A Functional Mimic of Vanadium Bromoperoxidase

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The unique marine metalloenzyme vanadium bromoperoxidase (V-BrPO) catalyzes the oxidation of chloride, bromide,2,3 and iodide4 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 halogen equivalent of hydrogen peroxide, producing dioxygen, which in the case of bromide is known to be in the singlet excited state (\(\text{O}_2^*\)) (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 \(\text{^1H NMR}\).5 Under conditions of excess TMB to H\(_2\)O\(_2\) (as in Figure 1), the overall stoichiometry of the reaction is consumption of 1 equiv each of H\(_2\)O\(_2\) and TMB per equivalent of Br-TMB produced.6 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\(_2\)SO\(_4\)) than V-BrPO, which functions maximally at pH 5-7, with a turnover rate of 4.7 \(\times\) 10\(^{13}\) mol of Br product/(mol of enzyme) h\(^{-1}\) at pH 6.5.5

The dioxovanadium(V)-catalyzed bromination of TMB was also followed by \(\text{\textsuperscript{51}V NMR}\) (Figure 2). The \(\text{\textsuperscript{51}V NMR}\) chemical shift of 0.5 mM dioxovanadium(V) in the reaction medium is -540 ppm (Figure 2a). Addition of 5 mM H\(_2\)O\(_2\) forms a mixture of monooxygenovanadium(V) [VO\(_2\)(OH)]\(^{2-}\), -687 ppm; Figure 2b] and dioxovanadium(V) [VO\(_2\)(OH)]\(^{2-}\)\(_2\), -687 ppm; Figure 2b] and dioxoperovovanadium(V) \([\text{VO}_2(O_2)^+]\), -529 ppm; Figure 2b] consistent with previously reported chemical shifts for these species.6 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 \(\text{\textsuperscript{51}V NMR}\) 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\(_2\)O\(_2\), 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\(_2\)O\(_2\) (Figure 3b) nor the reaction of Br\(^-\) and H\(_2\)O\(_2\) 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\(_2\)O\(_2\). 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 if H\(_2\)O\(_2\) is greater than [TMB].

Figure 1. GC analysis of the dioxovanadium(V)-catalyzed bromination of TMB. Conditions: 0.5 mM NH\(_4\)VO\(_3\), 6.66 mM TMB, 5.0 mM H\(_2\)O\(_2\), 0.43 M KBr in 0.05 M HClO\(_4\)/25% MeOH. (a) TMB in control reaction; (b) Br-TMB in catalytic reaction; (c) TMB in control reaction without VO\(_2\)^{2+}.

Figure 2. \(\text{\textsuperscript{51}V NMR}\) stack plot of the dioxovanadium(V)-catalyzed bromination of TMB. Conditions are the same as in Figure 1. (a) 0.5 mM NH\(_4\)VO\(_3\), in 0.05 M HClO\(_4\)/25% MeOH; (b) a + 5 mM H\(_2\)O\(_2\) + 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 \(\text{\textsuperscript{51}V}\) signal area is shown in parentheses.

Scheme I

\[\text{H}_2\text{O} + X \rightarrow \text{'intermediate'} (\text{X}' ; \\text{Enzyme-bound or released}) \]

\[\text{pH} = 5-7\]

\[\text{H}_2\text{O} + \text{X}' \rightarrow \text{\textsuperscript{51}V\text{ORG}} \]

\[\text{H}_2\text{O} + \text{X} + \text{H}_2\text{O} \rightarrow \text{O}_2 + \text{X} + \text{H}_2\text{O} \]

\(\text{V-BrPO}\)

(6) \(\text{\textsuperscript{31}P NMR}\) for Br-TMB (300 MHz) in CD\(_3\)OD: \(\delta = 6.172\) (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-TMB (M\(^+\)/m/e 324, 326, 328; correct relative intensities) is produced if [H\(_2\)O\(_2\)] is greater than [TMB].
(8) Campbell, N. J.; Dangel, A. C.; Griffith, W. P. Polyhedron 1989, 8, 1379-1386. Our \(\text{\textsuperscript{51}V NMR}\) chemical shifts of VO\(_2\)(OH)\(^{2-}\) and VO\(_2\)(OH)\(^{2-}\)\(_2\) 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.
vanadium(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.

Crystallographic and \(^{6}\)Li and \(^{18}\)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.\(^{12}\) Previously we reported highly enantioselective reactions involving deprotonation of cycloalkanes,\(^{13}\) aldo condensation,\(^{14}\) and alkylation\(^{15}\) by employing chiral bidentate lithium amides ((R)-1, \(Y = \text{OR, NR}_2\)). On the basis of our earlier studies on diastereoselective alkylation of chiral chelated lithium enolates,\(^{16}\) 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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Figure 3. Dioxygen formation in the bromide-assisted disproportionation of hydrogen peroxide. Conditions are the same as in Figure 1. O₂ was measured with a YSI Clark-type electrode. (a) VO₂⁺ + Br² + H₂O₂ (b) VO₂⁺ + H₂O₂(c) VO₂⁺ + Br⁻ + H₂O₂ + TMB. Note: The solubility of O₂ 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 H₂O₂ in acid. Peroxovanadium(V) species oxidize iodide; however, dioxovanadium(V) is reduced by iodide in acid,\(^{9}\) consistent with the observed decrease in the \(^{1}J\) 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⁺ 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 oxidative disproportionation of hydrogen peroxide, analogous to the reactivity of the V-BP0 enzyme (Scheme II; in this scheme, HOBr, Br² and Br⁻ are in rapid equilibration). In this biomimetic system, dioxovanadium(V) coordinates H₂O₂ 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-BP0 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 oxo-