

Expression, Purification and Characterization of Boophilin, a Thrombin Inhibitor from Cattle Tick *Boophilus microplus*

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B. microplus is responsible for an important decrease in meat, milk and leather production, due both to the cattle blood losses and to its role as a vector of potentially lethal anaplasmosis and babesiosis. *B. microplus* is a rich source of serine protease inhibitors, including BmTI-A (*B. microplus* trypsin inhibitor), BmTI-6, BmSI (*B. microplus* subtilisin inhibitor), and the recently described thrombin inhibitor boophilin (Macedo-Ribeiro et al. 2008). Boophilin is a double Kunitz-type thrombin inhibitor, with the unusual ability to form a ternary complex with a second serine protease molecule. In order to confirm this possibility, the aim of this work was to express, purify and characterize full-length boophilin (D1D2), as well as the individual domains (D1 and D2). All the constructions were done using a tick midgut cDNA preparation and oligonucleotides constructed based on sequence no. CAC82583. The DNA fragments were digested with restriction enzymes, and cloned into the pPICZaB vector. The expression yields of boophilin D1D2 and of boophilin D1 were 21 and 37.5 mg/L, respectively. The inhibitors were purified from the yeast culture supernatant by affinity chromatography on a trypsin-Sepharose column. Purified boophilin D1D2 inhibited trypsin (K_i 15 nM), neutrophil elastase (K_i 0.28 nM), bovine thrombin (K_i 0.20 nM) and plasmin (K_i 3.50 nM). Boophilin D1 only inhibited trypsin and plasmin with a K_i of 5.6 and 12 nM, respectively. We were so far unable to express boophilin D2. In the future we would like to elucidate the tridimensional structure of a thrombin:boophilin:trypsin ternary complex. *Supported by: FAPESP, CNPQ-GRICES and CNPq.*

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