CLEAVAGE OF SYNTHETIC SUBSTRATES WITH THE SEQUENCE OF KALLISTATIN BY HUMAN UROPEPSIN

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The aspartic peptidase uropepsin is found in acidified human urine. Aspartic peptidases have a highly conserved active center with two residues of aspartic acid. They exert their actions in acid pH, cleave peptide bonds between hydrophobic amino acid and are inhibited by Pepstatin A. The active peptide kinins - bradykinin, lysyl-bradykinin and methionyl-lysyl-bradykinin - are released from kininogen by enzymatic action. The aspartic peptidases act on kininogen and release methionyl-lysyl-bradykinin and methionyl-lysyl-bradykinin-serine, but the most important enzymes that release kinins are kallikreins, serine peptidases inhibited by kallistatin. Human kallistatin has two hydrophobic amino acids (Phe-Phe) in its reactive center loop what suggests that kallistatin may be cleaved by uropepsin. In this study we purified the human uropepsin using two ionic exchange chromatography: DEAE Bio Gel and Mono Q HR 5/5 and analyzed its activity on synthetic substrates with the sequence of kallistatin. Our results showed that uropepsin cleaves the substrates between the amino acids Phe-Phe. Kinetic parameters were Km=0,093 µM, Kcat=29,84 s^{-1} to the substrate Abz-Ala-Ile-Ala-Phe-Phe-Ser-Arg-Gln-Eddnp and Km=0,129 µM, Kcat=19,87 s^{-1} to the substrate Abz-Ala-Ile-Lys-Phe-Phe-Ser-Arg-Gln-Eddnp. In conclusion, purified human uropepsin interact with kallikrein-kinins system by releasing biologically active peptides from kininogen and possibly help kallikrein to release kinins inhibiting its inactivation by kallistatin.