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Research Note

PARTIAL CHARACTERIZATION OF LEISHMANIA CHAGASI PROMASTIGOTE PEPTIDASES

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Parasite peptidases (nomenclature of A. J. Barrett & J. K. McDonald, 1986, *Biochem. J., 237:* 935) have been demonstrated in protozoan, e.g. *Trichomonas, Plasmodium falciparum, Entamoeba histolytica, Giardia lamblia, Trypanosoma cruzi,* and also in some helminths as *Schistosoma mansoni* and *Ancylostoma caninum* (J. H. McKerrow, 1989, *Exp. Parasitol., 68:* 111-1150).

Recently, special attention has been adressed to the study of *Leishmania* proteolytic enzymes, mainly to the promastigote surface metallopeptidase Gp 63, which is present on pathogenic species and it seems to be involved in the infectivity of these microrganisms (R. Etges et al., 1986, *J. Biol. Chem., 261:* 9098-9101; J. Bouvier et al., 1987, *Mol. Biochem. Parasitol., 4:* 73-79; M. Kweider et al., 1989, *Parasit. Immunol., 11:* 197-209). Additionally, cysteine-peptidases were already described in amastigote and metaclycic forms of *Leishmania mexicana* by M. North & G. Coombs (1981, *Mol. Biochem. Parasitol., 3:* 293-300) and also by B. C. Lockwood et al., (1987, *FEMMS Microbiol. Lett. 48:* 345-350). In *L. mexicana* amastigotes, these enzymes could be distinguished not only by their physical properties, as well as due to their substrate specificities (C. D. Robertson & G. M. Coombs, 1990, *Mol. Biochem. Parasitol., 41:* 269-276). In this research note we report the partial characterization of *L. chagasi* promastigote peptidases through SDS-polyacrylamide gel electrophoresis containing co-polymerized gelatin as substrate.

To obtain *L. chagasi* detergent extract, promastigotes (MHOM/BR/79LI01 Imperatriz strain) after 7-day culture in the LIT medium supplemented with 10% fetal bovine serum and 1% of a 20x mixture of RPMI 1640 and medium 199 (M. Sadigursky & C. I. Brodskyn, 1986, *Am. Trop. J. Med. Hyg., 35:* 942-944), were thrice washed with cold PBS, pH 7.2, and lyzed in 6 mM CHAPS solution in 10 mM Tris-HCl buffer (pH 7.5) containing 150 mM NaCl, using 5 x 10^9 parasites ml^{-1}.

To evaluate the proteolytic activity, 20 µl of the extract were electrophoresed at 20 mA on a SDS-polyacrylamide mini-gel (0.5 mm thick, 7.5 cm wide and 5.0 cm long) containing 0.02% co-polymerized gelatin. The gel was twice washed in 2.5% Triton X-100 for 1 h at room temperature and tested for peptidases in different digestion buffers, at 37° for 18 h. Proteolytic enzymes were observed as clear digestion bands after staining with Coomassie Blue R-250 (B. C. Lockwood et al., 1987, *Mol. Biochem. Parasitol., 24:* 89-95).

Leishmania chagasi promastigote peptidases were better observed at neutral pH, using 100 mM citrate-phosphate digestion buffer (pH 7), without the reducing agent 2-mercaptoethanol. As showed in Fig. 1, four peptidases of molecular weight above 60 kDa could be demonstrated in the stained gel.

Inhibition experiments carried out with 1 mM PMSF, 1 mM TPCK, 5 mM EDTA, leupeptin and antipain (both 20 ug ml^{-1}) had no effect on gelatin proteolysis. The metallopeptidase inhibitor, 1,10-phenanthrolin, abolished the proteolytic activity at 66 kDa, suggesting the participation of the promastigote surface peptidase Gp 63 in these enzymatic reactions (not shown).

Experiments carried out to evaluate the proteolytic effect of *L. chagasi* promastigote peptidases on human IgG, using the immunoglobulin co-polymerized in SDS-PAGE as above, showed that only enzymes with molecular weight around 66 kDa digest this protein at neutral pH (Fig. 2).

At present we are investigating the *L. chagasi* peptidases involved in the IgG proteolysis, to elucidate their participation in possible immune evasion mechanisms.

