An Ectonucleotide ATP-diphosphohydrolase Activity in *Trichomonas vaginalis* Stimulated by Galactose and Its Possible Role in Virulence

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This work describes the ability of living *Trichomonas vaginalis* to hydrolyze extracellular ATP (164.0 ± 13.9 nmol Pi / h × 10^7 cells). This ecto-enzyme was stimulated by $ZnCl_2$, $CaCl_2$ and $MgCl_2$, was insensitive to several ATPase and phosphatase inhibitors and was able to hydrolyze several nucleotides besides ATP. The activity was linear with cell density and with time for at least 60 min. The optimum pH for the *T. vaginalis* ecto-ATPase lies in the alkaline range. D-galactose, known to be involved in adhesion of *T. vaginalis* to host cells, stimulated this enzyme by more than 90%. A comparison between two strains of *T. vaginalis* showed that the ecto-ATPase activity of a fresh isolate was twice as much as that of a strain axenically maintained in culture, through daily passages, for several years. The results suggest a possible role for this ecto-ATPase in adhesion of *T. vaginalis* to host cells and in its pathogenicity.

Introduction

The parasitic protozoan *Trichomonas vaginalis* causes human trichomoniasis, a common infection of the urogenital tract. This infection is globally considered one of the most frequent sexually transmitted diseases, with approximately 180 to 200 million cases annually (Petrin *et al.*, 1998). This disease presents various degrees of severity in women, from asymptomatic (nearly 50% of the cases) to extremely acerbic infections (Catterall, 1974). Women who are infected during pregnancy are predisposed to premature rupture of the placental membrane, premature labor, and low-birth-weight infants (Petrin *et al.*, 1998). Also linked to this disease are cervical cancer (Zhang and Begg, 1994), atypical pelvic inflammatory disease, infertility and enhanced HIV transmission (Sorvillo and Kerndt, 1998). *T. vaginalis* exerts its pathogenic effect when interacting with the surface of epithelial cells, although the mechanisms of the pathogenicity of *T. vaginalis* are not well defined (Alderete and Pearlman, 1984). Biochemical aspects on the surface membrane constituents of these parasites have been evaluated and may play an important role in the flagellate's mobility and cytoadhesion (Arroyo *et al.*, 1993).

Surface membrane interactions between parasites and their host cells are of critical importance for the survival of the parasite, from both the immunological and physiological viewpoints (Vannier-Santos *et al.*, 1995; Martiny *et al.*, 1996, 1999). The plasma membrane of cells 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 (Meyer-Fernandes *et al.*, 1997; Furuya *et al.*, 1998). Cell membrane ecto-ATPases are millimolar divalent cation-dependent, low specificity enzymes that hydrolyze all triphosphate nucleotides (Plesner, 1995; Zimmermann, 1999). The identity and the function of ecto-ATPases have been reviewed and the nomenclature of "E-type ATPases" was proposed to describe these enzymes (Plesner, 1995). Their physiological role is until unknown, however, several hypotheses have been suggested, such as (i) protection from cytolytic effects of extracellular ATP

(Steinberg and Di Virgilio, 1991), (ii) regulation of ectokynase substrate concentrations (Plesner, 1995), (iii) involvement in signal transduction (Dubyak and El-Moatassim, 1993; Clifford *et al.*, 1997) and (iv) involvement in cellular adhesion (Knowles, 1995; Kirley, 1997).

Ecto-ATPases have been described in some protozoan parasites such as *Toxoplasma gondii* (Asai *et al.*, 1995; Bermudes *et al.*, 1994; Nakaar *et al.*, 1998), *Entamoeba histolytica* (Barros *et al.*, 2000), *Tetrahymena thermophila* (Smith *et al.*, 1997), *Leishmania* sp. (Meyer-Fernandes *et al.*, 1997; Berrêdo-Pinho *et al.*, 2001), *Trypanosoma cruzi* (Bernardes *et al.*, 2000) and *Tritrichomonas foetus* (Jesus *et al.*, 2002). Here we show the presence of an ecto-ATPase on the cell surface of intact living *T. vaginalis*. We characterized the properties of this enzyme and demonstrate the effects of D-galactose, a carbohydrate exposed on the surface of host cells, involved in *T. vaginalis* adhesion. We also compared the ecto-ATPase activity of a strain maintained axenically for several years in culture with that of a fresh isolate of *T. vaginalis*.

