Cloning and Expression of Two Cystatins of the Blood-Sucking Bug *Triatoma infestans*

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Cystatins are tight-binding cisteine proteinase inhibitors. Several cystatins have been described in mammals, arthropods and plants. In arthropods such as the blood-sucking bug *Triatoma infestans*, its function is still unknown. Two cystatin genes were found in Expressed Sequence Tags (ESTs) sequenced from midgut and fat body cDNA libraries of *T. infestans*, respectively. Both *T. infestans* cystatins deduced amino acid sequence confirmed the presence of a Gly residue in the N-terminal region, a motif Gln-X-Val-X-Gly and a dipeptide PW. These structural features were described to be important for cystatin inhibitory activity. Moreover, it was also identified Cys residues, which probably form disulfide bonds by comparison to other cystatins. Thus, in the present work we cloned and expressed these cysteine proteinase inhibitors using pPICZα vector and GS115 *Pichia pastoris* strain. We also showed that the yeast supernatant (120 h of fermentation) midgut cystatin (gutcystatin) and fat body cystatin (fbcystatin) displayed inhibitory activity toward human cathepsin L. The expression levels of gutcystatin and fbcystatin were 0.55 mg/L and 2.4 mg/L, respectively. Preliminary results showed inhibition constants (Ki) for fbcystatin (0.1 nM) and gutcystatin (3.0 nM) toward cathepsin L. The expression scale-up of both cystatins have been done for further biochemical characterizations.

Keywords: Cystatin; fat body; midgut; *Triatoma infestans*.

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