Thesis Title Glycosidase Enzymes in the Cassava

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ABSTRACT

Cassava stem and petiole crude extract contained glycosidase enzyme with higher ability to hydrolyse PNP-β-monoglycosides than PNP-α-monoglycosides. Three major activity were found in both tissues are β-glucosidase, β-fucosidase and β-galactosidase, with higher activity being found at pH 6 than at pH 4 or pH 8. All three activities were precipitated in the 35%-65% saturated ammonium sulfate fraction, with β-galactosidase activity being lower than other enzyme in all tissues. Purified linamarase (EC.3.2.1.21) from cassava petiole, stem and root cortex were shown to have native Mr of about 600,000-2,000,000 on Sepharose 4B chromatography. Gel filtration showed that the high MW region possessed linamarase, β-glucosidase, β-fucosidase and β-galactosidase activities at the same position, and the subunit M_r of the enzyme was about 63,000 on SDS-PAGE. Another peak of β-galactosidase activity was found at the low molecular weight region, which did not contain a major band corresponding to M_r 63,000. Analysis of each fraction from Sepharose 4B chromatography on non-denaturing conditions showed ladder patterns for enzyme from the high MW region of all tissues, when stained for β-glucosidase, β-fucosidase and β-galactosidase activities. However, the pool from the low MW region gave a

single band staining only for β-galactosidase activity, suggesting that it is not linamarase enzyme. Linamarase from all three sources were glycoproteins. Root cortex linamarase had higher neutral sugar and sialic acid content than petiole and stem enzyme. Deglycosylation of linamarase with Peptide-N-Glycosidase F had no effect on size of linamarase from all three sources. Sialic acid removal by using neuraminidase from 2 sources caused no change in size and pI of linamarase from stem, petiole and root cortex, but had some effect on the extent of linamarase aggregation. Tryptic peptide mapping of linamarase both on paper and by HPLC showed similarities between enzymes from all three tissues, but each tissue also showed 1-3 distinctive peptides. Further structural analysis of these peptides will be required to determine whether these peptide differences are due to differences in amino acid sequence or glycosylation. Amino acid sequence analysis of linamarase from all three tissues with and without prior treatment with CNBr suggested that linamarase is blocked at the N-terminus.