Materials and Methods

Microorganisms and growth conditions

In this study we used two specimens of *Trichomonas vaginalis*, the JT strain, which has been maintained for several years in culture, as well as a fresh isolate, obtained from a clinically and pathologically confirmed case of human trichomoniasis. The parasites were axenically cultivated in TYM medium (Diamond, 1957), supplemented with 10% fetal calf serum, for 24 hours at 37 °C. The cells at late logarithmic phase of growth were collected by centrifugation at 1,400 × *g* for 5 min at 4 °C and washed three times with 50 mM Hepes pH 7.0, 5.5 mM D-glucose, 5.4 mM KCl and 116 mM NaCl. Cellular viability was assessed, before and after incubations, by mobility and the Trypan blue method (Dutra *et al.*, 1998). The viability of the cells was not affected under the conditions employed here.

Ecto-ATPase activity measurements

Intact living parasites were incubated for 1 h at 36 °C in 0.5 ml of a mixture containing, unless otherwise specified, 116.0 mM NaCl, 5.4 mM KCl, 5.5 mM D-glucose, 50.0 mM Hepes (N-[2-hydroxyethyl]piperazine-N′-[2-ethanesulfonic acid]) – Tris (tris[hydroxymethyl]aminomethane) buffer, pH 7.2, 5.0 mM ATP and 3.0×10^7 cells/ml. The ATPase activity was determined by measuring the hydrolysis of [γ-^{32}P]ATP (10^4 Bq/nmol ATP) (Saad-Nehme *et al.*, 1997). The experiments were started by the addition of living cells and terminated by the addition of 1.0 ml of a cold mixture containing 0.2 g charcoal in 1 M HCl. The tubes were then centrifuged at 1,500 × *g* for 10 min at 4 °C. Aliquots (0.5 ml) of the supernatant containing the released ^{32}Pi were transferred to scintillation vials. The ATPase activity was calculated by subtracting the nonspecific ATP hydrolysis measured in the absence of cells. The ATP hydrolysis was linear with time under the assay conditions used and was proportional to the cell number. In the experiments where other nucleotides were used, the hydrolytic activity measured under the same conditions described above was assayed spectrophotometrically by measuring the release of Pi from the nucleotides (Lowry and Lopez, 1946). The hydrolysis of other nucleotides was also calculated by subtracting the nonspecific nucleotide hydrolysis measured in the absence of parasites. The values obtained for the ATPase activities measured using both methods (spectrophotometric and radioactive) were exactly the same. All experiments were performed in triplicate, with similar results obtained in at least three separate cell suspensions.

Statistical analysis

All experiments were performed in triplicate, with similar results obtained in at least three separate cell suspensions. Apparent K_m and V_{max}. values were calculated using a computerized nonlinear regression analysis of the data to the Michaelis-Menten equation (Guilherme *et al.*, 1991). Statistical significance was determined by Student's *t* test. Significance was considered as $P < 0.05$.

Chemicals

All reagents were purchased from E. Merck (Darmstadt, Germany) or Sigma Chemical Co. (St. Louis, MO). (γ^{32}P) ATP was prepared as described by Glynn and Chappel (1964).

