Thesis Title Property and Functional Study of Rubber Latex Lectin.

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ABSTRACT

Lectin in B-serum of centrifuged rubber latex was purified to homogeneity with molecular weight of 38 kD dalton comprising two different subunits of 25.6 kD and 15.7 kD. The purified B-serum lectin was stable in the range of pH 6-10. The lectin showed wide spectrum of red blood cell hemagglutination. Sugar specificity study of B-serum lectin showed inhibition of hemagglutination by several monosaccharides and certain glycoproteins. It was also found to be a Ca^{2+}-dependent lectin.

Peroxidase enzyme was found in *Hevea* bark extract and purified to homogeneity with molecular weight of 50 kD as monomeric protein. The *Hevea* peroxidase enzyme display a very high specific activity, up to several thousand folds higher than the commercial enzyme. Optimal pH of the enzyme was found to be around 5.5 with thermal stability up to 50°C. The glycoprotein nature of the enzyme made it possible to be easily purified with Con A-Sepharose affinity chromatography.

Study on interaction between B-serum and *Hevea* bark extract showed the presence of lectin in bark extract which induced aggregation and precipitation of B-serum glycoproteins. Three specific glycoproteins in B-serum of molecular weight 31.6, 27.8 and 18 kD were precipitated by bark lectin. The bark lectin showed narrow

range of red blood cell hemagglutination which was different from the B-serum lectin. The lectin activity was lost upon protease treatment, confirming the protein nature of bark lectin. Sugar specificity study showed only glycoprotein fetuin and asialofetuin could inhibit hemagglutination. The bark lectin was found to be heat stable up to 60°C and 50% activity remaining at 70°C. It was also found to be stable over a broad pH range of 5-11.

Peroxidase-lectin, a bifunctional protein, was found in *Hevea* bark extract upon purification by DEAE and Bio-gel column chromatography. The co-purification of peroxidase and lectin confirmed that the two activities were present in the same protein molecule. Peroxidase-lectin was purified to homogeneity with native molecular weight of 141 kD. It was a tetrameric protein composing of the same monomer of molecular weight 36.5 kD. Peroxidase-lectin as a bifunctional protein displayed dual functions on the same entity. This was the first report on the discovery of peroxidase-lectin, an enzymic lectin. Study on kinetics of peroxidase-lectin revealed that it had different kinetics parameters from peroxidase enzyme. However, they were both sensitive to inhibition by cyanide and azide which indicated that they were both the heme proteins. The sensitivity to cyanide and azide inhibition was about the same when considering the K_i values from the Dixon plots. The results reflected the fact that both heme proteins were subjected to similar degree of sensitivity to the effect of inhibitors despite the finding that they were of different molecular nature.