ABSTRACT

Membrane bound Borassus flabellifer peroxidase was purified bysalting-out, salting-inand DEAE - Cellulose anion exchange chromatographic technique to apparent homogeneity. Relative molecular weight under denaturating condition was around 40 kDa. The preparation had single isoenzyme as evidenced under non denaturating condition. It was a glyco- and haemoprotein. It retained 100% activity for 120 hours at 70° C. pH optima with Benzidine, O-Dianisidine, Guaiacol and Tetra Methyl Benzidine were around 5.0. Kinetic studies showed it had higher affinity towards Tetra Methyl Benzidine than other three substrates. Gibb's free energy changes of the enzyme at 30°C with Benzidine, o-Dianisidine, Guaiacol, and Tetra Methyl Benzidine was -24,-17, -7 and -50KJ/mole respectively. It obeyed Ping-Pong kinetics. The fluorescence intensities of enzyme increased linearly as hydrogen peroxide concentration increased due to exposure of its hydrophobic moiety to the environment. Peel staining with Guaiacol substantiated it as membrane bound protein. BFP was ionically interacting with stone parts of its fruit. The apparently homogeneous membrane bound peroxidase was reversibly inhibited by various aromatic alcohols and its IC₅₀ values were determined. Dixon plot clearly showed mixed type of inhibition. k_i values of peroxidase-inhibitor complexes were determined. The homogenous peroxidase had non- covalently interacting triglycerides or triglyceride esterified phytosterols. This peroxidase was interacting with acid hydrolysable low density lipoprotein but not with high density lipoprotein. This may be one of the reasonsfor its stability, catalysis in organic solvents and non consumption by human beings.Immunoprecipitation experiments (IgY) indicated that the epitope present in BFP and HRP are same. Cibacron Blue G F3A interacted with BFP.Derivitization of BFP indicated the proper positioning of lysine and tyrosine that was essential for maintaining its catalytic activity. Further studies may prove it as lipophilic enzyme. These waste stone parts may be utilized in extracting phytosterols and fatty acids which have medicinal value.