Molecular Cloning of a Digestive Metalloproteinase from *Spodoptera frugiperda* Midgut Larvae

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Astacins are proteinases from the Metzicins family and are present in bacteria and animals. There are 3 types of astacins.The morphogenetically active proteins, the membrane-bound enzymes that hydrolyze biological active peptides and the astacins found in the digestive system of some invertebrades. In insects little is known about astacin, and it was detected only in the intestinal fluid of one Coleoptera. Screening of one *S. frugiperda* midgut cDNA expression library with antibodies raised against intestinal microvillar membrane proteins, we found a sequence putatively coding for astacin. Using specic primers designed from this sequence we suceeded in cloning a complete cDNA sequence. This sequence has 1008 base pairs that probably codes for a protein with 323 amino acids and a signal peptide of 13 amino acids. The canonical signature of the Metzincins is present, as the conserved motifs HEXXHXXGXXH and the Met-turn. The protein has a theoretical isoelectric point of 5.32 and a molecular weight of 37.6 kDa. The alignment of *S.frugiperda* astacin with the one from *Astacus astacus* reveals a potential cleavage site which leads to the enzyme activation, resulting in a mature protein of 29.6 kDa. The *S.frugiperda* astacin has highest identity with astacins from *Aedes aegypty* and *Anopheles gambiae* (32 and 28% identity, respectively). RT-PCR analysis shows that astacin transcripts are specific from the midgut, not being found in other larval tissues. In the midgut epithelium the enzyme seems to be produced in the anterior and middle parts. No glycosylation sites, transmembrane domains or GPI anchor could be predicted based on *in silico* analysis, suggesting that the enzyme is secreted into the midgut lumen.
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