Thesis Title Purification and Characterization of Bromoperoxidase from Thai seaweeds Polycarvernosa sp.

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ABSTRACT

Bromoperoxidase was purified from Thai red seaweeds Polycarvernosa sp. by alcohol precipitation, alkaline precipitation, DEAE cellulose column chromatography and Sephadex G-100 column chromatography. Two types of bromoperoxidase, peak I and peak II, were obtained; they were different in several properties, including kinetic property. Peak I bromoperoxidase had a molecular weight of 71,000 daltons whereas peak II enzyme had a molecular weight of 45,000 daltons as determined by Sephadex G-100 column chromatography. The isoelectric point of peak I and peak II bromoperoxidase were 4.7 and 6.8 respectively. The optimum pH of peak I bromoperoxidase was 5.0 whereas that of peak II enzyme was 5.8. Peak I bromoperoxidase was stable in an acidic pH range from 5 to 9 whereas peak II enzyme was stable in an alkaline pH range from 7 to 12. The optimum temperature of

peak I and peak II bromoperoxidase were 50 °C and 55 °C respectively. Peak I and peak II enzyme had similar thermal stability; 50 % of the activity was lost after incubation at 55 °C for 30 min. The spectrum of peak I bromoperoxidase showed the Soret band at 403 nm, a characteristic of hemeprotein. However, the Soret band was not found in the spectrum of peak II enzyme indicating that peak II bromoperoxidase was not a hemeprotein. Both peak I and peak II enzyme catalyzed the bromination of monochlorodimedone (MCD), phenol red and xylene cyanole FF. The Km values for MCD, phenol red and xylene cyanole FF of peak I were 6.9×10^{-6} M, 4.4×10^{-6}M and 3.1×10^{-6}M respectively. The Km values for MCD, phenol red and xylene cyanole FF of peak II were 6.4×10^{-5}M, 1.5×10^{-5}M and 2.7×10^{-5}M respectively. The Km values for H_2O_2 of peak II were in the range of 10^{-5}M for all three organic substrates and the Km values for KBr of peak II enzyme were in the range of 10^{-3}-10^{-4}M. Activities of both peak I and peak II bromoperoxidase were inhibited by azide and cyanide, however peak I was more susceptible to azide inhibition than peak II enzyme. Effect of some effectors on activities of both peak I and peak II bromoperoxidase were also studied.