

Thesis Title: Bromoperoxidase from Thai Seaweeds

Name: Bancha Veokeki

Degree: Master of Science (Biochemistry)

Thesis Supervisory Committee:

Bhinyo Panijpan

Pintip Ruenwongsa

Pichit Tosukhowong

Amaret Bhumiratana

Date of Graduation: May 23, 1988

#### ABSTRACT

Thai seaweeds were surveyed for haloperoxidase activity. Monochlorodimedone, C.I. 421 and dye No.143 were used as substrates. Only bromoperoxidase activity was found. The isolation and purification of bromoperoxidase from the marine red algae, *Polycarvernosa*, is described. By using a DEAE-cellulose column, 48% yield and 12 fold purification of bromoperoxidase was obtained. The bromoperoxidase can utilize bromide ions and iodide ions in the presence of hydrogen peroxide and a halogen acceptor.

Based on gel filtration by Sephadex G-100 column the molecular weight of bromoperoxidase was 54,000 and at least 4 isoenzyme bands were seen when isoelectric focusing was performed. It was found that partially purified bromoperoxidase was better kept at 4<sup>0</sup>C than at -20<sup>0</sup>C and lyophilization. The apparent Km value for monochlorodimedone

was  $2.2 \times 10^{-5} \text{M}$ , that for  $\text{H}_2\text{O}_2$  was  $9.1 \times 10^{-5} \text{M}$  and KBr was  $1.1 \times 10^{-2} \text{M}$ . For C.I.421 as substrate,  $K_m$  value was  $1.2 \times 10^{-5} \text{M}$  whereas the apparent  $K_m$  value for  $\text{H}_2\text{O}_2$  was  $7.2 \times 10^{-5} \text{M}$  and KBr was  $1.7 \times 10^{-2} \text{M}$ . In case of dye No. 143 as substrate, its apparent  $K_m$  value was  $5.1 \times 10^{-6} \text{M}$  while  $K_m$  for  $\text{H}_2\text{O}_2$  was  $1.3 \times 10^{-4} \text{M}$  and that for KBr was  $1.5 \times 10^{-2} \text{M}$ . Bromoperoxidase from *Polycarvernosa* was inhibited by hemoprotein inhibitors  $\text{NaN}_3$  and KCN. It was also inhibitable by high concentrations of substrates. Some properties of partially purified bromoperoxidase such as pH optimum, pH stability and heat stability are reported. The optimum pH of the enzyme was 5.8, and the enzyme was stable in a range from pH 6.0 to 11.0. The enzyme was heat-stable. It resisted denaturation at  $50^\circ\text{C}$ . Staining the activity of bromoperoxidase was performed by using dye No. 143.

For detecting the bromoperoxidase activity without spectrophotometry, the agarose gel and the filter paper were developed as the new convenient methods. Dye No.143 with agarose gel and filter paper were used for detecting the bromoperoxidase activity from various marine algae.