Characterization of Thrombin Inhibition Mechanism by the recombinant *Aedes aegypti* thrombin inhibitor (rAaTI)

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Aedes aegypti is an important vector of diseases such as yellow fever and dengue fever. *Ae. aegypti* molecular studies have been a strategy to generate tools for interfering in its interaction with host or virus, respectively. Serine protease inhibitors play crucial roles in controlling ectoparasite host physiological processes such as inhibition of target enzymes involved in these processes. Recently, we identify a putative Kazal type serine protease inhibitor in the *Ae. aegypti* salivary gland transcriptome. This molecule was named AaTI, and it was expressed in *Pichia pastoris* system and purified by affinity chromatography in trypsin-Sepharose and by gel-filtration chromatography in Superdex 75 columns. In order to better understand about thrombin inhibition mechanism of rAaTI, our aims were to produce and characterize rAaTI. Purified rAaTI inhibited only trypsin amidolytic activity with *Ki* in nM range, but it did not inhibit thrombin, fXa, tPA, urokinase, and plasma kallikrein. rAaTI was used in prothrombin time (PT), activated partial thromboplastin time (APTT) and thrombin time (TT) experiments, and it strongly prolonged the TT, this results allowed us to classify it as a thrombin inhibitor. The rAaTI truncated form (rAaTI?), without a charged tail, was also expressed and the acid tail was synthesized. Both molecules were able to prolong TT. rAaTI and peptide weakly inhibited platelet aggregation induced by thrombin. Our recently results showed that in competitive assays with hirudin and heparin rAaTI can binding in exosite I and II of thrombin, while rAaTI? interfered only in the heparin binding site. *Supported by: FAPESP and CNPq.*