Anticlotting Activity of a Cathepsin L-Like Protease from *Rhipicephalus microplus* Tick

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Introduction: *Rhipicephalus microplus* is an important parasite of cattle. New strategies for control depend on a better knowledge of its physiology and host-parasite relationship. Molecules involved in acquisition and digestion of blood meal are interesting targets. **Objectives:** to determine how BmGTI (*Boophilus microplus* Midgut Thrombin Inhibitor) inhibits coagulation. **Materials and Methods:** BmGTI was obtained from partially engorged females midguts homogenate through ion exchange, size exclusion and thrombin-affinity chromatographies. Thrombin inhibition activity was measured by thrombin-induced fibrinogen clotting assay. BmGTI preparation was checked by SDS-PAGE and analyzed by LC-MS/MS. BmGTI putative ORF was cloned in pPIC9 plasmid, expressed in *Pichia pastoris* and purified. Different molar ratios of thrombin:BmGTI were examined for inhibitory activity of thrombin upon fibrinogen cleavage at pH 7.5. E64 was used to block BmGTI active site and inhibitory activity was assayed. Different molar ratios of thrombin:BmGTI were incubated and analyzed by SDS-PAGE to verify protease-inhibitor interactions. BmGTI direct activity upon fibrinogen was analyzed by SDS-PAGE after incubation. **Results and Discussion:** Based on *m/z* of the BmGTI tryptic fragments it was identified as BmCL1, a cathepsin L-like proteinase active at acidic pH, but unable to hydrolyze synthetic substrate at pH ≥7.0. BmGTI/BmCL1 was expressed in *P. pastoris* as pro-enzyme and after activation the protease was purified. Different molar ratios of thrombin:rBmGTI/BmCL1 was assayed, and when rBmGTI/BmCL1 was 64-fold higher than thrombin concentration, thrombin residual activity was 37%. When rBmGTI/BmCL1 has its active site blocked with E-64, it was unable to inhibit thrombin activity upon fibrinogen. Analysis by SDS-PAGE of fibrinogen after incubation with rBmGTI/BmCL1 showed that fibrinogen α- and β-chains were hydrolyzed. **Conclusion:** Based on these results, we concluded that rBmGTI/BmCL1 inhibits blood coagulation through hydrolyzes of fibrinogen α- and β-chains.

Keywords: *R. microplus*, cathepsin L-like, fibrinogen
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