Identification of Serine Proteases from *Leishmania braziliensis*

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Leishmania (V.) braziliensis is one of the most important ethiologic agents of the two distinct forms of American tegumentary leishmaniasis (cutaneous and mucosal). The drugs of choice used in leishmaniasis therapy are significantly toxic, expensive and are associated with frequent refractory infections. Among the promising new targets for anti-protozoan chemotherapy are the proteases. In this study, serine proteases were partially purified from aqueous, detergent and extracellular extracts of *Leishmania braziliensis* promastigotes by aprotinin-agarose affinity chromatography. By zymography, the enzymes purified from the aqueous extract showed apparent activity bands of 60 kDa and 45 kDa; of 130 kDa, 83 kDa, 74 kDa and 30 kDa from the detergent extract; and of 62 kDa, 59 kDa, 57 kDa, 49 kDa and 35 kDa from the extracellular extract. All purified proteases exhibited esterase activity against N_α-benzoyl-L-arginine ethyl ester hydrochloride and N_α-*p*-tosyl-L-arginine methyl ester hydrochloride (serine protease substrates) and optimal activity at pH 8. 0. Proteases purified from the aqueous and extracellular extracts were effectively inhibited by benzamidine (trypsin inhibitor) and those from the detergent extract were inhibited by *N*-tosyl-L-phenylalanine chloromethyl ketone (chymotrypsin inhibitor) indicating that all these enzymes are serine proteases. These findings indicate that *L. braziliensis* serine proteases display some biochemical similarities with *L. amazonensis* serine proteases, demonstrating a conservation of this enzymatic class in the *Leishmania* genus. This is the first study to report the purification of a serine protease from *Leishmania braziliensis.*

Key words: Leishmania braziliensis, Serine Protease, Aprotinin-Agarose Affinity Chromatography