Ecto-Phosphatase Activities on the Cell Surface of the Amastigote Forms of *Trypanosoma cruzi*

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Live *Trypanosoma cruzi* amastigotes hydrolyzed *p*-nitrophenylphosphate (PNPP), phospho-amino-acids and ^{32}P-casein under physiologically appropriate conditions. PNPP was hydrolysed at a rate of 80 nmol·mg^{-1}·h^{-1} in the presence of 5 mM $MgCl_2$, pH 7.2 at 30 °C. In the absence of Mg^{2+} the activity was reduced 40% and we call this basal activity. At saturating concentration of PNPP, half-maximal PNPP hydrolysis was obtained with 0.22 mM $MgCl_2$. Ca^{2+} had no effect on the basal activity, could not substitute Mg^{2+} as an activator and in contrast inhibited the PNPP hydrolysis stimulated by Mg^{2+} (I_{50} = 0.43 mM). In the absence of Mg^{2+} (basal activity) the stimulating half concentration ($S_{0.5}$) for PNPP was 1.57 mM, while at saturating $MgCl_2$ concentrations the corresponding $S_{0.5}$ for PNPP for Mg^{2+}-stimulated phosphatase activity (difference between total minus basal phosphatase activity) was 0.99 mM. The Mg-dependent PNPP hydrolysis was strongly inhibited by sodium fluoride (NaF), vanadate and Zn^{2+} but not by tartrate and levamizole. The Mg-independent basal phosphatase activity was insensitive to tartrate, levamizole as well NaF and less inhibited by vanadate and Zn^{2+}. Intact amastigotes were also able to hydrolyse phosphoserine, phosphothreonine and phosphotyrosine but only the phosphotyrosine hydrolysis was stimulated by $MgCl_2$ and inhibited by $CaCl_2$ and phosphotyrosine was a competitive inhibitor of the PNPP hydrolysis stimulated by Mg^{2+}. The cells were also able to hydrolyse ^{32}P-casein phosphorylated on serine and threonine residues but only in the presence of $MgCl_2$. These results indicate that in the amastigote form of *T. cruzi* there are at least two ectophosphatase activities, one of which is Mg^{2+} dependent and can dephosphorylate phospho-aminoacids and phosphoproteins under physiological conditions.

Introduction

Chagas Disease is responsible for significant morbidity and mortality in Latin America. Its etiologic agent, *Trypanosoma cruzi* has a digenetic life cycle and in the insect vector exists extracellularly as epimastigotes, which upon differentiation become trypomastigote, non-dividing, circulating forms that enter cells and initiates infection in vertebrates (De Souza, 1984). The trypomastigotes differentiate into amastigotes which divide intracellularly in the mammalian host and it is assumed that the pathogenesis and maintenance of infection with *T. cruzi* depends mostly upon the amastigotes stage (De Souza, 1984).

The plasma membrane of cells may contain enzymes whose active sites face the external medium rather than the cytoplasm. The activities of these enzymes, referred to as ecto-enzymes, can be measured using intact cells (DePierre and Karnovsky, 1973; Fernandes *et al.*, 1997; Meyer-Fernandes *et al.*, 1997). Knowledge about interactions between

Abbreviations: CDTA, trans-1,2-diaminocyclohexane-N,N,N',N'-tetraacetic acid; HEPES, (N-[2-hydroxyethyl]-piperazine-N'-[2-ethanesulfonic acid; PNP, *p*-nitrophenol; PNPP, *p*-nitrophenylphosphate; PPO, 2,5-diphenyloxazole; POPOP, 1,4-bis[2-(5-phenyloxazoly)]-benzene); Tris, tris (hydroxymethyl)aminomethane.

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components of the external surface of the amastigotes and the cellular elements of the host is of obvious importance for the understanding of the complex pathology of Chagas' disease. The presence of membrane-bound acid phosphatases has been reported in *Trypanosoma cruzi* (Nakagura *et al.*, 1985), *Trypanosoma rhodesiense* (McLaughlin, 1986), *Trypanosoma congolense* (Tosomba *et al.*, 1996), *Trypanosoma brucei* (Fernandes *et al.*, 1997), and some *Leishmania* species (Lovelace *et al.*, 1986; Vannier-Santos *et al.*, 1995; Martiny *et al.*, 1996). In *Leishmania donovani* acid phosphatase activity was suggested as a marker of virulence (Katakura and Kobayashi 1988; Singla *et al.*, 1992). The characterization of a protein phosphatase in *Leishmania chagasi* provided evidence that this enzyme is conserved among *Leishmania* and a member of the four classes of eukaryotic serine/threonine protein phosphatase (Burns *et al.*, 1993).