Results and Discussion

In this paper we report the presence of an ecto-ATPase activity present on the external surface of *T. vaginalis*. Cellular integrity and viability were assessed, before and after the reactions, by motility and cell dye exclusion (Dutra *et al.*, 1998). The integrity of the cells was not affected by any conditions used in the assays. The time course of ATP hydrolysis by the ecto-ATPase present on the surface of *T. vaginalis* was linear for at least 60 min ($r^2 = 0.9984$). Similarly, in assays to determine the influence of cell density, the ATPase activity measured over 60 min was linear over a nearly 8-fold range of cell density ($r^2 = 0.9993$). The addition of $ZnCl_2$, $CaCl_2$ and $MgCl_2$, but not $MnCl_2$ or $SrCl_2$ stimulated the ATP hydrolysis (Fig. 1). To check the possibility that the observed ATP hydrolysis was the result of secreted soluble enzymes, as seen in other parasites (Bermudes *et al.*, 1994; Smith *et al.*, 1997), we prepared a reaction mixture with parasites that were incubated in the absence of ATP. Subsequently, the suspension was centrifuged to remove cells and the supernatant was checked for ATPase activity. This supernatant failed to show ATP hydrolysis (data not shown). This data also rules out the possibility that the ATPase activity here described could be from disrupted *T. vaginalis* cells.

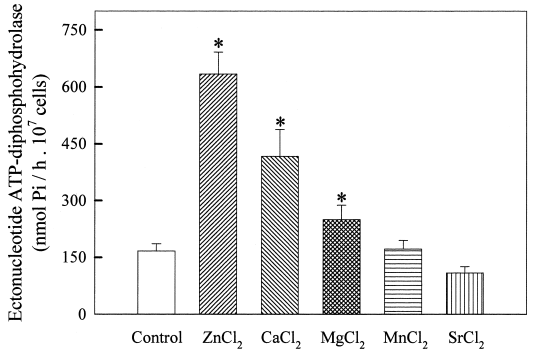

Fig. 1. Influence of different divalent cations on the ecto-ATPase activity of intact living *T. vaginalis* parasites. Cells were incubated for 1 h at 36 °C, in a reaction medium (final volume: 0.5 ml) containing 50 mM Hepes-Tris buffer, pH 7.2, 116 mM NaCl, 5.4 mM KCl, 5.5 mM D-glucose, 3.0×10^7 cells/ml, and 5 mM Tris-ATP (γ-^{32}P) ATP (specific activity = 10^4 Bq/nmol ATP), with the addition of 5 mM of each divalent cation. Data are means ± SE of three determinations, performed in triplicate, with different cell suspensions. Data analyzed by Student's *t* test.* Denotes statistically different from control parasites ($P < 0.05$).

The optimum pH for the ecto-ATPase lies in the alkaline range. In the pH range from 6.4 to 8.0, in which the cells were alive throughout the time course of reaction, the activity increased with the pH, reaching a value 45% higher at pH 8.0 as compared to pH 6.4. Similar results were obtained for *Leishmania tropica* (Meyer-Fernandes *et al.*, 1997), *Leishmania amazonensis* (Berrêdo-Pinho *et al.*, 2001) and *Entamoeba histolytica* (Barros *et al.*, 2000) ecto-ATPases. To discard the possibility that the ATP hydrolysis was due to phosphatase or other type of ATPases with internal ATP binding sites, different inhibitors for those enzymes were tested. Table I shows that the ecto-ATPase activity was insensitive to oligomycin and sodium azide, two inhibitors of mitochondrial Mg-ATPase (Meyer-Fernandes *et al.*, 1997); bafilomycin A_1, a

Table I. Influence of various agents on the ectonucleotide ATP-diphosphohydrolase activity of *T. vaginalis*.

