Thesis Title Haloperoxidases and Their Analytical Diagnostic Applications.

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ABSTRACT

Haloperoxidases are group of enzyme that catalyze the peroxidative halogenation reaction in the presence of hydrogen peroxide, a halide ion and a halogen acceptor. These enzymes can be classified into 3 major groups according to the range of halide ions that they utilize, chloroperoxidase, bromoperoxidase and iodoperoxidase, respectively. Iodoperoxidase utilizes iodide ion while bromoperoxidase can additionally use bromide ion. Chloroperoxidase usually accepts chloride, bromide and iodide ions as substrates.

In this group, the most popular enzyme applied in coupled enzyme assays and enzyme immunoassays is horseradish peroxidase. Chloroperoxidase from *Caldariomyces fumago* has been extensively studied for several applications as a synthetic tool and as an analytical diagnostic reagent.

Thai seaweeds naturally grown along the seacoast of the Gulf of Thailand contain bromoperoxidase activity. Bromoperoxidase from the seaweed *Gracilaria changii* was partially purified using 50 % ethanol precipitation and DEAE cellulose chromatography. The product was purified 20.11 fold to yield a specific activity of 12.27 U/mg protein. Chloroperoxidase from *C. fumago* was also partially purified using DEAE cellulose chromatography. This product was purified 4.17 fold to yield a specific activity of 1079.3 U/mg protein.

In studies of analytical applications of haloperoxidases, a spectrophotometric method for measuring bromide ion concentration was developed. The assay reaction was based on the change of color from yellow to purplish blue through the bromination of phenol red to bromophenol blue catalyzed by bromoperoxidase, chloroperoxidase and lactoperoxidase in the presence of bromide ion and hydrogen peroxide. The assay method using bromoperoxidase as the brominating enzyme gave an assay range between 25 - 400 μM and with % recovery and % coefficient of variation ranges of 96.4 - 101.1 % and 1.48 - 2.65 %, respectively.