

PROTEIN DIGESTION IN THE SCORPION *TITYUS SERRULATUS*: ISOLATION
OF MAJOR CISTEINE PROTEINASE

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Scorpions are ancient chelicerates that have changed little since the Silurian and are considered the oldest known terrestrial species, which makes this species a model to the study of digestion evolution in Arthropoda. Although their biological importance, only two enzymes involved in prey digestion are known in this group: an amylase and a lipase. Scorpion digestive glands, called hepatopancreas, occupy most of the abdominal region. In order to characterize protein digestion in scorpions, *Tityus serrulatus* hepatopancreas was isolated and homogenized. Protease activity was determined using hemoglobin, casein-FITC and Z-FR-MCA (carbobenzoxy-Phe-Arg-7amido4methyl coumarin) as substrates. Pepstatin, E-64, PMSF, and phenantroline were used as inhibitors allowing the classification of proteolytic activity. Low activity was observed on casein-FITC. The major proteolytic activity on hemoglobin and Z-FR-MCA present acidic activation, is completely inhibited by E-64 and is also dependent of EDTA and cysteine in the reaction medium. These activities presented acid pH optimum (3.0, 4.0 and 5.4), molecular weight of 60 kDa (non-activated sample) and 44 kDa (activated sample). Anionic exchange chromatography indicates the presence of at least three cysteine-proteinase involved in protein digestion on *Tityus serrulatus* (yield: 600%). Partial isolation of these enzymes were obtained with an affinity chromatography Arginine-Sepharose (yield: 150%, purification factor: 8x). A combination of both: affinity and anion exchange chromatography must complete the purification of cysteine-proteinases from *Tityus serrulatus*. Supported by: FAPESP.