

PRIMARY STRUCTURE CHARACTERIZATION OF A NEW TIL SERINE PROTEASE INHIBITOR FROM *BOOPHILUS MICROPLUS* EGGS

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In previous work we showed the purification and partial biochemical characterization of BmSI-7 and BmSI-6, two *Boophilus microplus* subtilisin inhibitors (BmSI) purified from eggs (SBBq/ Programa e Índices da XXXIII Reunião Anual; p.46). In the present work we show the partial amino acid sequencing of BmSI-7, the cloning and sequencing of *BmSI-7* cDNA fragment and its amino acid sequence comparison to other protease inhibitors. The inhibitors, BmSI-7 and BmSI-6, presented molecular masses of 7408 Da and 7271 Da, respectively. Both inhibited neutrophil elastase (K_i 0.4 and 0.3 nM), subtilisin A (K_i 1.4 nM for both inhibitors) and Pr1 proteases from *M. anisopliae* fungus, BmSI-7 (K_i 50 nM) and BmSI-6 (K_i 2.2 nM). In attempt to determinate the BmSI amino acid sequence, BmSI-7 was digested separately with two proteases, human neutrophil elastase (HNE) and Arg-C protease. Several peptides produced by these digestions were sequenced. Using the information from peptides sequences, two degenerated oligonucleotides were constructed and the cDNA fragment coding for BmSI-7 was obtained. The translated amino acid sequence of BmSI-7 allowed us to classify it in the Trypsin Inhibitory Like family. Based on our results, we can suggest that BmSIs could play a role in defense mechanism of *B. microplus* against entomopathogenic fungal or bacterial infections. **Supported by FAPESP, Pronex and CNPq.**