E2-27 Partial amino acid sequence of novel acid proteinase from *Nepenthes distillatoria* (Badura)

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Isolation, cleavage specificity and some of the enzymatic properties of acid proteinases of *Nepenthes* juice were reported previously. In this study the extended NH_2-terminal amino acid sequence and the 50% of the internal amino acid sequence of the enzyme and active site residues were determined at the protein level.

The NH_2-terminal amino acid sequence was determined by using an automated sequencer. To determine the partial internal sequence of the enzyme, Reduced and Carboxymethylated (RCM) protein was digested with endoproteinases and aliquots were subjected to HPLC. An aliquot of each HPLC peptide fraction was submitted to amino acid sequencer.

The extended NH_2-terminal amino acid sequence was determined up to the 32 residues as given below:

IGPSGVETTVYAGDGEYLMXLSIGTPAQPFSAI

PTH (Phenylthiohydantoin) amino acid was not detected at the 20th cycle of Edman degradation and this may be a probable glycosylation site as suggested earlier.

The NH_2-terminal amino acid sequence of 11, 8 and 6 cleavage peptide fragments obtained from HPLC of digestion mixture of RCM protein with *Staphylococcus aureus* V8 protease, endopeptidase Asp-N and trypsin, respectively, were determined and the results deduce the 50% of the internal amino acid sequence and putative active site aspartic acid residues of the enzyme. Computer homology search of the partial sequence with the protein data bank also indicates that the enzyme has much less homology with other known aspartic proteinases, suggesting a unique feature of the enzyme. Primers will be designed based on determined partial amino acid sequences and RT-PCR cloning will be used to isolate cDNA clone and to deduce primary structure of the enzyme.

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