Reversible phosphorylation of proteins is recognized to be a major mechanism for the control of intracelular events in eukaryotic cells. Phosphorylation-dephosphorylation of serine, threonine, and tyrosine residues triggers conformational changes in proteins that alter their biological properties (Cohen, 1989; Hunter, 1995). The regulation of the complex interactions required for differentiation and proliferation is mediated in part by protein phosphorylation in higher eukaryotes (Hunter, 1995), as well as in Trypanosomes (Parsons *et al.*, 1993). Such phosphorylations are reversible, and several phosphatases active towards phosphotyrosyl [Tyr(P)]-proteins (Swarup *et al.*, 1981) have been described as acid (Lau *et al.*, 1989) and alkaline phosphatases (Swarup *et al.*, 1981; Lau *et al.*, 1989). In various tissues and cells it has been described the presence of phosphotyrosyl protein phosphatase, which are also active toward low molecular weight, non-protein phosphoesters such as alkyl and aryl phosphates, including *p*-nitrophenylphosphate, *O*-phospho-L-tyrosine and D-glucose 6-phosphate. (Lau *et al.*, 1989; Zhang, 1995; Montserat *et al.*, 1996). More recently it has been demonstrated the presence of protein tyrosine phosphatase activities in *Leishmania donovani* (Cool and Blum, 1993), *Trypanososoma brucei* (Bakalara *et al.*, 1995a; 1995b) and *Trypanosoma cruzi* (Bakalara *et al.*, 1995a; Furuya *et al.*, 1998), however the modulation promoted by divalent cations has not been investigated.

In this work we show the presence of two ecto-phosphatase activities on the cell surface of *Trypanosoma cruzi* amastigotes that can be distinguished by their substrate specificity, ability to hydrolyze phosphorylated casein and their response to Mg^{2+}, Ca^{2+} and to inhibitors.

Material and Methods

Cell cultures

T. cruzi amastigote were obtained in axenic cultures using the procedure of Rondinelli *et al* (1988), with modifications described below. With this method it is possible to obtain the different morphological stages of the parasite, in amounts amenable to biochemical procedures and devoid of contaminating mammalian host cells. The cultures are initiated with epimastigotes (CL strain, CL 14 clone), which are routinely maintained in the laboratory (Oliveira *et al.*, 1993). In the log phase of culture the cells were washed twice and ressuspended in M16 medium (0.4% NaCl, 0.04% KCl, 0.8% Na_2HPO_4, 0.2% glucose, 0.125% tryptone, 2.5% bovine serum, 2.0% hemoglobin, pH 6.7), in a concentration of 2×10^7 cells/ml. Cell differentiation is completed by the 5th day of incubation at 29 °C, with a yield of 70–90% trypomastigotes. These were purified through a DOWEX E-52 column and immediately incubated with an equal volume of fresh mammalian plasma for 1h at 29 °C. After centrifugation at $2000 \times g$ for 10 min the pellet was resuspended in LIT medium (Oliveira *et al.*, 1993) and incubated at 29 °C. By the 5th day of culture, amastigotes clusters were collected by centrifugation at $2000 \times g$ for 1 minute and the pellet dispersed in LIT medium.

The amastigotes cultures were propagated by transference to new LIT medium every 48 h. Before enzymatic analyses, the cells were washed three times with a solution (150.8 mM NaCl, 2.7 mM KCl, 0.5 mM $MgCl_2$, 6 mM glucose, 2.7 mM $CaCl_2$, 10 mM Hepes-NaOH, pH 7.2). Since amastigotes are not motile, the viability of the cells were checked by trypan blue exclusion (Meyer-Fernandes *et al.*, 1997).