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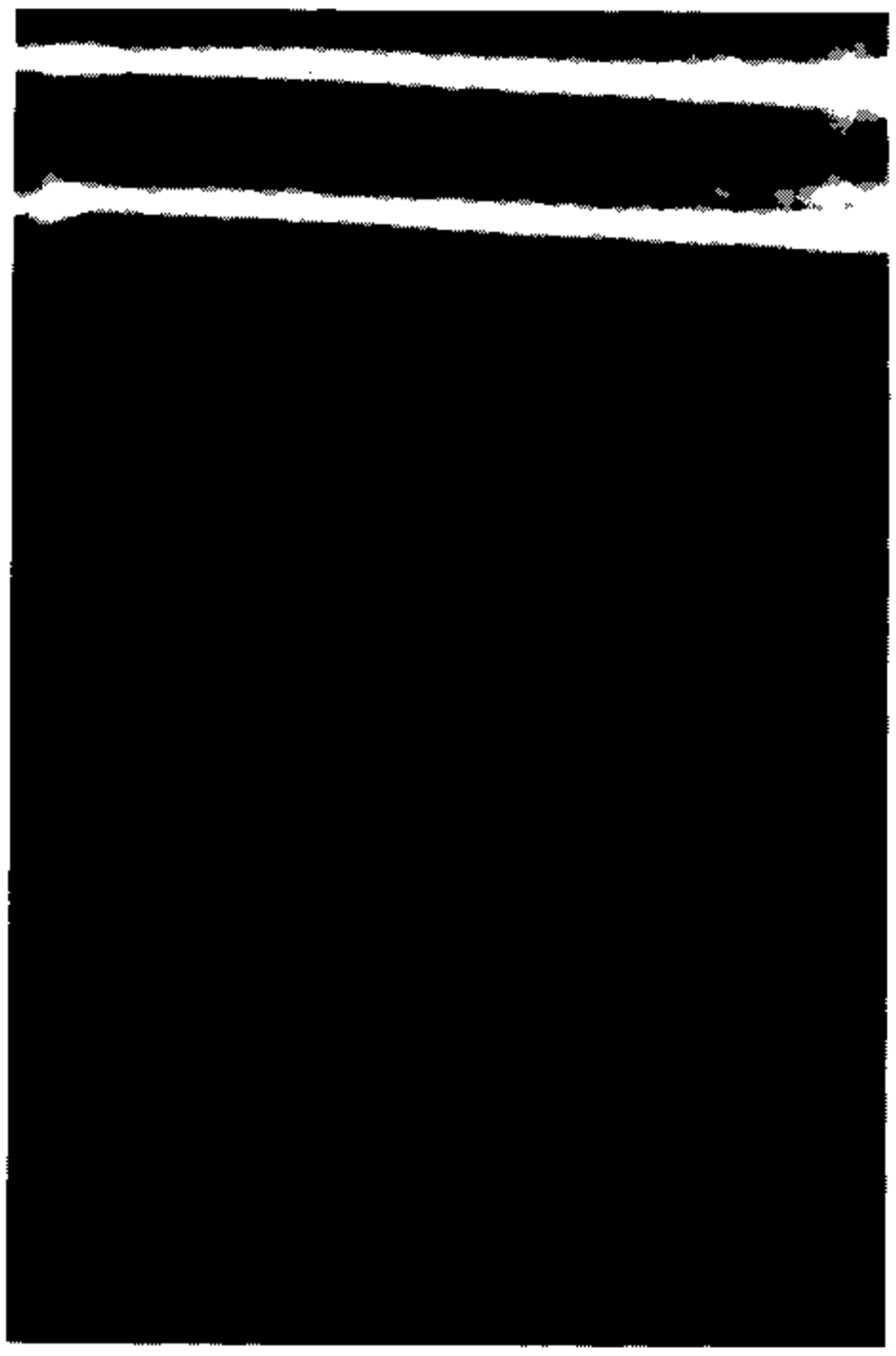

Fig. 1: proteolytic activity of *Leishmania chagasi* promastigote peptidases in sodium dodecyl sulfate-gelatin polyacrylamide gel (10% acrylamide gel).

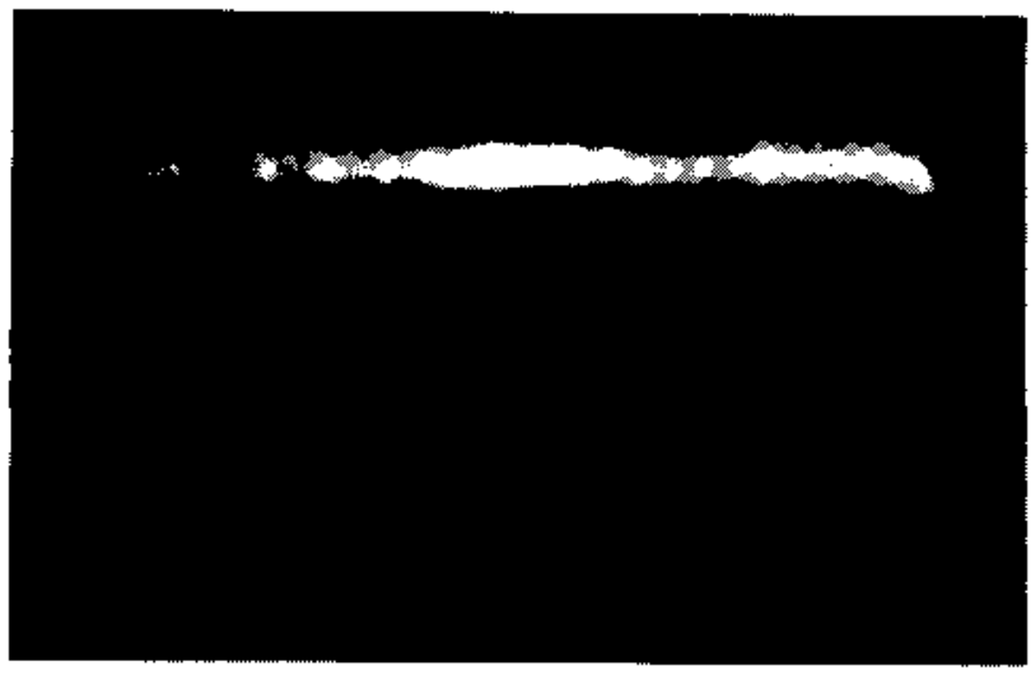

Fig. 2: proteolytic activity of *Leishmania chagasi* promastigote peptidases on co-polymerized human IgG (10% acrylamide gel).