

Spectroscopic and Functional Modeling Studies of Vanadium Bromoperoxidase

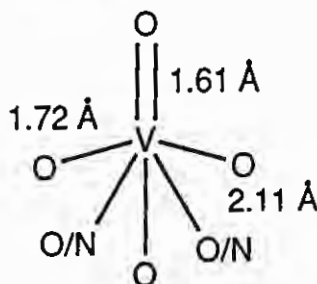
Alan Gengenbach

Literature Seminar

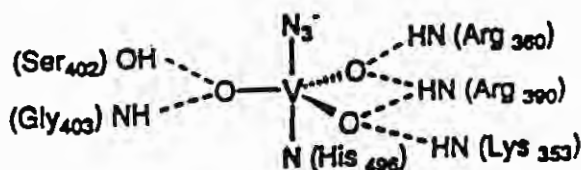
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Haloperoxidases have been isolated from numerous sources including a large number of marine organisms.¹⁻³ Numerous peroxidases are known to require heme as the essential cofactor. Recently, peroxidases isolated from some marine algae were found to be devoid of heme. A bromoperoxidase was isolated from *A. nodosum* and vanadium proved to be the essential metal for activity. Vanadium bromoperoxidase (V-BrPO) catalyzes the oxidation of bromide ions in the presence of hydrogen peroxide. The enzyme has been more recently shown to have a low specific activity for chloride oxidation.⁴ The final products in the reactions are brominated organic substrates or, in the absence of organic substrate, singlet dioxygen.^{5,6} Substituted indoles and other aromatic compounds are substrates for V-BrPO.⁷ Vanadium bromoperoxidase and the even more recently discovered vanadium dependent nitrogenase are the only known examples of metalloenzymes requiring vanadium. Spectroscopic and functional modeling studies have been performed to address the nature and function of the vanadium site in V-BrPO.

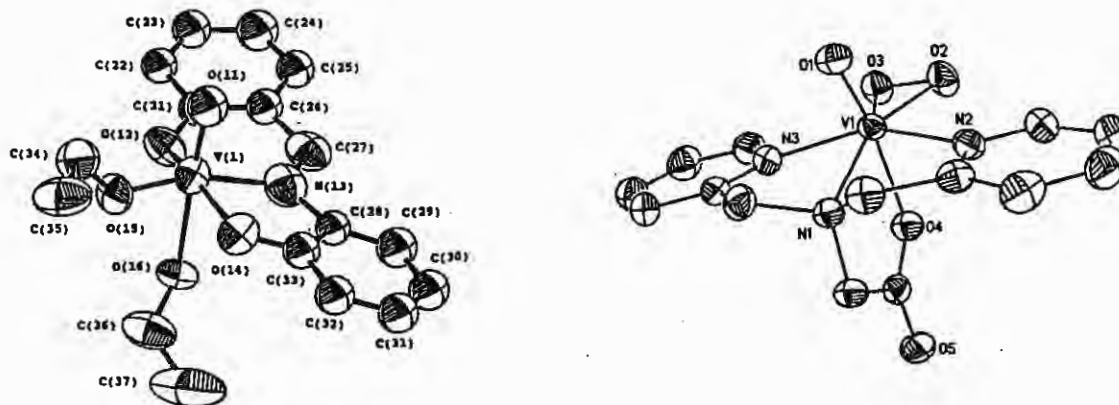
Vanadium bromoperoxidase has been studied by EPR, XANES, EXAFS, and ⁵¹V NMR spectroscopy. The native form of V-BrPO is EPR silent and no EPR signal is observed under turnover conditions, suggesting that the active form of the enzyme contains V(V) and that vanadium is not reduced in the catalytic cycle. The EPR spectra of reduced V-BrPO contains 16 lines and is consistent with a V(IV)-oxo species.⁸ The XANES spectra contain the intense pre-edge feature characteristic of vanadium-oxo species.⁹ EXAFS data of the reduced and native forms of the enzyme show the presence of oxygen and nitrogen donors.⁹ ⁵¹V NMR spectroscopy reveals a novel chemical shift for vanadium and is explained by coordination of vanadium by six or seven oxygens.¹⁰



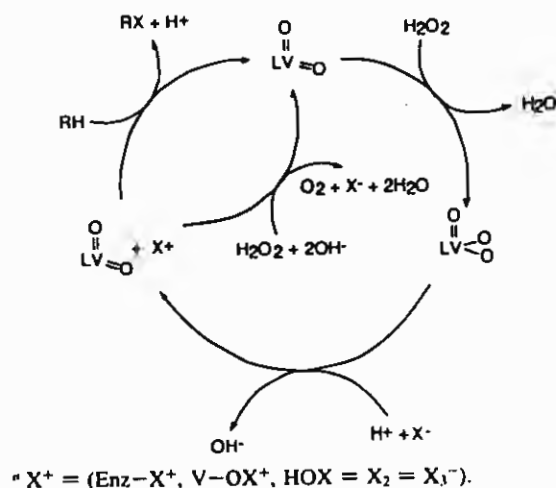
Single crystal X-ray structural information is not available for V-BrPO, although preliminary structural analysis data have been reported.^{11,12} The crystal structure of the azide form for a related vanadium chloroperoxidase (V-CIPO) from *Curvularia Inaequalis* has been solved.¹³ This structure reveals the coordination sphere of vanadium to consist of three non-protein oxygen atoms, a nitrogen donor from the bound azide and a single histidine residue. Preliminary data on the azide free form of V-CIPO suggest that the apical position is replaced by an oxygen atom in the resting state. Similarities in both reactivity, spectroscopy, and the conservation of critical active site residues of V-BrPO and V-CIPO suggest the structure of the active sites should also be similar.^{3,13}



Functional modeling studies have been successful in mimicking the reactivity observed in the natural system. Cis-dioxovanadium is a catalyst precursor for the oxidation of bromide in acidic solution.^{14,15} The active oxidant was shown to be the dioxotriperoxovanadium dimer. Although a good functional model, cis-dioxovanadium is a poor structural model because the active site is not dimeric. Vanadium complexes of Schiff-base ligands also catalyze bromide oxidation.¹⁶ The complexes have been structurally and spectroscopically characterized and their reactivity is consistent with generation of an electrophilic oxidized bromine intermediate. A series of monoperoxo complexes exhibiting bromoperoxidase activity are known and have been structurally characterized.^{17,18} These peroxide complexes are model compounds for the proposed peroxide intermediate in the catalytic cycle of V-BrPO.



A mechanism for V-BrPO based on enzyme kinetic studies, spectroscopic studies and the functional model compounds has been proposed.¹⁸ The proposed mechanism is consistent with all the data present for V-BrPO and the functional model systems.



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