Preparation of ^{32}P-labeled casein

Phosphorylated ^{32}P-labeled casein was prepared by mixing 5 mg/ml of previously dephosphorylated casein with casein kinase II obtained from Rhodnius *prolixus* oocytes (Silva-Neto and Oliveira, 1993). The following reaction medium was used: 50 mM Tris-HCl pH 8.0, 10 mM $MgCl_2$, 150 mM NaCl, 1 mM NaF, 1.2 mM EDTA, 1.2 mM EGTA, 100 μM ^{32}P-[ATP] 10^5 Bq/nmol). When phosphorylation was completed, aliquots were analyzed for incorporation of phosphate (Silva-Neto and Oliveira, 1993). The reaction mixture was then passed through spin columns, equilibrated in 20 mM Tris-HCl, pH 8.0 in order to remove remaining ^{32}P-[ATP]. ^{32}P-labeled casein was then adjusted to 4.6 mg/ml using the following buffer: 20 mM Tris-HCl, 15 mM NaCl, 10 mg/ml casein, 2 mM benzamidine and 100 μM PMSF, pH 8.0.

p-Nitrophenylphosphate hydrolysis

The standard assay for *p*-nitrophenylphosphate (PNPP) hydrolysis, unless otherwise specified in legends of figures and tables, was measured as follows. Reaction mixtures (0.5 ml) contained 10 mM PNPP, 50 mM Tris-HCl pH 7.2 and 1 mg/ml of protein from intact amastigotes. The reaction was initiated by the addition of cells, incubated during 1 hour at 30 °C and terminated by the addition of 1 ml of 1 N NaOH. The phosphatase activity was calculated by subtracting the nonspecific PNPP hydrolysis measured in the absence of cells. The PNPP hydrolysis was linear with time under the conditions used and was proportional to the protein concentration. The released *p*-nitrophenol was determined spectrophotometrically at 425 nm using the extinction coeficient of 1.75×10^4 M^{-1} cm^{-1}). The values shown represent averages ± SE of three experiments with different cell suspensions.

Phosphoamino acids hydrolysis

The phosphorylated aminoacids hydrolysis was measured in the same conditions of PNPP hydrolysis except that PNPP was replaced by phosphoamino acids. In these conditions the reaction was also linear with time under the conditions used and were proportional to the protein concentration. The free phosphate released was determined in the end of reaction (Furuya *et al.*, 1998). The values shown represent averages ± SE of three experiments with different cell suspensions.

Phosphoprotein hydrolysis

Hydrolysis of ^{32}P-labeled casein was measured in the same conditions used for PNPP hydrolysis. The reactions were initiated by the addition of cells and terminated by the addition of 0.5 ml 20% trichloacetic acid. The ^{32}P released was measured as a phosphomolybdate complex using a mixture of benzene and isobutyl alcohol (Vieyra *et al.*, 1985). The organic supernatant (0.5 ml) was added to 9 ml of scintilation liquid (2 g PPO, 1 g POPOP in 1 l toluene) and counted in a liquid scintilation counter. The values shown represent averages ± SE of three experiments with different cell suspensions.

Chemicals

All chemicals were obtained from Merck S. A. (São Paulo, S. P.) or Sigma Chemical Co. (St. Louis, MO). Distilled water deionized by the MilliQ system of resins (Millipore Corp., Bedford, MA) was used in the preparation of all solutions.

Statistical analysis

All experiments were performed in triplicate, with similar results obtained at least in three separate cell suspensions. Apparent K_m for PNPP was calculated using a computerized nonlinear regression analysis of the data to the Michaelis-Menten equation (Meyer-Fernandes *et al.*, 1997). I_{50} is the concentration of inhibitors that gives half-maximal inhibition. Differences were evaluated for statistical significance by using Student's *t* test for paired or unpaired data, as required.

Results

Living *T. cruzi* amastigotes at pH 7.2 were able to hydrolyse *p*- nitrophenylphosphate (PNPP) at a rate of 30 $nmol \cdot mg^{-1} \cdot h^{-1}$ in the presence of 1 mM CDTA (basal activity) which was stimulated to more than double, reaching 80 $nmol \cdot mg^{-1} \cdot h^{-1}$. In the presence of 5 mM $MgCl_2$. At saturating concentration of PNPP, half maximal stimulation of PNPP hydrolysis was obtained with 0.22 mM $MgCl_2$ (Fig. 1A). This stimulatory activity was not observed when Mg^{2+} was replaced by Ca^{2+}. The calcium modulation of the phosphatase activities is shown in Figure 1B, where the basal phosphatase activity was insensitive to $CaCl_2$, while the phos-

