Thesis Title Gluosyltransferase from cassava (*Manihot esculenta* Crantz.)

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ABSTRACT

A glucosyltransferase which catalyzes the conjugation of acetone cyanohydrin to glucose in the last step of linamarin biosynthesis was isolated from young leaves of cassava (*Manihot esculenta* Crantz.). The glucosyltransferase was found to be a soluble enzyme and was partially purified by 40-80% ammonium sulphate fractionation followed by Sephadex G-200 column and Mono Q HR 5/5 column chromatography. These steps resulted in a purification of 6 folds. The partially purified glucosyltransferase possessed negligible linamarase and little UDP-glucose hydrolysis activity. The native molecular weight of cassava glucosyltransferase was 46,000. The partially purified preparation consisted of five major bands of 13,000; 21,000; 25,000; 31,000 and 39,000 separated by polyaccrylamide gel electrophoresis in the presence of sodium dodecyl sulphate. The enzyme activity was unstable but could be kept at -20^{o}C for 17 days with 50% loss of the activity.

Catalytically, the partially purified glycosyltransferase showed a pH optimum of 9 to 10 and a temperature optimum of 37°C. The substrate specificities were also analyzed. UDP-glucose was the best sugar donor with a K_m of 0.15 mM which ADP-glucose was not and UDP-galactose was only half as good as UDP-glucose. Among the sugar acceptors tested, acetone cyanohydrin was the best and better than butanone cyanohydrin. Other aliphatic alcohols were poorer sugar acceptors in the following order: isopropanol, butanol, ethanol and glycerol. Phenol was unable to serve as the sugar acceptor for this enzyme.

The enzyme was unable to catalyze the transfer of glucose from UDP-glucose to starch, linamarin or simple sugars i.e. glucose, galactose and sucrose. However, a high glucosyltransferase activity was detected in the presence of UDP-glucose and fructose with a K_m for fructose of 4.4 mM. This activity could be due either to a contaminating UDP-glucose: D-fructose-glucosyltransferase or to cassava glucosyltransferase having two activities, one for linamarin synthesis and the other for sucrose synthesis.