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Investigation of Vanadium Bromoperoxidase: Kinetics and Mechanism

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Helena Setyarini Soedjak

Committee in charge:

Professor A. Butler, Chairperson

Professor P.C. Ford

Professor T.M. Hooker

Professor S.M. Parsons

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Abstract

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Vanadium bromoperoxidase (V-BrPO) was purified from the marine brown algae *Ascophyllum nodosum*, *Fucus distichus* and *macrocystis pyrifera*. V-BrPO catalyzes the oxidation of bromide or chloride by hydrogen peroxide, resulting in the bromination or chlorination of certain organic acceptors or the formation of dioxygen. This study provides the first evidence for a nonheme haloperoxidase of marine origin to catalyze the oxidation of chloride. Chloride is a competitive inhibitor with respect to bromide. The steady-state kinetics of bromide- or chloride-assisted dioxygen formation catalyzed by V-BrPO is consistent with Bi Bi Ping Pong mechanism. V-BrPO can also use peracids (i.e., peracetic acid, phenyl peracetic acid, m-chloroperoxy benzoic acid and p-nitroperoxy benzoic acid) as the source of peroxide, but not alkyl hydroperoxides.

At low H_2O_2 concentrations, the rates of MCD halogenation and dioxygen formation are similar, indicating that both processes proceed through a common intermediate formed in a rate limiting step. The stoichiometry of H_2O_2 consumed to dioxygen produced or monochlorodimedone reacted is 2 or 1, respectively. $H_2^{18}O_2$ -labelling experiments show that the oxygen atoms in dioxygen originate from the same molecule of H_2O_2 . At higher concentrations of H_2O_2 , H_2O_2 competes with monochlorodimedone for the intermediate. The degree of competition increases with increasing H_2O_2 concentration and pH. At

high concentrations, H_2O_2 inhibits V-BrPO by a noncompetitive type of mechanism. The inhibition is stronger at higher pH values and is reversible. An ionizable group with a pK_a between 6.5-7.0 is involved in the inhibition. Similar to H_2O_2 , bromide also inhibits V-BrPO by a noncompetitive-type of mechanism. The inhibition for V-BrPO from *M. pyrifera* occurs most strongly at pH 5.0-5.5 and for V-BrPO from *F. distichus* at pH 5.5-6.0.

V-BrPO is inactivated by phosphate due to a substitution of vanadate by phosphate. The rate of inactivation increases with decreasing pH. The inactivation can be prevented by H_2O_2 , probably due to its coordination to vanadium-bound BrPO. In addition, azide irreversibly inactivates V-BrPO by a mechanism-based inactivation process.

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