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Peroxidative Halogenation Catalyzed by Vanadium Bromoperoxidase: Mechanism and Reactivity

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Gretchen Eileen Meister Winter

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Committee in charge:

Professor Alison Butler, Chairperson
Professor Hellmut Eckert
Professor William Kaska
Professor Marcello DiMare

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Abstract

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Peroxidative halogenation catalyzed by vanadium bromoperoxidase (V-BrPO) from the marine alga *Ascophyllum nodosum* was investigated using a broad range of experimental approaches.

V-BrPO is irreversibly inactivated under turnover conditions at pH 4.0. Inactivation is concurrent with oxidation of histidine to 2-oxohistidine. At low pH, HOBr reacts with N^{α}-benzoylhistidine to form N^{α}-benzoyl-2-oxohistidine. V-BrPO no longer coordinates vanadium upon inactivation. Inclusion of a bromoperoxidase substrate, i.e., monochlorodimedone, protects against inactivation. Functional mimics of V-BrPO, i.e. VO_2^+, oxidize N^{α}-benzoylhistidine in the presence of hydrogen peroxide and bromide to N^{α}-benzoyl-2-oxohistidine.

Oxodiperoxomolybdenum(VI) and oxodiperoxotungsten(VI) are effective catalysts for the oxidation of bromide by hydrogen peroxide in acid. The reactions are first order in metal complex and bromide, and independent of hydrogen peroxide. Catalysis mediated by molybdenum(VI) is inhibited by chloride.

The major products of the reaction of luminol, V-BrPO, H_2O_2, and KBr are brominated luminols. Luminol is preferentially brominated by V-BrPO over phenol red. Luminol decreases the rate of dioxygen evolution by V-BrPO. V-BrPO quenches the fluorescence of luminol at pH 6. A binding constant of $2.62 \times 10^5\ M^{-1}$ for luminol and V-BrPO was calculated from Stern-Volmer analysis. Bromination of luminol by V-BrPO at pH 8.0 results in luminescence with a λ_{max} at 425 nm which shifts over time to 441 nm.

The major product of bromination of 1,3-di-*tert*-butylindole at pH 6.0 catalyzed by V-BrPO is the 2-oxo derivative, while reaction of 1,3-di-*tert*-butylindole with HOBr also results in the formation of other brominated products. V-BrPO prevents bromination on the sterically preferred benzene ring of the indole.

Photoaffinity labeling of V-BrPO with 5-azido-2-phenylindole results in specific crosslinking to the enzyme. Amino acid analysis of photolabeled V-BrPO shows losses of phenylalanine, leucine, and isoleucine relative to native V-BrPO.

Irradiation of dilute (nM) V-BrPO at 308 nm results in vanadium-dependent and time-dependent inactivation. The enzyme loses affinity for vanadium upon irradiation, and no cleavage is observed by SDS-PAGE. Oxygen is not required for inactivation. Amino acid analysis shows losses of glycine, alanine, and proline, and gains in aspartate, glutamate, and threonine in photolyzed V-BrPO relative to native V-BrPO.