Activities of Human Pepsin and Cathepsin D using Synthetic Substrates with Human Kallistatin Sequence

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The aspartic peptidases act on kininogen and release kininis (methionyl-lysyl-bradykinin and methionyl-lysyl-bradykinin-serine), but the most important enzymes that release kinins are kallikreins, serine peptidases inhibited by kallistatin. In this way, we supposed that the aspartic peptidases may cleave the human kallistatin between its hydrophobic amino acids (Phe-Phe) in reactive center loop, the peptide bond cleaved by these enzymes. In this study we purified the human pepsin and human cathepsin D from gastric mucosa and spleen, respectively, using chromatographic steps. It is analyzed the activity on synthetic substrates with the original sequence of kallistatin and three modifications at the P_3 position (Abz-Ala-Ile-Lys-Phe-Phe-Ser-Arg-Gln-Eddnp; Abz-Ala-Ile-Ala-Phe-Phe-Ser-Arg-Gln-Eddnp; Abz-Ala-Ile-Leu-Phe-Phe-Ser-Arg-Gln-Eddnp and Abz-Ala-Ile-Ser-Phe-Phe-Ser-Arg-Gln-Eddnp). Our results showed, by kinetic parameters, that the best substrate to pepsin are Abz-Ala-Ile-Lys-Phe-Phe-Ser-Arg-Gln-Eddnp with Km= 6.44 µM, Kcat= 232.5 s^{-1} and to catepsin D the best substrate was Abz-Ala-Ile-Ser-Phe-Phe-Ser-Arg-Gln-Eddnp with Km= 4.63 µM, Kcat= 111.93 s^{-1}. In conclusion, it was clear from the analysis of the kinetic parameters for hydrolysis of these substrates that the modifications at the P_3 position play significant roles in the interaction and catalytic processes of aspartic peptidases on the kallistatin reactive-center loop. Moreover, purified human pepsin and cathepsin D interact with kallikrein-kinins system by releasing biologically active peptides from kininogen and possibly help kallikrein to release kinins inhibiting its inactivation by kallistatin.

Key words: *aspartic peptidases, kallistatin, kinins, synthetic substrates.*
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