E2-24 Cleavage specificity of novel acid proteinase from *Nepenthes distillatoria* (Badura)

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Plant aspartic proteinases had little attention in contrast to the well characterized mammalian aspartic proteinases. Isolation and some of the enzymatic properties of acid proteinases of *Nepenthes* juice were reported previously. In this report cleavage specificity of *Nepenthes* acid proteinase is compared with that of other members of the aspartic proteinase family.

The B chain of oxidized insulin was digested at 37°C and pH 3.0 with *Nepenthes* acid proteinase. For comparison the B chain of oxidized insulin was digested at 37°C with other members of aspartic proteinases at respective optimum pHs. Aliquots were subjected to HPLC. The peptides were eluted and aliquots of each peptide was submitted to amino acid analyzer.

The B chain of oxidized insulin was cleaved rapidly and extensively by the *Nepenthes* acid proteinase as other aspartic proteinases at the acidic pHs. The HPLC pattern of *Nepenthes* acid proteinase was significantly different from that of other proteinases. Several peptide bonds, especially Phe_{24}-Phe_{25}, Tyr_{16}-Leu_{17}, Leu_{15}-Tyr_{16}, and Glu_{13}-Ala_{14} bonds were simultaneously cleaved to a marked extent by *Nepenthes* acid proteinase. The extents of cleavage of these bonds were estimated to be 80%, 35%, 39% and 55% respectively by *Nepenthes* acid proteinase. The total extents of hydrolysis relative to that of pepsin A-5 (taken as 100%) were 94% by *Nepenthes* acid proteinase, 81% by cathepsin E, 70% by cathepsin D and 61% by pepsin C.

Peptide bonds in between aromatic amino acids and hydrophobic amino acids are the preferential sites for *Nepenthes* acid proteinase. Cleavage pattern of *Nepenthes* acid proteinase resembles that of major human gastric digestive enzyme pepsin A-5. Therefore *Nepenthes* acid proteinase will be a good supplementary enzyme to provide with diet to facilitate the protein digestion of patients with partial gastrectomy.