BIOCHEMICAL CHARACTERIZATION OF ASPARTIC AND CYSTEINE PROTEINASES INVOLVED IN HEMOGLOBIN DIGESTION IN THE GUT OF THE TICK *BOOPHILUS MICROPLUS*

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An antimicrobial peptide (Hb33-61) derived from the alpha-chain of bovine hemoglobin has been purified from the gut contents of the tick *Boophilus microplus* (J. Biol. Chem. 274: 25330-25334, 1999). We report on the purification and biochemical characterization of aspartic and cysteine proteinases responsible for the generation of this antimicrobial peptide. Enzyme activities were determined with two chemically synthesized fluorogenic substrates containing the amino acid sequences 29-35 and 57-67 of the alpha-chain of bovine hemoglobin. Aspartic proteinase purification was performed with total gut homogenates through ion exchange chromatography followed by hydrophobic interaction chromatography and gel filtration in FPLC system. This purification yielded one aspartic proteinase activity, which was also confirmed by thermal inactivation. This aspartic proteinase has a molecular weight of approximately 45 kDa, determined by gel filtration. Additionally, a light and heavy chain was observed through silver-stained SDS-PAGE. Aminoacid sequencing of this enzyme is underway. More than one cysteine proteinase was identified in total gut preparations. These proteinases were purified through ion exchange and hydrophobic interaction chromatography. Currently we are characterizing these enzymes. Hemoglobin processing *in vitro* using these purified proteinases yielded peptides active against *Micrococcus luteus*. Supported by FAPESP and CNPq.