A-09: Purification and characterization of an acid proteinase from *Nepenthes distillatoria* (Badura)

Senarath B P Athauda[1], Sarath Malavipathirana[1],
Ponnamperuma A J Perera[1], Kenji Takahashi[2]
([1]Dept of Biochemistry, Faculty of Medicine, Univ of Peradeniya, [2]Dept of Biophysics & Biochemistry, Faculty of Medicine, Univ. of Tokyo, Hongo, Japan)

Plant aspartic proteinases have received little attention in contrast to the well characterized mammalian aspartic proteinases. Insectivorous plants, such as *Nepenthes distillatoria*, have evolved the capability to nourish directly on insects and other small invertebrates captured and thereby supplement its nutrition. The type of proteinases involved in this process and their mode of action is not clear.

The acid proteinase from *Nepenthes distillatoria* was purified and the physicochemical and enzymatic characteristics of the enzyme studied. An

assay procedure was developed to measure the proteolytic activity, using denatured haemoglobin as a substrate.

The proteinase was purified by elution through successive, columns of DEAE-Cellulose, Sephacryl S-200, Pepstatin-Sepharose and Mono Q to obtain a 217 fold purification with a specific activity of 864 units per mg of the proteinase. The molecular mass of the purified proteinase was estimated to be 68.0 Kd by SDS-PAGE under reducing conditions and 75.0 Kd by gel filtration.

The enzyme preferentially hydrolysed denatured bovine haemoglobin at pH 2.5, releasing soluble peptides, but not free amino acids. Pepstatin strongly inhibited the proteolytic activity of the enzyme suggesting that it belongs to the family of aspartic proteinases.