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Ecto-phosphatases in protozoan parasites: possible roles in nutrition, growth and ROS sensing

Daniela Cosentino-Gomes · José Roberto Meyer-Fernandes

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Abstract The cellular plasma membrane contains 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 living cells. Ecto-phosphatases are ecto-enzymes that presumably hydrolyze extracellular phosphorylated substrates, releasing free inorganic phosphate. Although, several alternative functions have been suggested for these enzymes, such as participation in proliferation, differentiation, adhesion, virulence, and infection, little is known about the physiological roles of these enzymes in protozoa parasites. In this review, we discuss the principal features of ecto-phosphatases in protozoan parasites that are causative agents of important diseases such as Chagas' disease, leishmaniasis, amoebiasis, giardiasis, trichomoniasis and, sleeping sickness.

Keywords Ecto-phosphatase · Protozoan parasites · Ecto-enzymes · Membrane-bound acid phosphatase

Protein phosphorylation/dephosphorylation is an important mode of regulation of cellular activities such as differentiation and proliferation (Bakalara et al. 1995, 2000; Meyer-Fernandes et al. 1999; Gomes et al. 2006; Fonseca-de-Souza et al. 2008, 2009; Dick et al. 2010). The plasma membrane of cells may display 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 by assaying intact cells (Meyer-Fernandes 2002; Lemos et al. 2002; Jesus et al. 2002; Fernandes et al. 2003; Peres-Sampaio et al. 2008). The reversible phosphorylation of extracellular proteins can be controlled by the coupled action of two classes of enzymes: ecto-phosphatases and ecto-kinases (Andreeva and Kutuzov 2008; Szöör 2010). The phosphorylation and dephosphorylation of serine, threonine, and tyrosine residues in ectodomains of functionally important surface proteins and/or soluble external substrates are involved in processes such as cell growth inhibition (Friedberg et al. 1995; Bakalara et al. 2000) and parasite-host interactions (Vannier-Santos et al. 1995; Martiny et al. 1996, 1999; Zhong et al. 1998; Bakalara et al. 2000; Fernandes et al. 2003; Kneipp et al. 2004; Olivier et al. 2005; Collopy-Junior et al. 2006; Kiffer-Moreira et al. 2007; Portela et al. 2010).

Phosphotyrosyl protein phosphatases, have been described (Lau et al. 1989; Zhang 1995; Montserat et al. 1996) as being active toward low molecular weight nonprotein phosphoesters, such as alkyl and aryl phosphates (including *p*-nitrophenylphosphate). Ecto-phosphatase activity with high affinity for phosphotyrosine substrates (ecto-PTPase) has been demonstrated in several protozoan parasites, including *Trypanosoma brucei* (Bakalara et al. 2000; Fernandes et al. 1997, 2003); *Trypanosoma cruzi* (Furuya et al. 1998; Meyer-Fernandes et al. 1999); *Trypanosoma rangeli* short epimastigote forms (Gomes et al. 2006); *Phytomonas* (Dutra et al. 2000, 2001), *Entamoeba histolytica* (Anaya-Ruiz et al. 2003; Aguirre-García et al. 2003; Pinheiro et al. 2007); *Giardia lamblia* (Amazonas et al. 2009); and *Leishmania* species (Remaley et al. 1985; Cool and Blum 1993; Wiese et al.

D. Cosentino-Gomes · J. R. Meyer-Fernandes (✉)
Instituto de Bioquímica Médica, Universidade Federal do Rio de Janeiro, CCS, Bloco H, Cidade Universitária,
Ilha do Fundão,
21941-590, Rio de Janeiro, RJ, Brazil
e-mail: meyer@bioqmed.ufrj.br

D. Cosentino-Gomes · J. R. Meyer-Fernandes
Instituto Nacional de Ciência e Tecnologia de Biologia Estrutural e Bioimagem (INCTBEB), CCS, Bloco H, Cidade Universitária,
21941-590, Rio de Janeiro, RJ, Brazil

1996; Aguirre-García et al. 2006; de Almeida-Amaral et al. 2006). These ecto-enzymes are usually reported to have optimum activities in the acidic pH range, and they are therefore also known as membrane-bound acid phosphatases (McLaughlin 1986; Lovelace and Gottlieb 1986; Tosomba et al. 1996; De Jesus et al. 2002a). The low optimum pH and the surface location of these enzymes suggest a role in an acidic microenvironment and/or a close relationship with lysosomal digestion, possibly reflecting an adaptation of the parasite to the intracellular or phagosomal environment. (Gottlieb and Dwyer 1981; Shakarian et al. 2002; Aguirre-García et al. 2003, 2006).