Additions[b]	Relative activity[a]
None	100.4 ± 12.7
Levamizole[c] (1.0 mM)	103.7 ± 10.6
Sodium orthovanadate (1.0 mM)	95.4 ± 10.1
Ammonium molybdate (0.1 mM)	100.5 ± 11.6
Sodium tartrate (1.0 mM)	90.4 ± 10.7
Sodium fluoride (1.0 mM)	108.9 ± 12.3
Ouabain (1.0 mM)	101.5 ± 9.7
Sodium azide (10.0 mM)	90.3 ± 11.9
Bafilomycin A_1 (1 μM)	105.5 ± 9.2
Oligomycin (1 μg/ml)	95.2 ± 9.7
Furosemide (1.0 mM)	91.6 ± 10.1
Dipyridamole[d] (10 μM)	92.1 ± 11.4
DIDS (1 mM)	37.4 ± 4.4

[a] The ectonucleotide ATP-diphosphohydrolase activity was measured in the standard assay described under Material and Methods section. ATPase activity is expressed as a percentage of that measured under control conditions, i.e., without other additions. The ectonucleotide ATP-diphosphohydrolase activity (157.4 ± 13.8 nmol Pi/h × 10^7 cells) was taken as 100%. The standard errors were calculated from the absolute activity values of three experiments, performed in triplicate, with different cell suspensions and converted to percentage of the control value.

[b] The final concentrations of the different agents were the highest ones in which there was no alteration in the parasite integrity.

[c] Levamizole (L[-] 2,3,5,6–tetrahydro 6-phenylimidazo [2,1-b] thiazole) is an inhibitor of alkaline phosphatase (Van Belle, 1976).

[d] dipyridamole (2,6–bis (diethanolamino)–4,8–dipiperidinopyrimido–[5,4–d] pyrimidine) is a nucleoside transporter antagonist (Lemmens *et al.*, 1996).

V-ATPase inhibitor (Browman *et al.*, 1988); ouabain, a Na^++K^+-ATPase inhibitor (Caruso-Neves *et al.*, 1998a); furosemide, a Na^+-ATPase inhibitor (Caruso-Neves *et al.*, 1998b); sodium fluoride and ammonium molybdate, two potent inhibitors of acid phosphatase activity (Dutra *et al.*, 1998) and sodium orthovanadate, a potent inhibitor of P-ATPases and acid phosphatases (Fernandes *et al.*, 1997; Dutra *et al.*, 1998; Meyer-Fernandes *et al.*, 1999). Levamizole, an inhibitor of alkaline phosphatase (Van Belle, 1976), and dipyridamole, a nucleoside transporter antagonist (Lemmens *et al.*, 1996) also failed to inhibit the ATPase activity (Table I). Since we used intact cells for measuring the enzyme activity in all the experiments performed in this work, it is likely that the ATPase activity is an ectoenzyme. To confirm this, we applied the criterion that an authentic ectoenzyme should be inhibited by an added extracellular impermeant inhibitor such as 4, 4′-diisothiocyanostilbene 2′–2′–disulfonic acid (DIDS) (Barros *et al.*, 2000; Meyer-Fernandes *et al.*, 2000; Berrêdo-Pinho *et al.*, 2001). Agreeably, this ATPase activity was inhibited by 63% in the presence of 1 mM DIDS (Table I). For these reasons we assign an ectolocalization of the ATPase activity described here (Plesner, 1995; Meyer-Fernandes *et al.*, 1997, 2000; Berrêdo-Pinho *et al.*, 2001). The dependence on ATP concentration shows a normal Michaelis-Menten kinetics for this ATPase activity and the values of V_{max} and apparent K_m for ATP were 182.5 ± 2.63 nmol Pi/h × 10^7 cells and 0.015 ± 0.0013 mM, respectively (Fig. 2). It has been shown that the mechanism of nucleotide hydrolysis by ecto-ATPases is strongly dependent on the interaction of the transmembrane domains with the active site and solubilized ecto-ATPases have lower catalytic activity than membrane-bound ecto-ATPases (Wang *et al.*, 1998).

