E2-28 Proteolytic activities of aspartic proteinases at neutral pH

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Most of the aspartic proteinases (pepsins, gastricsins and cathepsin D) are functioning *in vivo* at the acidic environment and their physiological function is well established. In addition, the presence of significant amounts of pepsinogen C & A in serum, pepsinogen C in seminal plasma and pepsinogen A in urine were reported.

Under some pathological conditions, increase of aspartic proteinases in the extra cellular fluids was also reported. However physiological importance of localization of these proteinases in the tissues with neutral pH environment and the clinical significance of release of aspartic proteinases under some pathological conditions are not clear.

In the present study, proteolytic activities of aspartic proteinases towards the B chain of oxidized insulin were investigated at different pH values.

Human pepsin A-5 and pepsin C were obtained by activating the corresponding purified pepsinogens and isolating the resulting mature enzyme from the activation mixture. Cathepsin D and rhizopuspepsin were purchased from Sigma and Seikagaku Kogyo Co., respectively.

The B chain of oxidized insulin (100 nmol) was digested at 37°C with 0.2 nmoles of the following enzymes (pepsin A-5, pepsin C, cathepsin D and rhizopuspepsin) separately in 300 µl of the buffers at pH 2.0, 3.0, 5.5, 6.5 and 7.4. Aliquots of 100 µl were removed at appropriate time intervals and subjected to high performance liquid chromatography (HPLC). An aliquot of each peptide fraction dissolved in distilled water was subjected to amino acid analysis using an Applied Biosystems automated derivatizer analyzer A.
The B chain of oxidized insulin was cleaved rapidly and extensively by the above proteinases at the acidic pH (2.0 - 3.0). It was also hydrolyzed to a significant extent at pH 5.5 and 6.5. In addition, the change in HPLC patterns at different pHs with a particular enzyme was notable.

At pH 2.0-5.5, several peptide bonds, especially Phe-X, Tyr-X and Leu-X were cleaved to marked extents by the 4 proteinases. In addition the Glu_{13}-Ala_{14} peptide bond was cleaved significantly by pepsin C at pH 2.0 and cathepsin D at pH 3.0. Further the Gln_4-His_5 peptide bond was cleaved to a significant extent by pepsin A-5 at pH 2.0 and rhizopuspepsin at pH 5.5. At pH 6.5, the Leu_{15}-Tyr_{16} peptide bond was hydrolyzed fairly selectively and to a significant extent by all 4 proteinases and the Phe_{24}-Phe_{25} bond was cleaved selectively by rhizopuspepsin. The total percentage of hydrolysis were 100,27,8 & 0 for pepsin A-5; 100, 52, 19 & 0 for pepsin C; 100,96, 42, & 5 for rhizopuspepsin and 100, 82, 12, & 0 for cathepsin D, respectively at the pH of 2.0, 5.5, 6.5 & 7.4. These results confirm the significant proteolytic activities of the 4 different aspartic proteinases at neutral pH range (5.5-7.5).

Significant proteolytic activities of all 4 proteinase tested were observed at the neutral pH range although their maximum activities were observed at acidic pH. These findings suggest that in addition to renin and HIV proteinase, some of the other members of aspartic proteinase family also may function *in vivo* at neutral pH range. Further pH dependence of the activities of the proteinase from the same family and isozymes depend on the type of substrate and enzyme used.