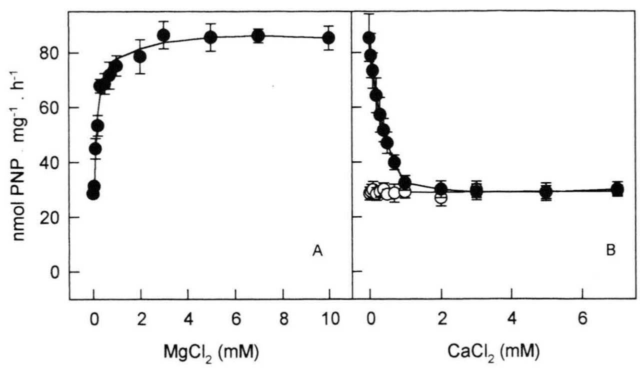

Fig. 1A. Dependence of $MgCl_2$ concentration on the ecto-phosphatase activity. Cells were incubated for 1 h at 30 °C in a reaction medium containing 50 mM Tris-HCl pH 7.2, 10 mM PNPP, 1 mg/ml of protein from *T cruzi*, intact cells and increasing concentration of $MgCl_2$ as shown on the abscissa. Curve for Mg^{2+}-phosphatase activity represents the fit of experimental data by linear regression using the Michaelis-Menten equation as described under Materials and Methods.
Fig. 1B. Effects of $CaCl_2$ on ecto-phosphatase activities in intact cells. Conditions of the assay are identical to those described in Figure 1, panel A with increasing concentration of $CaCl_2$ as shown on the abscissa, in the absence (○) or in the presence of 5 mM $MgCl_2$ (●).

phatase activity stimulated by physiological concentrations of $MgCl_2$ was inhibited by $CaCl_2$, in a dose-dependent manner (I_{50} = 0.43 mM) (Fig. 1B). It is important to note that the inhibitory calcium concentration is well above the mammalian cytoplasmic values, where amastigotes reside within the host cell, thus suggesting that the Mg-dependent ecto-phosphatase would be active under those conditions. To check the possibility that the observed activities could be the result of secreted soluble enzymes, we incubated the cells in reaction mixture without PNPP. Subsequently, the cells were removed by centrifugation and the supernatant was assayed for phosphatase activities. This supernatant failed to show PNPP hydrolysis either in the absence or presence of $MgCl_2$ (data not shown).

As can be seen in Fig. 1A the Mg^{2+} stimulated phosphatase activity (difference between total minus basal phosphatase activity) was higher than the basal phosphatase activity. In Fig. 2 it is shown that the affinity of the two phosphatases for PNPP were also different. In the absence of Mg^{2+} (1 mM CDTA) the apparent K_m for PNPP was 1.57 mM and at saturating $MgCl_2$ concentrations, the corresponding apparent K_m for PNPP for Mg-stimulated phosphatase activity (difference between total minus basal phosphatase activity) was 0.99 mM.

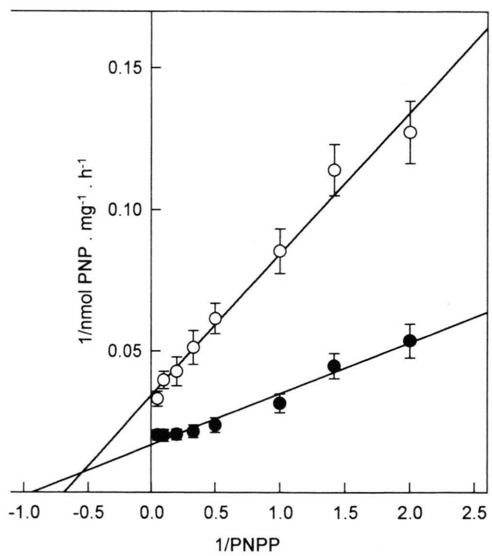

Fig. 2. Double reciprocal plot of the dependence of PNPP concentration of *T. cruzi* amastigote ecto-phosphatase activities. Cells were incubated for 1 h at 30 °C in a reaction medium containing 50 mM Tris-HCl pH 7.2 and 1 mg/ml of protein from *T cruzi* intact cells, in the absence (○) or in the presence of $MgCl_2$ (●).

In addition, these enzymes were able to hydrolyze phosphoaminoacids (Fig. 3). This figure shows that the cation requirement for PNPP and P-tyrosine hydrolysis is the same, namely Mg^{+2}-

activated, Ca^{+2}-inhibited activity and a Ca^{+2}-insensitive basal phosphatase activity (Fig. 3). The enzyme which dephosphorylated P-treonine and P-serine do not display the same Ca^{+2} and Mg^{+2} modulation (Fig. 3). These results suggest that at least two phosphatase activities are present on the amastigotes surface, implying that the same enzyme was active against PNPP and P-tyrosine.

