under catalytic conditions (data not shown). Further, we have found that dioxovanadium(V) catalyzes the oxidation of chloride by hydrogen peroxide, and the formation of chlorotrimethoxybenzene (Cl-TMB; M^+ *m/e* 202, 204), although the chlorination rate is slower than that for bromination.

Thus we have shown that dioxovanadium(V) catalyzes the bromination of TMB and the bromide-assisted disproportionation of hydrogen peroxide, analogous to the reactivity of the V-BrPO enzyme (Scheme II; in this scheme, HOBr, Br_2 and Br_3^- are in rapid equilibration). In this biomimetic system, dioxovanadium(V) coordinates H_2O_2 forming the monoperoxo or diperoxo species, in ratios dependent on the hydrogen peroxide and acid concentrations (Figure 2).[8] The peroxovanadium(V) species then oxidize the halide, although the detailed mechanism of this reaction is not yet known: the halide could be oxidized directly by bound peroxide, retaining the vanadium(V) moiety, or the halide could coordinate or reduce vanadium(V), before reduction of bound peroxide occurs. The two main differences between the biomimetic system and V-BrPO are the rate of catalysis and the pH dependence, with a much greater enzyme rate at higher pH. Further mechanistic studies are in progress to address the role of the protein in the enzymatic activity by investigating the role of oxovanadium(V) ligands in mediating the rate of halide oxidation and the pH dependence.

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Supplementary Material Available: Listing of details of experimental procedures for Figures 1-3 (2 pages). Ordering information is given on any current masthead page.

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Crystallographic and ^6Li and ^{15}N NMR Studies of a Chiral Bidentate Lithium Amide. An Effect of Aggregation States on an Enantioselective Deprotonation Reaction

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Asymmetric synthesis using chiral lithium amides has received much attention in recent years.[2] Previously we reported highly enantioselective reactions involving deprotonation of cycloalkanones,[3] aldol condensation,[4] and alkylation[5] by employing chiral bidentate lithium amides ((*R*)-**1**, Y = OR, NR_2). On the basis of our earlier studies on diastereoselective alkylation of chiral chelated lithio enamines,[6] we designed (*R*)-**1** using the following three hypotheses: (1) five-membered chelated structures will be formed as shown in (*R*)-**2**; (2) a chiral amide nitrogen will be formed efficiently since a bulky alkyl group on the amide nitrogen should be exclusively trans to the bulky phenyl group on the chiral carbon for steric reasons, i.e., the lone pair on amide nitrogen will be fixed cis to the phenyl group; and (3) the aggregation state of (*R*)-**1** in solution can be controlled by adding an external ligand such as HMPA (hexamethylphosphoric triamide) to satisfy the

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