Ecto-Nucleoside Triphosphate Diphosphohydrolase Activities in Trypanosomatids: Possible Roles in Infection, Virulence and Purine Recycling

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Abstract: Ecto-nucleoside triphosphate diphosphohydrolases (ecto-NTPDases), also known as ecto-ATPases and/or ecto-apyrases, are integral membrane glycoproteins or soluble enzymes that are dependent on divalent cations. These ecto-enzymes are important ecto-nucleotidases that are characterized by the ability to hydrolyze nucleoside triphosphates and nucleoside diphosphates to the monophosphate form. The hydrolysis of nucleoside monophosphates to nucleosides such as adenosine may then be catalyzed by the action of ecto-5´nucleotidases. The present study reviews the sequential hydrolysis of ATP → ADP → AMP → adenosine catalyzed by these ecto-enzymes from different trypanosomatids. These reactions participate in the salvage of purines in these parasites and simultaneously interfere with the establishment of infection and changes in the host immune response.

Keywords: Ecto-nucleoside triphosphate diphosphohydrolases, trypanosomatids, virulence, adenosine acquisition.

Surface membrane interactions between parasites and their host cells are of critical importance for the survival of the parasite, from both immunological and physiological viewpoints [1-4]. Plasma membranes of cells may contain enzymes that are oriented with their active sites facing the external medium rather than the cytoplasm. The activities of these enzymes can be measured using living cells [5-8]. Ecto-nucleoside triphosphate diphosphohydrolases are glycoproteins present in the plasma membrane with their active sites facing the external environment, which suggests that these enzymes may be involved in surface membrane interactions between parasites and their host cells. Ecto-nucleoside triphosphate diphosphohydrolases have been described in several protozoa parasites including *Toxoplasma gondii* [9-15], *Tetrahymena thermophila* [16], *Leishmania* sp, [17-25], *Entamoeba histolytica* [26], *Acanthamoeba* sp [27], *Balamuthia mandrillaris* [28], *Trichomonas vaginalis* [29-31], *Trichomonas foetus* [32], *Trichomonas gallinae* [33], *Giardia lamblia* [34], *Crithidia deanei* [35], *Herpetomonas* sp [36, 37] and *Trypanosoma* sp [38-44]. These enzymes are divalent cation-dependent. In trypanosomatide parasites such as *Leishmania tropica* [21], *Leishmania amazonensis* [20], *Crithidia deanei* [35], *Trypanosoma cruzi* [40] and *Trypanosoma rangeli* [39], the ecto-ATPase activities are stimulated by magnesium and manganese, but not by calcium [20, 21, 40]. In *Trypanosoma brucei* the ecto-ATPase activity is stimulated by magnesium and manganese, and also by calcium and zinc [38]. The ecto-nucleoside triphosphate diphosphohydrolases from trypanosomatids are membrane bound enzymes and do not secreted enzymes as observed in the apicomplexan parasite, *Toxoplasma gondii* [13].

Trypanosomatids are protozoan parasites that cannot synthesize purines *de novo* [20, 38, 45]. It has been postulated that these ecto-nucleoside triphosphate diphosphohydrolases could play a role in the salvage of purines from the host in *Leishmania amazonensis* [18, 20], *Trypanosoma cruzi* [40] and *Trypanosoma brucei* [38]. The ability of these trypanosomatids to hydrolyze ATP, ADP and AMP to generate adenosine (Fig. **1**) was confirmed by HPLC analyses [18, 38]. It has also been demonstrated that when these protozoa are grown in the presence of adenosine, they have lower ecto-ATPase activity than in the absence of adenosine [18, 20, 35, 38]. This negative modulation of the ecto-ATPase activity is associated with the lower expression of the enzyme, as confirmed by flow cytometry analysis of *Leishmania amazonensis* incubated with different anti-NTPDase antibodies [18]. In *Trypanosoma brucei*, it was recently shown that the E-NTPDase and ecto-5'nucleotidase activities sequentially dephosphorylate ATP to adenosine: ATP → ADP → AMP → adenosine, making adenosine available to *T. brucei* [38]. The inhibition of E-NTPDase activity but not the ecto-5'nucleotidase activity by ferrous iron and heme suggests that E-NTPDase catalyzes the rate-limiting step in the generation of adenosine from ATP in this protozoa [46].

In trypanosomatids, the localization of ectoATPases in the plasma membrane with their active sites facing the external environment suggests that these enzymes may be involved in virulence and infection [47,48]. Interestingly, exogenous carbohydrates involved with cellular recognition and adhesion of these parasites with their hosts stimulated ecto-ATPase activities from different trypanosomatids.