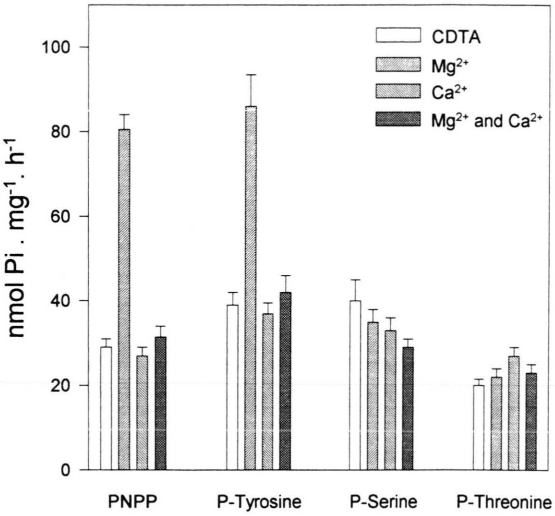

Fig. 3. Substrate specificity of *T. cruzi* amastigote ecto-phosphatase activities. Cells were incubated for 1 h at 30 °C in a reaction medium containing 50 mM Tris-HCl pH 7.2, 1 mg/ml of protein from *T cruzi*, intact cells in the presence of different substrates in different conditions (1 mM CDTA, 5 mM $MgCl_2$, 5 mM $CaCl_2$, and 5 mM $MgCl_2$ plus 5 mM $CaCl_2$).

To test this hypothesis competition studies were done and the results the Fig. 4 are showing that P-tyrosine was a competitive inhibitor of the PNPP hydrolysis Mg^{+2}-activated. In the presence of 10 mM of P-tyrosine the $S_{0.5}$ for PNPP was increased to 3.5 mM. These data suggested that the Mg^{2+} dependent ecto-phosphatase was also a P-tyrosine phosphatase. To prove this we studied the sensitivity to known P-tyrosine phosphatase inhibitors, such as vanadate, Zn^{+2} and sodium fluoride (Lau *et al.*, 1989). As shown in Fig. 5 the Mg^{2+}-dependent phosphatase activity was strongly inhibited by micromolar concentrations of vanadate (I_{50} = 3.3 μM; Fig. 5A, closed circle) and $ZnCl_2$ (I_{50} = 260.6 μM; Fig. 5B, closed circle), while the Mg^{2+}-insensitive phosphatase activity was less in-

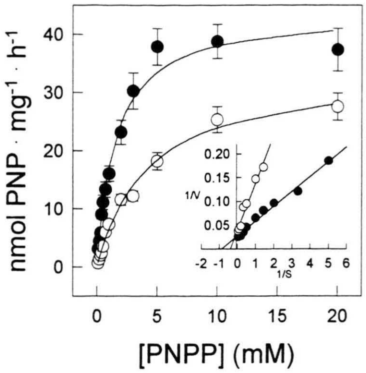

Fig. 4. Influence of phospho-tyrosine on PNPP hydrolysis catalyzed by Mg^{2+}-dependent ecto-phosphatase activity. Cells were incubated for 1 h at 30 °C in a reaction medium containing 50 mM Tris-HCl pH 7.2, 1 mg/ml of protein from *T cruzi*, intact cells and increasing concentration of PNPP as shown on the abscissa, in the absence (●) or in the presence of 10 mM P-tyrosine (○). Inset shows double reciprocal plot of the inhibition of *p*-tyrosine.

hibited by vanadate (I_{50} = 94.2 μM; Fig. 5A, open circle) and $ZnCl_2$ (I_{50} = 821.6 μM; Fig. 5B, open circle). Furthermore NaF was also a inhibitor of the Mg^{2+} dependent phosphatase (I_{50} = 923. 3 μM; Fig. 5C, closed circle), having no effect on the basal phosphatase activity (Fig. 5C, open circle). No inhibition was observed in both activities when levamizole, an alkaline phosphatase inhibitor (Fernandes *et al.*, 1997), or tartrate, a secreted phosphatase inhibitor (Lovelace and Gottlieb, 1986) were added to the reaction medium (data not shown).