The nucleoside triphosphate hydrolyse (NTPase) purified from *T. gondii* was shown to be a mixture of two isozymes, termed NTPase I and NTPase II. A primary difference between these isozymes is that NTPase II hydrolyzes nucleoside triphosphate and diphosphate substrates at almost the same rate, whereas NPTase I was almost exclusively limited to nucleoside triphosphate hydrolyzis (Asai *et al.*, 1995). Recently it has been shown that avirulent *T. gondii* strains express only NTPase II, whereas virulent strains express both NTPase I and NTPase II (Nakaar *et al.*, 1998). We analyzed the specificity of this ecto-ATPase activity for other nucleotides. Table II shows that this ecto-ATPase hydrolyzed ATP, ADP, ITP, TTP, GTP, UTP and CTP at high rates, indicating that it is an ectonucleoside triphosphate diphosphohydrolase, described for other cells (Wang and Guidotti,

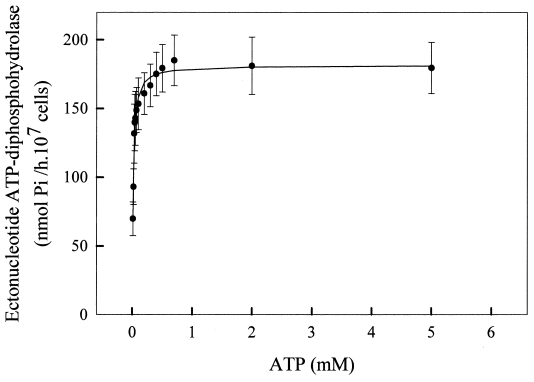

Fig. 2. Dependence on ATP concentrations on the ecto-ATPase activity of intact living *T. vaginalis* parasites. Cells were incubated for 1 h at 36 °C in the same reaction medium (final volume: 0.5 ml) as that described in the legend of Fig. 1, which corresponds to ATP concentrations varying as shown on the abscissa. Curves represent the fit of experimental data by nonlinear regression using the Michaelis-Menten equation as described under Material and Methods. Data are means ± SE of three determinations, performed in triplicate, with different cell suspensions.

Table II. Substrate specificity of the ectonucleotide ATP-diphosphohydrolase activity of *T. vaginalis*.

Nucleotides	Relative activity[a]
ATP	100.0 ± 12.2
ITP	94.8 ± 8.4
TTP	91.1 ± 9.3
GTP	73.9 ± 8.2
UTP	54.4 ± 5.7
CTP	47.1 ± 3.2
ADP	103.4 ± 14.6

[a] The ectonucleotide ATP-diphosphohydrolase activity was measured in a standard assay described under Material and Methods section with the nucleotides listed (5 mM). The ATP hydrolysis (162.5 ± 12.6 nmol Pi/h × 10^7 cells) was taken as 100%. The standard errors were calculated from the absolute activity values of three experiments, performed in triplicate, with different cell suspensions and converted to percentage of the control value. In these experiments, ATP hydrolysis was measured using the same calorimetric assay of Pi release from other nucleotides as that described under Material and Methods section.

1996; Barros *et al.*, 2000; Meyer-Fernandes *et al.*, 2000).

Carbohydrates exposed on the surface of mammalian cells play an important role in the interaction of those cells with *T. vaginalis* (Bonilha *et al.*, 1995). Adhesins with lectin properties comparable to those reported for *E. histolytica* and *Giardia lamblia* have also been implicated in the cytoadherence of *Tritrichomonas mobilensis* to mammalian cells (Demes *et al.*, 1989). We have previously shown that D-galactose stimulates a Mg^{2+}-dependent ecto-ATPase activity of *E. histolytica* (Barros *et al.*, 2000). Accordingly, the ecto-ATPase of *T. vaginalis* was stimulated by more than 90% by 50 mM D-galactose (Fig. 3). On the other hand, 50 mM D-mannose and 50 mM D-glucose did not significantly stimulate this ATPase activity. Recently, we have shown that the invasive amoebae *E. histolytica* presents a much higher ecto-ATP diphosphohydrolase activity than both the non-invasive amoebae *E. histolytica* and the free-living amoebae *E. moshkovskii* (Barros *et al.*, 2000). D-galactose, a sugar moiety known to be an important adhesion molecule between mammalian host cells and protozoan parasites (Ravdin *et al.*,1989), promoted a twofold stimulation of this E-type ATPase in *E. histolytica* (Barros *et al.*, 2000). Adhesion of *T. vaginalis* has also been related to the presence of D-galactose exposed on the surface of its host cells (Bonilha *et al.*, 1995). The ectonucleotide ATP-diphosphohydrolase of *E. histolytica* (Barros *et al.*, 2000) and that here described for *T. vaginalis* share several characteristics, such as the sensitivity to the impermeant inhibitor DIDS (Table I), as well as the similar responses to the pH variation and to the stimulatory effect of D-galactose (Fig. 3). These enzymes may have similar functions in those parasites and they could be considered pathogenesis markers for them. Accordingly, the ecto-ATPase activity of a fresh isolate of *T. vaginalis* was almost threefold higher that that of a strain axenically maintained in culture, through daily passages, for several years (data not shown), which suggests an involvement of this ectonucleotide ATP-diphosphohydrolase in the pathogenicity of *T. vaginalis.*

