

A Serine Protease from a Detergent-soluble Extract of *Leishmania* (*Leishmania*) *amazonensis*

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Proteases mediate important crucial functions in parasitic diseases, and their characterization contributes to the understanding of host-parasite interaction. A serine protease was purified about 43-fold with a total recovery of 60% from a detergent-soluble extract of promastigotes of *Leishmania amazonensis*. The purification procedures included aprotinin-agarose affinity chromatography and gel filtration high performance liquid chromatography. The molecular mass of active enzyme was 110 kDa by native gel filtration HPLC and by SDS-PAGE gelatin under non-reducing conditions. Under conditions of reduction using SDS-PAGE gelatin analyses the activity of enzyme was observed in two proteins of 60 and 45 kDa, suggesting that the enzyme may be considered as a dimer. The *Leishmania* protease was not glycosylated, and its isoelectric point (pI) was around 4.8. The maximal protease activity was at pH 7.0 and 28 °C, using α -N- ρ -tosyl-L-arginyl-methyl ester (L-TAME) as substrate. Assays of thermal stability indicated that this enzyme was totally denatured after pre-treatment at 42 °C for 12 min and preserved only 20% of its activity after pre-treatment at 37 °C for 24 h, in the absence of substrate. Hemoglobin, bovine serum albumin (BSA), ovalbumin and gelatin were hydrolyzed by *Leishmania* protease. Inhibition studies indicated that the enzyme belonged to a serine protease class because of a significant impediment by serine protease inhibitors such as benzamidine, aprotinin, and antipain. The activity of the present serine protease is negatively modulated by calcium and zinc and positively modulated by manganese ions. This is the first study that reports the purification of a protease from a detergent-soluble extract of *Leishmania* species.

Key words: *Leishmania* (*Leishmania*) *amazonensis*, Serine Protease, Characterization