Molecular Studies of Trypsin-like Enzymes Present in Midgut of *Aedes aegypti* Larvae

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Aedes aegypti is the most important vector of human arboviral diseases and responsible for the transmission of dengue and urban yellow fever. Trypsin-like enzymes play very important role in insect digestion. These enzymes were already described in *Ae. aegypti* adult and larval midguts. The aim of this work was the characterization of trypsin-like enzymes present in the *Ae. aegypti* larval midgut. Small cDNA fragment library of *Ae. aegypti* trypsins was constructed and eighty-one clones were sequenced. Two sequences identified as AAEL5607 and AAEL6371 showed to be more expressed representing 50% and 32.6% of frequency, respectively. Specific oligonucleotides were constructed based on the AAEL5607 and AAEL6371 sequences and used in a semi quantitative PCR of *Ae. aegypti* different life stages. The AAEL5607 transcript was present in all larval instars but AAEL6371 appeared only in 3rd and 4th larval instars. In order to confirm the transcription data, trypsin-like enzyme was purified from *Ae. aegypti* midgut 4th instar larvae. A midgut crude extract was prepared and applied on a BPTI-Sepharose column, and further purified in ionic exchange and reversed-phase chromatographies. Purified trypsin presented molecular mass of 28 kDa by SDS-PAGE. Its partial amino acid sequence allowed suggesting that it is the product of AAEL5607 sequence, which corroborates the expression data. Purified trypsin (AAEL5607) showed K_m for tosyl-Gly-Pro-Arg-pNa of 36.4 µM and was strongly inhibited by AaTI and HiTI, both trypsin inhibitors, with K_i of 0.94 pM and 160 pM, respectively. In conclusion, the trypsin enzyme AAEL5607 is the major digestive enzyme of 4th larval instar of *Ae. aegypti*.

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