E2-02: NH_2-terminal amino acid sequence and enzymatic properties of novel acid proteinase from *Nepenthes distillatoria* (Badura)

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Proteinases participate in a wide variety of biological processes and most of these still remain to be clarified. Plant proteinases have received little attention in contrast to the well characterized mammalian proteinases. Insectivorous plant *Nepenthes* is available in Sri Lanka 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 and purification procedure of an acid proteinase from *Nepenthes* juice were reported last year. In this paper enzymatic properties and NH_2-terminal amino acid sequence of purified enzyme are presented.

Nepenthes acid proteinase was purified as reported previously. Effect of pH and temperature on proteolytic activity was investigated by determining activity of enzyme toward haemoglobin at different pHs and temperatures. Thermal stability of the enzyme was investigated by incubating the enzyme solution at different temperatures (30-90^{0}C) for about 1 h and determining the remaining activity of the enzyme. Susceptibility towards proteinase inhibitors was investigated by preincubating the enzyme with inhibitiors at 37^{0}C for about 10 min and determining the remaining activity. The NH_2-terminal amino acid sequence of the ensyme was analysed by submitting the purified protein onto automated gas phase sequencer (Applied Biosystem 477A).

Upon SDS-PAGE of the purified enzyme followed by the periodic acid Schiff staining, protein band was stained, suggesting the presence of carbohydrate.

Maximum activity of the enzyme was observed at pH 2.0-3.0 and significant activity was not detected at pH above 5.5, suggesting major proteinase present in *Nepenthes* juice is an acid proteinase. Optimum activity of the proteinase was observed at 50-60°C. Reduction of enzyme activity was not observed with 1h incubation at 30 - 55°C and gradual decreasing of activity was observed above 60°C. The complete loss of activity was observed with 1h incubation at 80°C. The proteolytic activity of enzyme was completely inhibited by 0.1 mM pepstatin at pH 3.0 However significant effect was not observed with other proteinase inhibitors. Further, enzyme was stable at the neutral pH where pepsin loses its activity. These results suggest that this enzyme belongs to the family of non pepsin type aspartic proteinase. The NH_2-terminal amino acid sequence was determined up to the 24th residue as follows:

IGPSGVETTVYAGDGEYLMXLSIG

Only a single residue was identified at each position of the sequence analysed except 20th residue. PTH amino acid was not detected at the 20th cycle of Edman degradation. This may be a probable glycosylation site. Computer homology search of this NH_2-terminal sequence with protein bank showed that it has less homology with other known proteins, suggesting *Nepenthes* proteinase may be a novel enzyme.

Nepenthes proteinase possesses characteristic properties: optimum temperature, optimum pH, thermal stability, stability at neutral pH, presence of carbohydrate and NH_2-terminal amino acid sequence suggesting this proteinase is a novel member of the family of non pepsin type aspartic proteinase. Investigation of substrate specificity, structure(primary, secondary and tertiary) and physiological function of this novel enzyme will provide information to understand structure function relationship and its application in medicine.