To determine whether these ecto-phosphatases were able to hydrolyse phosphate residues in proteins, we prepared a phosphorylated casein (Methods) to use in our assay. When ^{32}P-casein, a protein phosphorylated in serine and threonine residues was used as a substrate, its dephosphorylation was observed only in the presence of $MgCl_2$ (data not shown). These intact cells in the presence of 5 mM $MgCl_2$ were able to hydrolyze ^{32}P-casein at a rate of 63.3 pmol $^{32}Pi \cdot mg^{-1} \cdot h^{-1}$. To insure that no proteolytic residues were being measured, we took special care to extract the TCA supernatant with phosphomolybdate complex using a benzene and isobutyl alcohol mixture (Methods).

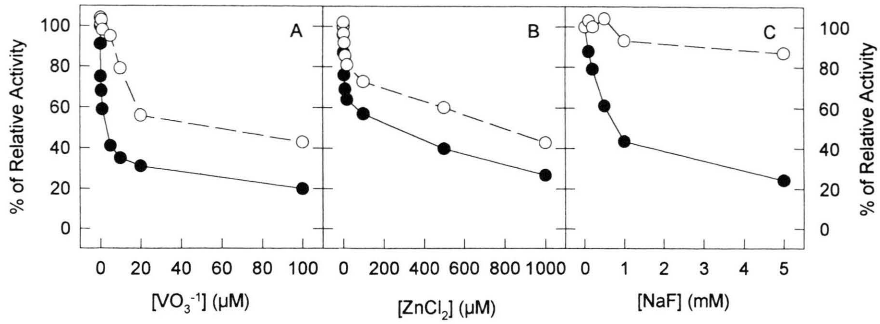

Fig. 5. Inhibition of *T. cruzi* amastigote ecto-phosphatases activities by phospho-tyrosine phosphatase inhibitors. Cells were incubated for 1 hour at 30 °C in a reaction medium containing 50 mM Tris-HCl pH 7.2, 10 mM PNPP, 1 mg/ml of protein from *T. cruzi*, intact cells and increasing concentration of vanadate (A), $ZnCl_2$ (B) and NaF (C) in the absence (○) or in the presence of $MgCl_2$ (●).

Discussion

In *Leishmania* an acid phosphatase (AcP) localized on the external surface can also hydrolyze phospho-aminoacids, but the hydrolysis is not stimulated by divalent cations such as Mg^{+2} (Lovelace *et al.*, 1986). This membrane-bound phosphatase activity was described as a virulence marker in *Leishmania donovani* (Katakura and Kobayashi 1988; Singla *et al.*, 1992). This process may involve the hydrolysis of phosphatidylinositol, phospholipids and phosphoproteins (Das *et al.*, 1986). Recently, it has been shown that Leishmanial AcP modulates attachment to macrophages (Vannier-Santos *et al.,* 1995) and it has been suggested that signal transduction networks, involving ecto-enzymes with tyrosine kinase and phosphatase activities may modulate crucial events during *Leishmania* infection (Martiny *et al.*, 1996). Recently it has been suggested an important role for protein tyrosine phosphorylation (Favoreto *et al.*, 1998) and dephosphorylation (Zhong *et al.*, 1998) in the invasion of host cells by *Trypanosoma cruzi.*

In this work we demonstrated that in external cell surface of the amastigote form of *T. cruzi* there are two phosphatase activities that can hydrolyse phosphoaminoacids and phosphoproteins, distinguished by their substrate specificity (Fig. 3) and their responses to inhibitors and activator cations (Figs. 1, 5). It is known that most phosphotyrosyl protein phosphatases can hydrolyze both phosphotyrosine residues and PNPP (Lau *et al.*, 1989). The stimulation by Mg^{+2} of phosphatase activity (Figs. 1, 3) and its inhibition by micromolar range concentrations of Ca^{+2} (Fig. 1B) could be indicating that in amastigote form of *T. cruzi* there is a phosphoprotein phosphatase activity able to hydrolyze phosphoamino acids and phosphoproteins under physiological conditions. It has been described a Mg^{2+}-dependent, Ca^{2+}-inhibitable serine/threonine protein phosphatase in bovine brain, but this activity is not inhibited by vanadate (Wang *et al.*, 1995). The high sensitivity to vanadate (Fig. 5, panel A) and $ZnCl_2$ (Fig. 5, panel B), two known potent and specific phosphotyrosyl protein phosphatases inhibitors (Swarup *et al.*, 1981; Lau *et al.,* 1989), suggest that this Mg^{2+}-dependent phosphatase has similarities with the tyr/ser protein phosphatase present in *vaccinia* virus (Guan *et al.*, 1991) and might dephosphorylate phosphoproteins phosphorylated in tyrosine and serine residues on host cell. The reason for the no complete inhibition of the Mg^{2+}-dependent phosphatase activity by vanadate (Fig. 5, panel A), $ZnCl_2$ (Fig. 5, panel B) and NaF (Fig. 5, panel C) remains unclear. It is possible that the supposed selective action of these inhibitors depends on the catalytic mechanism of the enzymes, substrate specificity and association with possible specific regulatory subunits. Other protein phosphatases such as the receptor protein tyrosine phosphatase (RPTP) were shown to have an important role in the process of homophilic cell-cell adhesion (Fischer *et al.*, 1991; Gebbink *et al.*, 1993). We suggest that these phosphatases present on the surface membrane which externally dispose the active sites, are active within the range of physiological pH and able to hydrolyze phosphoproteins may have physiological role in the interactions between parasite and host cells.

