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TITLE

FUNTIONAL CHARACTERIZATION OF THE RECOMBINANT ELASTASE FROM SCHISTOSOMA MANSONI AND ITS ROLE IN IMMUNOMODULATION

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ABSTRACT

Shistosoma mansoni parasite has a complex life cycle and the host infection occurs by the active penetration of cercariae through the skin. This penetration process starts a several immune mechanisms which are different between individual who has been infected for the first time and individual who had previous contact with the parasite antigens. Among the S. mansoni antigens, the protein involved in the penetration process: serine protease elastase (SmCE) has been identified as the most abundant and most important protease in the process. Despite that, the trigger to the initial response against infection remains unknown. So the present study aims to produce and characterize the Schistosoma mansoni recombinant elastase enzyme and evaluate its importance in the development of the immune response to the parasite in healthy individuals and previously infected ones. The heterologous production of rSmCE was conduct in different Escherichia coli strains: pLys, BL21 and Rosetta. The plasmid containing SmCE coding sequence was transformed into each strain and the protein production was induced using IPTG. Bacterial lysates were analyzed by SDS-PAGE. Following the protein production protocol we used the soluble supernatant to purify the rSmCE through affinity chromatography. The samples were evaluated by Western blot. Also performed an enzymatic assay with the chromatogenic substrate for 24h in some different pHs and protein concentrations. With these results we did another incubation, change the substrate concentration to determinate the Vmax and Km of the enzyme. Human peripheral blood mononuclear cells were incubated for 48 hours with the SmCE and a viability test was made, the supernatant was used to determinate the optimal and sub-optimal concentration of the protein for cytokines assays as well. All bacterial strains tested were able to produce the protein after a 24-hours induction; however, Rosetta presented a better yield. The purification of the protein extract resulted in a purified SmCE. The enzyme was also identified by Western blot. The best protease activity was found in the concentration of 650µg/mL at pH 9, and its Vmax and Km was 0,02568 mM/min and 0, 04089mM respectivily. The test of viability shows about 52% of viable cells in the presence of 100 µg/mL of SmCE and the optimal concentration was 6.2µg/mL for IL-10 and INF-Ϫ and 12,5 µg/mL for IL-5. This experiment concluded so far that in higher concentration the SmCE protein is cytotoxic to cells, thus the protein seams to modulate the immune responses creating satisfactory levels of IL-10 and cytokines from the Th1 responses. (FAPESB, CNPq 403336/2021-0).

KEYWORDS

S. mansoni; Immunology; Elastase

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