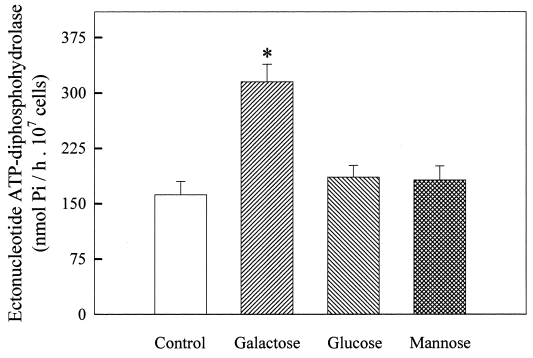

Fig. 3. Effects of carbohydrates on the ecto-ATPase activity of intact living *T. vaginalis* parasites. Cells were incubated for 1 h at 36 °C in the same reaction medium (final volume: 0.5 ml) as that described in the legend of Fig. 1, in the absence (control) or in the presence of 50 mM of the following carbohydrates: D-galactose, D-glucose and D-mannose. Data are means ± SE of three determinations, performed in triplicate, with different cell suspensions. Data analyzed by Student's *t* test.* Denotes statiscally different from control parasites ($P < 0.05$).

The physiological role of ecto-ATPases is still unknown, but it has been suggested a possible involvement of these enzymes in cellular adhesion (Knowles, 1995; Kirley, 1997; Meyer-Fernandes *et al.*, 2000; Peres-Sampaio *et al.*, 2001). Ongoing studies in our group pursue further knowledge on the participation of the ectonucleotide ATP-diphosphohydrolase of *T. vaginalis* in the relationship between these parasites and mammalian epithelial cells.

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Alderete J. T. and Pearlman E. (1984), Patogenic *Trichomonas vaginalis* cytotoxic to cell culture monolayers. Br. J. Vener. Dis. **60**, 99–105.

Arroyo R., Gonzáles-Robles A., Martinez-Palomo A. and Alderete J. F. (1993), Signaling of *Trichomonas vaginalis* for amoeboid transformation and adhesin synthesis follows cytoadherence. Mol. Microbiol. **7**, 299–309.

Asai T., Miura S., Sibley L. D., Okabayashi H. and Takeushi T. (1995), Biochemical and molecular characterization of nucleoside triphosphate hydrolase isozymes from the parasitic protozoan *Toxoplasma gondii*. J. Biol. Chem. **270**, 1191–1197.

Barros F. S., De Menezes L. F., Pinheiro A. A. S., Silva E. F., Lopes A. H. C. S., De Souza W. and Meyer-Fernandes J. R. (2000), Ectonucleotide diphosphohydrolase activities in *Entamoeba histolytica*. Arch. Biochem. Biophys. **375**, 304–314.

Bermudes D., Peck K. R., Afifi M. A., Beckers C. J. M. and Joiner K. A. (1994), Tandemly repeated genes encoded nucleoside triphosphate hydrolase isoforms secreted into parasitophorous vacuele of *Toxoplasma gondii*. J. Biol. Chem. **269**, 2952–2960.