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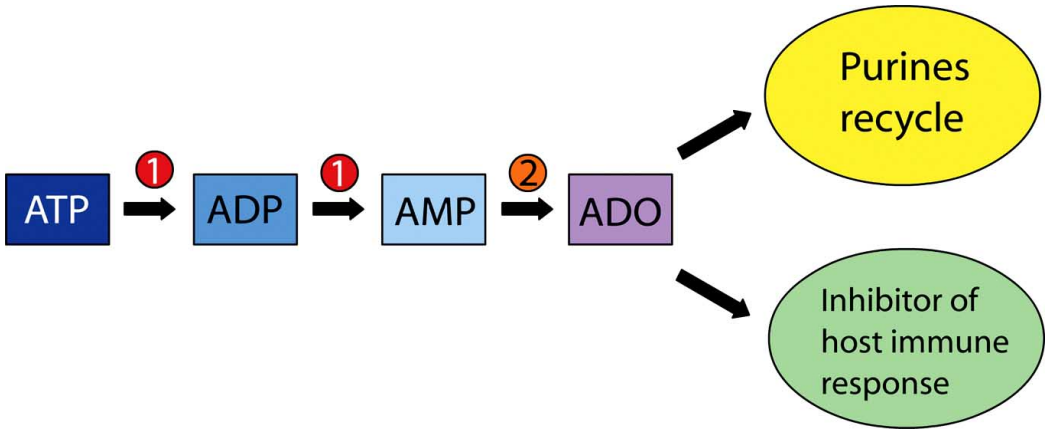

Fig. (1). Partial reactions catalyzed by ecto-nucleoside triphosphate diphosphohydrolase (1) and ecto-5´nucleotidase (2). Extracellular ATP can originate from stressed or injured cells. This mechanism triggers the host inflammatory and immune response. NTPDase of parasites modulate the level of extracellular nucleotides by the sequential hydrolysis of ATP to AMP. AMP is then dephosphorylated by the action of an ecto-5'-nucleotidase to release adenosine (ADO). Adenosine may be used in the salvage of purines or as a mediator of immune suppression by the parasites.

Galactose was a good activator of *T. cruzi* ecto-ATPase [40] while fructose was a good activator of *T. rangeli* ecto-ATPase [39]. Similar findings were also observed with other protozoan parasites: galactose stimulated ecto-ATPase from *Entamoeba histolytica* [26], *Tritrichomonas foetus* [32], *Trichomonas vaginalis* [31], and *Balamuthia mandrilaris* [28] while mannose stimulated the ecto-ATPase from *Acanthamoeba* [27]. Many other studies have shown the participation of ecto-ATPases from trypanosomatids parasites in the infection of the host cells. Ecto-ATPase activity is higher in the infective forms of *T. cruzi* (trypomastigote) than in non-infective forms (epimastigotes) [40, 41, 43]. Suramin and 4,4'diisothiocyanostylbene 2,2'-disulfonic acid, inhibitors of the *T. cruzi* ecto-ATPase, reduced the number of parasites attaching to mouse peritoneal macrophages [41]. ATP, the substrate for these enzymes, protected *T. cruzi* ecto-ATPase activity from inhibition by suramin and 4,4'diisothiocyanostylbene 2,2'-disulfonic acid [40], increasing parasite-infected macrophages [41]. Recently, it was also shown that suramin and other inhibitors of *T. cruzi* ecto-ATPase activity also promoted a marked inhibition of trypomastigotes infectivity [44]. Mice infected with ecto-ATPase-inhibited trypomastigotes had lower levels of parasitemia and higher host survival than non-inhibited control parasites [44]. In *Leishmania amazonensis*, ecto-ATPase activity was also higher in the amastigote stage than in axenic promastigotes [18]. The ecto-ATPase activity of *L. amazonensis* was increased when the parasites were submitted to heat shock [17], which may play a fundamental role in parasites during infection [17]. In species of the genus *Leishmania*, further evidence for a role of ecto-ATPase in virulence comes from the observation that pretreatment of the parasites with anti-NTPDase antibodies reduced the interaction of the promastigotes with mouse peritoneal macrophages [18].

The ecto-nucleoside triphosphate diphosphohydrolases could also play a role in modulating inflammation and the immune response by affecting the extracellular concentration of ATP [22]. ATP, released in the extracellular milieu by injured or pathogen-stimulated cells, participates in many aspects of the establishment of an inflammatory response such as cytokine secretion and cellular migration [22, 47]. The presence of ecto-ATPases and ecto-5´nucleotidases in trypanosomatids suggests the possibility that the coordinated action of these enzymes on extracellular ATP hydrolysis and adenosine production could interfere with the immune response of the host (Fig. **1**). It has been demonstrated that the presence of increased levels of adenosine early in infection by *Leishmania braziliensis* causes an increase in lesion size and parasitism and delayed lesion control [22]. However, it is not yet clear whether only the increase of extracellular adenosine concentration and/or a decrease of ATP concentrations are involved in *Leishmania* infection. Studies on the selective inhibition by ferrous iron and heme of E-NTPDase and ecto-5'nucleotidase activities from *T. brucei* [46] could clarify the role of extracellular ATP and adenosine in several infectious diseases involving Trypanosomatids, opening new perspectives for rational drug design.

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