Characterization of Proteinases from Subcellular Fractions of *Leishmania (Viannia) braziliensis*

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We performed a combination of proteinase assays, either in solution or immobilized (gelatin-SDS-PAGE), to detect and quantify proteinases of two *Leishmania (V.) braziliensis* subcellular fractions: membrane fraction (MF) and flagellar fraction (FF). The proteolytic activity in these fractions were characterized using chromogenic substrates and specific inhibitors. The MF showed proteinase bands of 78kDa, 69kDa, 60kDa and 50 kDa in all assayed pH (3.5, 5.5, 7.5 and 8.0), while FF showed bands of 60kDa, 50kDa, and 38kDa (at pH 3.5 and 8.0) and bands of 78kDa, 69kDa, 60kDa and 50kDa (at pH 5.5 and 7.5). The activity of the 50kDa band from the MF was sensitive to the presence of aspartic-proteinase inhibitor, while the 50kDa band from FF was sensitive to inhibitors for cysteine-, serine- and metallo-proteinases. The 60kDa band from FF was also sensitive to metallo-proteinase inhibitor. Moreover, we have examined proteinase activity by hydrolysis of a panel of peptides specific for the main proteinase classes. We have demonstrated that the substrate tNa-p-Tosyl-L-Arg methyl ester was hydrolyzed by MF (6.5±0.9 nmoles second^{-1} mg of protein^{-}1) and FF (5.3±0.16 nmoles second^{-1} mg of protein^{-}1), suggesting a predominance of esterase activity. We have also verified a lower proteolitic activity (=0.1) over substrates pGlu-Phe-Leu p-nitroanilide and N-Cbz-Pro-Phe-His-Leu-Leu-Val-Tyr-Ser b-naphthylamide, in the assayed preparations. These findings contribute to improve the knowledge on parasite physiology associate to the cellular distribution of proteinases.

Key words: *Leishmania (Viannia) braziliensis;* proteinases; Subcellular fraction

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