Bernardes C. F., Meyer-Fernandes J. R., Saad-Nehme J., Peres-Sampaio C. E., Vannier-Santos M. A. and Vercesi A. E. (2000), Effects of 4,4′-diisothyocyanatostilbene-2,2′-disulfonic acid on *Trypanosoma cruzi* proliferation and Ca^{2+} homeostasis. Int. J. Biochem. Cell Biol. **32**, 519–527.

Berrêdo-Pinho M., Peres-Sampaio C. E., Chrispim P. P. M., Belmont-Firpo R., Lemos A. P., Martiny A., Vannier-Santos M. A. and Meyer-Fernandes J. R. (2001), A Mg-dependent ecto-ATPase in *Leishmania amazonensis* and its possible role in adenosine acquisition and virulence. Arch. Biochem. Biophys. **391**, 16–24.

Bonilha V. L., Clivaglia M. C., De Souza W. and Silva Filho F. C. (1995), The involvement of terminal carbohydrates of the mammalian cell surface in the cytoadhesion of Trichomonads. Parasitol. Res. **81**, 121–126.

Browman E. J., Siebers A. and Altendorf K. (1988), Bafilomycin: A class of inhibitors of membrane ATPases from microorganisms, animal cells, and plant cells. Proc. Natl. Acad. Sci. USA **85**, 7972–7976.

Caruso-Neves C., Meyer-Fernandes J. R., Saad-Nehme J. and Lopes A. G. (1998a), Osmotic modulation of the ouabain-sensitive $(Na^+ + K^+)$ATPase from malpighian tubules of *Rhodnius prolixus*. Z. Naturforsch. **53c**, 911–917.

Caruso-Neves C., Meyer-Fernandes J. R., Saad-Nehme J., Proverbio F., Marín R. and Lopes A. G. (1998b), Ouabain-insensitive Na^+-ATPase activity of malpighian tubules from *Rhodnius prolixus*. Comp. Biochem. Physiol. B **119**, 807–811.

Catterall R. D. (1974), Trichomonal infection of the genital tract. Med. Clin. North Am. **56**, 1203–1209.

Clifford E. E., Martin K. A., Dalal P., Thomas R. and Dubyak G. R. (1997), Stage-specific expression of P2Y receptors, ecto-apyrase and 5′-nucleotidase in myeloid leukocytes. Am. J. Physiol. **42**, C973-C987.

Demes P., Pindak F. F., Wells D. J. and Gardner W. A. Jr. (1989), Adherence surface properties of *Tritrichomonas mobilensis*, an intestinal parasite of the squirrel monkey. Parasitol. Res. **75**, 589–594.

Diamond L. S. (1957), The establishment of various trichomonads of animals and men in axenic culture. J. Parasitol. **43**, 488–490.

Dubyak G. R. and El-Moatassim C. (1993), Signal transduction via P_2-purinergic receptors for extracellular ATP and other nucleotides. Am. J. Physiol. **265**, C577-C606.

Dutra P. M. L., Rodrigues C. O., Jesus J. B., Lopes A. H. C. S., Souto-Padrón T. and Meyer-Fernandes J. R. (1998), A novel ecto-phosphatase activirty of *Herpetomonas muscarum muscarum* inhibited by platelet-activating factor. Biochem. Biophys. Res. Commun. **253**, 164–169.

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.

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 forms. Mol. Biochem. Parasitol. **92**, 339–348.

Glynn I. M. and Chappel J. B. (1964), A simple method for the preparation of ^{32}P-labelled adenosine triphosphate of high specific activity. Biochem. J. **90**, 147–149.

Guilherme A., Meyer-Fernandes J. R. and Vieyra A. (1991), Reversible inhibition by 4,4′-Diisothiocyanatostilbene-2,2′-disulfonic acid of the plasma membrane $(Ca^{2+}+Mg^{2+})$ATPase from kidney proximal tubules. Biochemistry **30**, 5700–5706.

