# Cloning and Expression of Triapsin, a Serine Protease from Saliva of Chagas Disease Vector, *Triatoma infestans.*

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Triapsin, a trypsin-like serine protease is expressed in the D2 salivary gland of Chagas disease vector, the hematophagous insect, *T. infestans.* This enzyme is synthesized as an inactive precursor and, at bite moment, it is released in its active form, suggesting that triapsin has a role in blood feeding, but its physiological roles are not understood. The aim of this work was to clone pro-triapsin and pro-peptide, express and perform kinetic studies in order to study its specificity using protease inhibitor and phage display library. Pro-triapsin cDNA was synthesized using total mRNA from D2 salivary glands with ImProm-II™ Reverse Transcription System (Promega®). Pro-triapsin was cloned in pGEM–T Easy Vector (Promega®) and sequenced. The pro-peptide region and the pro-enzyme were sub-cloned in PGEX-4T2 vector (GE Healthcare®) to be expressed as a Glutathione-S-Transferase (GST) fusion protein. The protein and peptide expression were carried out for 24h and the bacterial lysate was achieved by French Press. The fused protein and peptide were purified with a gluthatione-Sepharose affinity resin (Amersham Biosciences®) and to analyze the expression, we used protein electrophoresis (SDS-PAGE) and measured pro-triapsin proteolytic activity by hydrolysis of a chromogenic substrate (HD-Ile-Pro-Arg-pNA) after treatment with thrombin, for pro-triapsin cleavage from GST, and trypsin, to generate triapsin active form. In SDS-PAGE it were identified two bands in reduction conditions (~60 kDa and ~25 kDa) of pro-enzyme and pro-peptide proteins, respectively. The enzymatic activity assay showed a low amidolytic activity. These preliminary results showed that the cloning was successful and rather the protease and peptide are being expressed.

*Keywords:* triapsin, *Triatoma infestans*, serine protease, protein expression.

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