

IDENTIFICATION AND CHARACTERIZATION OF EXCRETORY/SECRETORY PRODUCTS OF *Giardia duodenalis* TROPHOZOITES

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ABSTRACT: One of the major questions concerning *Giardia* is the understanding of pathophysiological processes associated with small intestine abnormalities. There are evidences that *Giardia* trophozoites contain and/or release proteolytic enzymes that may be implicated in the host intestinal epithelium. The present investigation was undertaken to examine the protease activity in excretory/secretory (E/S) products of *Giardia duodenalis* trophozoites of an axenic Brazilian strain (BTU-11) and the reference strain Portland 1 (P1). E/S products from trophozoites of each strain in conditioned medium were tested with sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) for the protein profiles, and the protease activity was analyzed using substrate-impregnated SDS-PAGE (gelatin and collagen) and hemoglobin assay. The proteases characterization was based on inhibition assays including synthetic inhibitors. Electrophoresis analysis of E/S products revealed a banding pattern composed by few bands (4 to 6 bands) in the migration region of 123 to 28 kDa. Proteolytic products were detected in the conditioned medium by trophozoites of both assayed strains. In the gels containing copolymerized gelatin and collagen, E/S products promoted substrate degradation and the most evident proteolysis zones were distributed in the migration regions of 77 to 18 kDa and 145 to 18 kDa, respectively, in the patterns of gelatinolytic and collagenolytic activities. Degradation of hemoglobin was also observed, and the pattern of hydrolysis was similar in both E/S products assayed. Inhibitor assays showed that the main proteolytic activity in both E/S products is due to cysteine proteases, although the presence of serine proteases was also indicated. Degradation of substrates including

collagen and hemoglobin could lead us to speculate different functions of *Giardia* excreted/secreted proteases *in vivo*, but to confirm this possibility and to elucidate its implication on host-parasite interactions, further experiments applying protocols for the purification of proteases are necessary. Even so, our observations are relevant and hold the perspective for the understanding about protease activity in *Giardia* trophozoites of axenic strain isolated in an endemic area.

KEY WORDS: *Giardia duodenalis*, trophozoites, excretory/secretory products, proteases, protein substrates.

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