Thesis Title	Cassava α-Hydroxynitrile lyase
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Abstract

α-Hydroxynitrile lyase (EC 4.1.2.10) is an enzyme involved in cyanogenesis which is responsible for cyanide toxicity associated with the consumption of cassava (*Manihot esculenta* Crantz.). It catalyses the dissociation of acetone cyanohydrin from linamarin into acetone and the toxic hydrogen cyanide. The enzyme was purified 35.6 folds from petioles of cassava to apparent homogeneity with 42.7 % recovery. The purification steps consist of 60-80% ammmonium sulfate precipitation, followed by gel filtration chromatography using a Sephadex G-200 column and anion-exchange fast protein liquid chromatography using a Mono Q HR 5/5 column. The native molecular weight for the enzyme was found to be 102,000 by gel filtration and the subunit molecular weight was

estimated to be 25,600 by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate so the enzyme was a homotetramer. The enzyme was highly stable at basic condition (pH 6-11) and had a narrow pH optimum of 5.0 with a K_m value for acetone cyanohydrin of 3.1 mM. An optimal temperature of the enzyme was found to be 40°C. The isoelectric point (pI) of the enzyme was estimated by polyacrylamide isoelectrofocusing to be 4.7. Several substrate analogs with alcohol or aldehyde groups were found to inhibit the enzyme activity. By kinetic analysis, the strong inhibitors were chlorobutanol (K_i = 3.0 mM), 2-methyl-2-butanol (K_i = 4.8 mM), isobutyraldehyde (K_i = 0.2 mM), and 2-methylbutyraldehyde (K_i = 1.6 mM). Acetone and methyl ethyl ketone were also inhibitors. By kinetic analysis, the alcohols and ketones were competitive inhibitors while the aldehydes were non-competitive inhibitors. By K_i analysis, the most potent inhibition required a 4-carbon structure. The inhibitory effect decreased gradually when the inhibitor had fewer than 4 carbons or more than 4 carbons.