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Bakalara N., Seyfang A., Davis C. and Baltz T. (1995), Characterization of a life-cycle-stage-regulated membrane protein tyrosine phosphatase in *Trypanosoma brucei*. Eur.J. Biochem. **234**, 871–877.

Bakalara N., Seyfang A., Baltz T. and Davis C. (1995), *Trypanosoma brucei* and *Trypanosoma cruzi*: life cycle-regulated protein tyrosine phosphatase activity. Exp. Parasitol. **81**, 302–312.

Burns J. M., Parsons M., Rosman D. E. and Reed S. G. (1993), Molecular cloning and characterization of a 42-kDa protein phosphatase of *L. chagasi*. J. Biol. Chem. **268**, 17155–17161.

Cohen P. (1989), The structure and regulation of protein phosphatases. Annu. Rev. Biochem. **58**, 453–508.

Cool D. E. and Blum J. J. (1993), Protein tyrosine phosphatase activity in *Leishmania donovani*. Mol. Cell Biochem. **127**, 143–149.

Das S., Saha A. K., Remaley A. T., Glew R. H., Dowling J. N., Kajiyoshi M. and Gottlieb M. (1986), Hydrolysis of phosphoproteins and inositol phosphates by cell surface phosphatase of *Leishmania donovani* Mol. Biochem. Parasitol. **20**, 143–153.

DePierre J. W. and Karnovsky M. L. (1973), Plasma membrane of mammlian cells. A review of methods for their characterization and isolation. J. Cell Biol. **56**, 275–286.

De Souza W. (1984), Cell biology of *Trypanosoma cruzi*. Int. Rev. Cytol. **26**, 197–283.

Fernandes E. C., Meyer-Fernandes J. R., Silva-Neto M. A. C. and Vercesi A. E. (1997), *Trypanosoma brucei*: ecto-phosphatase activity on the surface of intact procyclic forms. Z. Naturforsch. **52c**, 351–358.

Fischer E. H., Charbonneau H. and Tonks N. K. (1991), Protein tyrosine phosphatase: A diverse family of intracellular and transmembrane enzymes. Science **253**, 401–406.

Furuya T., Zhong L., Meyer-Fernandes J. R., Lu H-G., Moreno S. N. J. and Docampo R. (1998), Ecto-protein tyrosine phosphatase activity in *Trypanosoma cruzi* infective stages. Mol. Biochem. Parasitol. **92**, 339–348.

Gebbink M. F. B. G., Zondag G. C. M., Wubbolts R. W., Beijersberge R. L., Van Etten I. and Moolenaar W. H. (1993), Cell-cell adhesion mediated by a receptor-like protein tyrosine phosphatase. J. Biol. Chem. **268**, 16101–16104.

Guan K. and Dixon J. E. (1991), A tyr/ser protein phosphatase encoded by vaccinia virus. Nature **350,** 359–362.

Hunter T. (1995), Protein kinases and phosphatases: the yin and yang of protein phosphorylation and signaling. Cell **80**, 225–236.

Katakura K. and Kobayashi A. (1988), Acid phosphatase activity of virulent and avirulent clones of *Leishmania donovani* promastigotes. Infect. Immun. **56**, 2856–2860.

Lau K.-H. W., Farley J. R. and Baylink D. J. (1989), Phosphotyrosyl protein phosphatases. Biochem. J. **257**, 23–36.

Lovelace J. K. and Gottlieb M. (1986), Comparison of extracellular acid phophatases from various isolates of *Leishmania*. Amer. J. Trop. Med. Hyg. **35**, 1121–1128.

