

4136783 SCBC/M : MAJOR : BIOCHEMISTRY ; M. Sc (BIOCHEMISTRY)

KEY WORD : PEROXIDASE, CASSAVA

KANJANA SURIYAPROM : CHARACTERIZATION OF PEROXIDASE FROM CASSAVA LEAVES. THESIS ADVISOR : MONTRI CHULAVATNATOL, Ph. D., M.R. JISNUSON SVASTI, Ph. D., NUANCHAWEE WETPRASIT, Ph.D. 94 p. ISBN 974-664-247-2

Peroxidase (EC 1.11.1.7) is an ubiquitous plant enzyme that catalyzes the oxidation of cellular components by H_2O_2 . A peroxidase was purified from the leaves of cassava (*Manihot esculenta* Crantz.) 74 folds to a specific activity of 386 U/mg. The purification procedure consisted of 60-80% ammonium sulfate precipitation, followed by affinity chromatography using a concanavalin A Sepharose 4B column and gel filtration chromatography using a Sephadex G-200 column. The native molecular weight for the enzyme was found to be 112 kD by gel filtration and the subunit molecular weight was estimated to be 56 kD by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. So the enzyme was a homodimer. The isoelectric point of the purified enzyme was estimated by polyacrylamide isoelectrofocusing. It existed in two forms with pI values of 6.4 and 6.25. The enzyme contained a higher amount of the amino acids (GLX +ASX) than the basic amino acids. It was shown to be heme proteins with a Soret band at 404 nm. The enzyme was stable in a broad pH range of 4-11 and had a slightly acidic optimum pH of 6. An optimal temperature of the enzyme activity was 60°C. The enzyme retained about 70% of its activity during incubation at temperature upto 65°C for 24 hr. The cassava leaf peroxidase catalyzed the oxidation of the following substrates: coniferyl alcohol ($K_m = 0.003$ mM), o-dianisidine ($K_m = 0.037$ mM), quercetin ($K_m = 0.054$ mM), syringaldazine ($K_m = 0.077$ mM), 3,3'-diaminobenzidine ($K_m = 0.022$ mM), pyrogallol ($K_m = 0.89$ mM) and guaiacol ($K_m = 5.52$ mM). KCN, NaN_3 and thiourea were inhibitory to the enzyme.