Acid protein digestion in the spider *Nephilengys cruentata*

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Protein digestion in spiders has been sporadically studied and there are few reports on literature. Our group characterized protein digestion in the hepatopancreas of the spider *Nephilengys cruentata*. Studies with natural protease substrates and specific inhibitors indicated two distinct activities: an alkaline activity already characterized as an astacin-like enzyme and an acidic activity. In order to classify the acidic peptidases, *Nephilengys cruentata* females were fed, dissected and the isolated hepatopancreas was homogenized in Milli-Q water. Chromatographic separation and specific assay showed two different peptidases in acidic conditions: an aspartic endopeptidase, inhibited by pepstatin and a cysteine-endopeptidase inhibited by E-64. Assays using quenched fluorescent substrates indicated that the cysteine-endopeptidase is the major acidic activity and that this is probably a cathepsin-L like enzyme hydrolyzing preferentially substrates with a Phe residue at P2. This activity was partially isolated in a combination of two cation-exchange chromatographies a Hitrap S and a Resource S both equilibrated in 0.05 M citrate-phosphate pH 5.0 buffer and eluted with a linear (0 – 1 M) NaCl gradient. Thus the cathepsin-L like was characterized. This enzyme presented a pH optimum of 3.6, a molecular weight of 30.4 kDa determined by SDS-PAGE, it is mainly stable in acidic conditions (pH 3.0 to 6.0) at 4°C and 30°C presented a half-life of 13 minutes at 60°C. The KD determined is in agreement of data for other arthropod cathepsin-L like enzymes. This enzyme will be isolated to homogeneity for detailed specificity studies and in order to characterize its ability to hydrolyze spider silk. Supported by: FAPESP 06/03474-0 and 05/02486-1