Chemical and Enzymatic Stability of Phytohemagglutinin (PHA) Purified From Kidney Beans

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Crude phytohemagglutinin (PHA) was ammonium sulfate precipitated from milled kidney bean (CV. Montcalm) and purified by concanavalin A-agarose affinity chromatography. The purified PHA fraction, maintained in physiologically buffered saline, was exposed to the following treatments: (I) Chemical agents (120 minutes): 1) 2M NaCl; 2) 5M Urea; 3) 5% mercaptoethanol; 4) pH 3.0 and 5) pH 12.0. (II) Enzymatic digestions (incubated 180 minutes, 0.05 mg/ml): 1) pepsin (3200 units/mg protein); 2) trypsin (14,600 BAEE units/mg protein); 3) chymotrypsin (58 units/mg protein); 4) peptidase (100 units/g solid); 5) protease (6.0 units/g solid); 6) pancreatin (4 x NF grade); 7) alanine amino- peptidase (6.5 units/mg protein); 8) α-amylase (1900 units/mg protein); 9) β-amylase (850 units/mg protein); 10) α -mannosidase (17 units/mg protein); 11) and neuraminidase (19 units/mg protein).

Following treatment, sample mixtures were immediately assayed for hemagglutinating activity and subjected to electrophoretic analysis. Three replicates of this experiment were performed. The hemagglutinating activity of the purified and treated lectin was determined using the cell counting method of Coffey et al. (1985). Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was performed (10% acrylamide gel concentration) to resolve the component peptides according to their molecular weight (Weber and Osborne, 1969).

Results of these treatments are presented in Figure 1. Exposure to strong alkaline conditions (pH 12.0) resulted in significant decrease in PHA activity (38.8% of native activity) and changed the elution profile. There was a lack of the 0.30 band and the presence of stained areas from 0.40 to 0.60 and from 0.73 to 0.94. This indicated an increase in the concentration of lower molecular weight species. The additional band at 0.95 to 0.98 may be due to condensation of smaller proteins. All proteinases resulted in significant reduction in PHA activity (range, 1.5-11.0% of native activity). Digestion with proteolytic enzymes is characterized by a general lack of high molecular components in the gel patterns. Peptidase caused a broad diffuse staining from 0.68 to 1.0 along with the band at 0.58 and a minor band at 0.12 indicating reduction in molecular weight of the component peptides. Alanine amino peptidase treatment caused an increase in the number of higher molecular weight bands. Digestion with other proteolytic enzymes yielded similar patterns. Carbohydrases did not significantly reduce PHA activity. Incubation of the PHA with amylases and α-mannosidase yielded patterns that were identical to that of the untreated PHA. Neuraminidase treatment also yielded a pattern similar to the PHA except for the exclusion of the 0.3 band. Neuraminidase specifically cleaves the terminal sialic acid residue in the oligosaccharide moiety of the glycoprotein. The elimination of the Rₐ 0.3 band (58,000 Daltons) suggests that this is involved in the interchain association of the native PHA. Disassociation of the quaternary structure under these conditions would likely result in disruption of a 58,000 Dalton dimer.

Large decreases in the hemagglutinating activity of purified PHA may correspond to a decrease in the enterai toxicity therefore; the use of alkaline cooking or protease incubation treatments may be possible approaches for improving bean nutrient availability.
Figure 1. SDS-PAGE analysis of PHA (purified from kidney bean) exposed to selected chemical and enzymatic treatments. PHA activity (%) expresses percentage of native activity retained following treatment.