To date, ecto-protein phosphatases have been cloned and purified in some parasites. In *T. brucei*, the enzyme may be regulated with respect to life cycle stages, because it is expressed in bloodstream forms but not in the insect procylic form. Moreover, the T. brucei enzyme may represent a new ecto-phosphatase class lacking homology to other known phosphatases (Bakalara et al. 2000). Purified tartrate-resistant acid phosphatase of *L. donovani* was characterized as an integral membrane glycoprotein that accounts for more than 70% of the total cellular acid phosphatase activity (Remaley et al. 1985). Later, this fact was confirmed by the identification of the gene that encoding this enzyme (Shakarian et al. 2002). An ecto-phosphatase was also cloned and purified in *L. mexicana*, where it was located in the endosomal/lysosomal compartment between the flagellar pocket and the nucleus in wild-type promastigotes, and the overexpression of this protein leads to its abundant exposure on the cell surface (Menz et al. 1991; Wiese et al. 1996). The same was seen with membrane-bound acid phosphatase from the bloodstream form of *T. brucei*, where the enzyme is supposed to participate in the maintenance of endocytosis/exocytosis and in differentiation to the insect stage (Engstler et al. 2005). The wide distribution of acid phosphatases on the cell may reflect some physiological adaptation for parasite survival within the host. Its surface localization was also confirmed by electron microscopic examination in *T. congolense* and trypanosomatid parasites of plants such as *Phytomonas françai* and *Phytomonas mcgheei*, *L. mexicana*, *L. donovani*, *T. rangeli*, *E. histolytica* and *T. vaginalis* (Gottlieb and Dwyer 1981; Tosomba et al. 1996; Wiese et al. 1996; Dutra et al. 1998, 2000; De Jesus et al. 2002b; Gomes et al. 2006; Pinheiro et al. 2007).

The modulation of ecto-phosphatase activity by divalent metals has been described for some parasites. In *T. cruzi*, about one third of the Y-strain ecto-phosphatase activity is Mg^{2+}-dependent (Dutra et al. 2006). Likewise, in *T. rangeli* and *T. brucei* this divalent ion exerts almost the same effect (Fernandes et al. 2003; Gomes et al. 2006; Fonseca-de-Souza et al. 2008). In all cases, it seems that the Mg^{2+}-dependent ecto-phosphatase activity is due to a different enzyme, because several biochemical features are distinct. Moreover, $MnCl_2$ and $CaCl_2$ modulate the phosphatase activities of *T. cruzi*, *T. brucei* and *T. rangeli* in a different manner depending on the life stage of the cells (Meyer-Fernandes et al. 1999; Bakalara et al. 2000; Fernandes et al. 2003; Gomes et al. 2006). In *Trichomonas vaginalis* and *Tritrichomonas foetus*, the ion Fe^{2+} has been ascribed a pivotal role in enhancing the expression of ecto-phosphatase activities (De Jesus et al. 2006). In its oxidized form (Fe^{3+}) it is able to stimulate *E. hystolitica* phosphatase (Anaya-Ruiz et al. 2003).

Although the physiological functions of ecto-phosphatases are not well established in protozoan parasites, the pretreatment of cells with inhibitors suggests a role in cell proliferation and adhesion. The incubation of *L. amazonensis* promastigotes with sodium orthovanadate, an inhibitor of acid and phosphotyrosine protein phosphatases, increases parasite adhesion to macrophages and promotes intracellular survival within macrophages (Martiny et al. 1996; 1999). Corroborating this fact, Ghosh and Chakraborty (2002) have shown that macrophage tyrosine phosphorylation is involved in parasite entry. Inhibition of macrophage protein phosphatase increases the uptake of *L. donovani* promastigotes. Conversely, the addition of sodium orthovanadate to the culture medium completely inhibits the proliferation of *L. amazonensis* (de Almeida-Amaral et al. 2006). A similar result was shown for *T. rangeli* proliferation (Fonseca-de-Souza et al. 2009). In *G. lamblia*, ecto-phosphatase activity is higher during the time course of encystation, and incubation of *G. lamblia* cells with sodium orthovanadate inhibits this process, but it does not modify the proliferation of trophozoites (Amazonas et al. 2009). The disruption of the actin cytoskeleton in HeLa cells mediated by the ecto-phosphatase of *E. histolytica* is abolished in the presence of ammonium molybdate, an acid phosphatase inhibitor (Anaya-Ruíz et al. 2003). The distinct features of ecto-phosphatases may reflect a broad range of functions for these enzymes, that are dependent on the cell type and the environment. The possible functions described for ecto-phospahates in protozoan parasites are summarized in Table 1.

Parasite life cycles are highly regulated and include significant changes in morphology, biochemical and signal transduction pathways, gene expression and structural alterations to surface molecules (De Souza 2002). A role for phosphotyrosine phosphatase activity in the metacyclogenesis of *T. cruzi* (Nagakura et al. 1985; Bakalara et al. 1995; Meyer-Fernandes et al. 1999) and *T. brucei* (Bakalara et al. 1995; Fernandes et al. 1997) has been suggested. The same was suggested for *L. donovani* which undergoes phosphotyrosine modification in the process of cell differentiation (Cool and Blum 1993).

