A *Rhipicephalus (Boophilus) microplus* Cathepsin with a Dual Activity: Peptidase and Antimicrobial

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This study describes the Vitellin-Degrading Cysteine endopeptidase (VTDCE): a novel dual-property cathepsin L-like protein with enzymatic and antimicrobial activity. Native VTDCE was obtained from *Rhipicephalus microplus* egg and its cathepsin L activity was characterized. The hypothesis of a possible dual activity emerged after determination of VTDCE amino acid sequence. Amino acid sequence comparison with sequences from data bank revealed that the VTDCE sequence has a higher similarity with antimicrobial peptides than with other cysteine proteases. Here we present results about cloning and expression of recombinant VTDCE (rVTDCE) and its immunological, biochemical and antimicrobial properties. A fragment of 285 base pairs, covering the sequence of the enzyme without pro-peptide, was cloned into pGEM-T and pET32a vectors. *Escherichia coli* strain BL21(DE3) Star was used for the expression of rVTDCE, that was purified by affinity chromatography. rVTDCE was used to immunize rabbits and cattle, and was immunogenic for both species. The polyclonal sera were tested and confirmed the cross-reactivity between the native and recombinant forms. The recombinant enzyme showed activity upon substrate N-CBZ-Phe-Arg-MCA and was sensitive to inhibitors E-64 and leupeptin. Also, the rVTDCE inhibited the growth of Gram-positive bacterium *Staphylococcus epidermidis*, showing a bacteriostatic effect against this pathogen. Experimental results indicated that antimicrobial and enzymatic are two independent activities. The removal of fusion protein (TRX and histidin tag) of recombinant protein increased its enzymatic and antimicrobial activities. Further studies about VTDCE will be useful to understand the real role of this cathepsin, with an unusual antimicrobial property, in tick physiology.

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