Thesis Title	Effects of Chemical Modification on Enzyme Activity and Stability of Bromoperoxidase from A Thai Seaweed
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ABSTRACT

Bromoperoxidase was extracted from Thai red seaweed, *Polycarvernosa* sp. and purified by using 0-60% ammonium sulphate precipitation, DE 52 column chromatography and fast protein liquid chromatography by using MonoQ column and Superose12 column. Two isoenzymes, BPO1 and BPO2 were separated in the purification step of DE52 column chromatography. After purification by Superose12 column the specific activity of BPO1 and BPO2 were 61.86 and 330.58 mU/mg, respectively. BPO1 and BPO2, were non-heme enzymes that could be activated 2.5 and 3.5 fold by V_2O_5. Chemical modification of BPO1 by 2,4,6 -trinitrobenzenesulfonic acid and iodoacetamide caused an enhancement in the enzyme activity and stability while the modification by N-acetylimidazole, diethylpyrocarbonate, 5, 5'-dithiobis (2-nitrobenzoic acid) and 1,2-cyclohexanedione did not cause any change in enzyme activity and stability. The modification of BPO1 by N-bromosuccinimide, 2-hydroxy-5-nitrobenzyl bromide, carbodiimide and iodoacetic acid caused a decrease in

the enzyme activity. Chemical modification of BPO2 by 2,4,6-trinitro-benzenesulfonic acid, N-bromosuccinimide, 2-hydroxy-5-nitrobenzyl bromide, diethylpyrocarbonate, carbodiimide, iodoacetic acid and iodoacetamide inactivated the enzyme activity while modification by N-acetylimidazole, o-nitrophenylsulfenyl chloride and 1,2-cyclohexanedione did not change the enzyme activity. None of the modifying agent used in this study increased the enzyme activity or stability. The modification of BPO1 by iodoacetamide enhanced the enzyme activity about 500% and enhanced thermostability in both acidic and alkaline condition. K_m values of iodoacetamide-modified BPO1 for monochlorodimedone, potassium bromide and hydrogen peroxide were 1.43×10^{-5}, 1.54×10^{-2}, and 9.09×10^{-7} M respectively while the K_m values of the native enzyme were 2.94×10^{-5}, 2.17×10^{-4} and 1.00×10^{-4} M, respectively.

BPO1 contained two isoenzymes, BPO1.1 and BPO1.2 were isolated by using non-denaturing electrophoresis. From amino acid analysis of iodoacetamide-modified BPO1.1 and BPO1.2, the modified lysyl residues were 14 and 16 residues of the total lysyl residues of 42 and 49.5 residues, respectively at 18 hours of incubation at 35°C pH 7.0 while the modified histidyl residues were 6 and 9.5 of the total histidyl residues of 30 and 21.6 residues, respectively. The results suggested that the modified lysyl and histidyl residues might be involved in the enhancement of the enzyme activity and stability of BPO1.