Because of its extracellular active site and ability to hydrolyze phosphomonoesters, ecto-phosphatases may en-

Table 1 Possible functions of ecto-phosphatase in protozoan parasites

Functions	Organisms	References
Cell differentiation	*T. brucei; G. lamblia; T. cruzi; L. donovani*	Nagakura et al. 1985; Cool and Blum 1993; Bakalara et al. 1995; Fernandes et al. 1997; Meyer-Fernandes et al. 1999; Engstler et al. 2005; Amazonas et al. 2009
Parasite-host interaction	*L. amazonensis; T. cruzi; T. brucei; E. histolytica*	Vannier-Santos et al. 1995; Martiny et al. 1996, 1999; Zhong et al. 1998; Bakalara et al. 2000; Anaya-Ruíz et al. 2003; Aguirre-García et al. 2003
Cell proliferation	*L. amazonensis; T. rangeli*	de Almeida-Amaral et al. 2006; Fonseca-de-Souza et al. 2009
Nutrition	*L. donovani; T. rangeli*	Gottlieb and Dwyer 1981; Fonseca-de-Souza et al. 2009
Inhibition of superoxide anions production	*L. donovani*	Remaley et al. 1984
ROS sensing	*T. rangeli; E. histolytica*	Pinheiro et al. 2007; Cosentino-Gomes et al. 2009

able organisms to obtain necessary nutrients from organic phosphates in their environment (Gottlieb and Dwyer 1981). Recently, it has been demonstrated that ecto-phosphatase activities are extremely important to inorganic phosphate (Pi) acquisition by *T. rangeli*. At low Pi concentrations in the medium, the growth of *T. rangeli* is inhibited. Interesting, when the culture medium is supplemented with β-glycerophosphate (a substrate for ecto-phosphatase), *T. rangeli* recover normal proliferation (Fonseca-de-Souza et al. 2009). It seems that in Pi-limiting condition, *T. rangeli* expresses two distinct phosphatase activities, and yet the concentration of Pi in the growth medium of living *T. rangeli* influences the ability of this parasite to colonize the anterior midgut of its insect vector (Dick et al. 2010). These findings suggest that ecto-phosphatase activity plays roles in parasite nutrition and survival. Proteins implicated in phosphate metabolism are generally encoded by genes belonging to the so-called *pho* regulon family and are transcriptionally activated in response to extracellular inorganic phosphate starvation (Oshima et al. 1996). The transport of inorganic phosphate through the cytoplasmic membrane is performed by the Pst (phosphate specific transport) importer (Surin et al. 1985; Takemaru et al. 1996). In *S. cerevisiae*, transcription of genes encoding acid and alkaline phosphatases and the Pi transporter are coordinately repressed and derepressed depending on the Pi concentration in the culture medium (Oshima et al. 1996). The regulation of this adaptive response is very complex, involving several genes that signal Pi starvation (Ogawa et al. 2000). Most of the phosphatases synthesized under Pi-limiting conditions are either located on the extracellular medium or are associated with the plasma membrane or cell wall (Metzenberg 1979; Kneipp et al. 2004; Dick et al. 2010).

Many studies have pointed to the importance of the mechanisms by which protozoan survive and multiply in host cells. Ecto-phosphatase isolated from *L. donovani* promastigotes inhibits the production of superoxide anions in intact human neutrophils (Remaley et al. 1984). This activity could contribute to the survival of the parasite within the host: parasites with greater ecto-phosphatase activity would be more resistant to oxidative bursts from the host's immune system. *L. donovani* is an obligate intracellular protozoan parasite that resides and multiplies within the hydrolytic milieu of mammalian macrophage phago-lysosomes. Ecto-phosphatase activity from *L. donovani* and *T. cruzi* promastigotes seems to be more resistant to H_2O_2 than that from *T. rangeli*, an apathogenic parasite of mammalian hosts (Saha et al. 1985; Cosentino-Gomes et al. 2009). In addition, reactive oxygen species (ROS) sensing is likely to be an important mechanism for the adaptation and interaction of trypanosomatids with their environment (Steenkamp 2002). Phosphatases that utilize a nucleophilic cysteine residue in catalysis have been shown to be mediators of redox signaling through the reversible oxidative inactivation of their active site (Leslie et al. 2004; Tonks 2005; den Hertog et al. 2005). The Ecto-phosphatase of *T. rangeli* was described to be an important molecule in the detection of H_2O_2 because it can be readily inhibited by either external addition of H_2O_2 or endogenous mitochondrial production of H_2O_2. Indeed, enzymatic or non-enzymatic antioxidants can reverse this inhibition and stimulate the ecto-phosphatase activity (Cosentino-Gomes et al. 2009). Similar results have been shown with respect to antioxidants and E. *histolytica* ecto-phosphatase activity (Pinheiro et al. 2007). More studies are necessary to elucidate the potential of ecto-enzymes to regulate cellular signaling pathways through oxi-reduction reactions.

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