

E2-25 Isolation, purification and enzymatic properties of a minor acid proteinase from *Nepenthes distillatoria* (Badura)

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Insectivorous plants have evolved the capability to nourish directly on insects and other small invertebrates captured and thereby supplement its nutrition.

Insectivorous plant *Nepenthes* is available in plenty in Sri Lanka, and will be a good source of proteolytic enzymes and is an appropriate model to study mechanisms involved in regulation of synthesis, secretion and degradation of these enzymes. Isolation, purification procedure, cleavage specificity and enzymatic properties of acid proteinases of *Nepenthes* juice were reported previously.

The minor acid proteinase of *Nepenthes* was purified by elution through successive, columns of DEAE-cellulose, Sephacryl-S-200, Pepstatin-Sepharose and Mono Q to a homogeneous form or single band at the SDS-PAGE. About 0.2mg of the minor enzyme was purified from 30 l of the *Nepenthes* juice. Elution positions of the minor enzyme from DEAE-cellulose 52, Mono Q chromatography were different from those of the major one. The purified enzyme had a specific activity of 907 units/mg of enzyme towards the substrate haemoglobin. The molecular mass of the purified proteinase was estimated to be 35 kd and 43 kd by SDS-PAGE under reducing conditions and gel filtration, respectively and different from that of major acid proteinase (45-58Kd). Upon SDS-PAGE followed by periodic acid-Schiff's base staining, the protein band of major acid proteinase was stained well, but not the protein band of the minor enzyme, suggesting the presence of carbohydrate only in the major enzyme. The minor enzyme preferentially hydrolyzed denatured bovine haemoglobin at pH 2.0-3.0, releasing soluble peptides, but not free amino acids. Further significant activity was not observed above pH 7.0, suggesting in addition to the major proteinase, a minor proteinase present in *Nepenthes* juice is also an acidic proteinase. The proteolytic activity of minor enzyme was completely inhibited by 0.1mM pepstatin at pH 3.0, like major acid proteinase. However a significant effect was not observed with other proteinase inhibitors. Further, minor enzyme was stable at neutral pH, where pepsin loses its activity. These results suggest that minor proteinase of *Nepenthes* juice belongs to the family of non-pepsin type aspartic proteinase.