Lovelace J. K., Dwyer D. M. and Gottlieb M. (1986), Purification and characterization of the extracellular acid phosphatase of *L. donovani*. Mol. Biochem. Parasitol. **20**, 243–251.

Martiny A., Vannier-Santos M. A., Borges V. M., Meyer-Fernandes J. R., Asseruy J. Cunha e Silva N. L. and de Souza W. (1996), *Leishmania*-induced tyrosine phosphorylation in the host macrophage and its implication to infection. Eur. J. Cell Biol. **71**, 206–215.

McLaughlin J. (1986), The association of distinct acid phosphatases with the flagella pocket and surface membrane fractions obtained from bloodstream forms of *T. rhodesiense*. Mol. Cell Biochem. **70**, 177–184.

Meyer-Fernandes J. R., Dutra P. M. L., Rodrigues C. O., Saad-Nehme J. and Lopes A. H. C. S. (1997), Mg-dependent ecto-ATPase activity in *Leishmania tropica*. Arch. Biochem. Biophys. **341**, 40–46.

Montserat J., Chen L., Lawrence D. S. and Zhang Z-Y. (1996), Potent low molecular weight substrates for protein-tyrosine phosphatase. J. Biol. Chem. **271**, 7868–7872.

Nakagura K. H., Tachibana H. and Kaneda Y. (1985), Alteration of the cell surface acid phosphatase concomitant with morphological transformation in *Trypanosoma cruzi*. Comp. Biochem. Physiol. **81B**, 815–817.

Oliveira M. M., Rocha E. D. Rondinelli E., Arnholdt A. V. and Scharfstein J. (1993), Signal transduction in *Trypanosoma cruzi*: opposite effects of adenylcyclase and phospholipase C systems in growth control. Mol. Cell. Biochem. **124**, 91–99.

Parsons M., Valentine M. and Carter V. (1993), Protein kinases in divergent eukaryotes: Identification of protein kinase activities regulated during trypanosome development. Proc. Acad. Sci. USA **90**, 2656–2660.

Rondinelli E, Silva R., Carvalho J. F. O., Soares C. M. A., Carvalho E. P. and Castro F. T. (1988), *Trypanosoma cruzi*: an *in vitro* cycle of cell differentiation in axenic culture. Exp. Parasitol. **66**, 197–204.

Silva-Neto M. A. C. and Oliveira P. L. (1993), Protein phosphorylation in *Rhodnius prolixus* oocytes: indentification of a type II casein kinase. Insect. Biochem. Mol. Biol. **23**, 815–823.

Singla N., Khuller G. K. and Vinayak V. K. (1992), Acid phosphatase activity of promastigotes of *L. donovani*:a marker of virulence. FEMS Microbiol. Lett. **94**, 221–226.

Swarup G., Cohen S. and Garbers D. L. (1981), Selective dephosphorylation of proteins containing phosphotyrosine by alkaline phosphatases. J. Biol. Chem. **256**, 8197–8201.

Tosomba O. M., Coetzer T. H. T. and Lonsdale-Eccles J. D. (1996), Localization of acid phosphatase activity on the surface of bloodstream forms of *Trypanosoma congolense*. Exp. Parasitol. **84**, 429–438.

Vannier-Santos M. A., Martiny A., Meyer-Fernandes J. R. and de Souza W. (1995), Leishmanial protein kinase C modulates host cell infection via secreted acid phosphatase. Eur. J. Cell. Biol. **67**, 112–119.

Vieyra A., Meyer-Fernandes J. R. and Gama O. B. H. (1985), Phosphorolysis of acetyl phosphate by orthophosphate with energy conservation in the phosphoanydride linkage of pyrophosphate. Arch. Biochem. Biophys. **238**, 574–583.

Wang Y., Santini F., Qin K. and Huang C. Y. (1995), A Mg^{2+}-dependent, Ca^{2+}-inhibitable serine/threonine protein phosphatase from bovine brain. J. Biol. Chem. **270**, 25607–25612.

Zhang Z-Y. (1995), Are protein-tyrosine phosphatases specific for phosphotyrosine? J. Biol. Chem. **270**, 16052–16055.

Zhong L., Lu H-G., Moreno S. N. L. and Docampo R. (1998), Tyrosine phosphate hydrolysis of host proteins by *Trypanosoma cruzi* is linked to cell invasion. FEMS Microbiol. Lett. **161**, 15–20.