Jesus J. B., Lopes A. H. C. S. and Meyer-Fernandes J. R. (2002), Characterization of an ecto-ATPase of *Tritrichomonas foetus*. Vet. Parasitol **103**, 29–42.

Kirley T. L. (1997), Complementary DNA cloning and sequencing of the chicken muscle ecto-ATPase. J. Biol. Chem. **272**, 1076–1081.

Knowles A. F. (1995), The rat liver ecto-ATPase/C-CAM cDNA detects induction of carcinoembryonic antigen but not the mercurial-insensitive ecto-ATPase in human hepatoma Li-7A cells treated by epidermal growth factor and cholera toxin. Biochem. Biophys. Res. Commun. **207**, 529–535.

Lemmens R., Vanduffell L., Teuchy H. and Culic O. (1996), Regulation of proliferation of LLC-MK_2 cells by nucleosides and nucleotides: the role of ecto-enzymes. Biochem. J. **316**, 551–557.

Lowry H. O. and Lopez M. (1946), The determination of inorganic phosphate in the presence of labile phosphate esters. J. Biol. Chem. **162**, 421–428.

Martiny A., Vannier-Santos M. A., Borges V. M., Meyer-Fernandes J. R., Asrreuy 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.

Martiny A., Meyer-Fernandes J. R., De Souza W. and Vannier-Santos M. A. (1999), Altered tyrosine phosphorylation of ERK1 MAP kinase and other macrophage molecules caused by *Leishmania* amastigotes. Mol. Biochem. Parasitol. **102**, 1–12.

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.

Meyer-Fernandes J. R., Lans-Mendoza H., Gondim K. C., Willott E. and Wells M. A. (2000), Ectonucleotide diphosphohydrolase activities in hemocytes of larval *Manduca sexta*. Arch. Biochem. Biophys. **382**, 152–159.

Meyer-Fernandes J. R., Silva-Neto M. A. C., Soares M. S., Fernandes E., Vercesi A. E. and Oliveira M. M. (1999), Ecto-phosphatase activities on the cell surface of the amastigotes forms of *Trypanosoma cruzi*. Z. Naturforsch. **54c**, 977–984.

Nakaar V., Beckers C. J. M., Polotsky V. and Joiner K. A. (1998), Basis for substrate specificity of the *Toxoplasma gondii* nucleotide triphosphate hydrolase. Mol. Biochem. Parasitol. **97**, 209–220.

Peres-Sampaio C. E., Palumbo S. T. and Meyer-Fernandes J. R. (2001) An ecto-ATPase activity present in *Leishmania tropica* stimulated by dextran sulfate. Z. Naturforsch. **56c**, 820–825.

Petrin D., Delgaty K., Bhatt R. and Garber G. (1998), Clinical and microbiological aspects of *Trichomonas vaginalis*. Clin. Microbiol. Rev. **11**, 300–317.

Plesner L. (1995), Ecto-ATPases: Identities and functions. Int. Rev. Cytol. **158**, 141–214.

Ravdin J. I., Stanley P., Murphy C. F. and Petri W. A. (1989), Characterization of cell surface carbohydrate receptors for *Entamoeba histolytica* adherence lectin. Infect. Immun. **57**, 2179–2186.

Saad-Nehme J., Bezerra A. L., Fornells L. A. M., Silva J. L. and Meyer-Fernandes J. R. (1997), A contribution of the mitochondrial adenosinetriphosphatase inhibitor protein to the thermal stability of the F_0F_1ATPase complex. Z. Naturforsch. **52c**, 459–465.

Smith T. M. and Kirley T. L., Hennessey T. M. (1997), A soluble ecto-ATPase from *Tetrahymena thermophila*: purification and similarity to the membrane-bound ecto-ATPase of smooth muscle. Arch. Biochem. Biophys. **337**, 351–359.

Sorvillo F. and Kerndt P. (1998), *Trichomonas vaginalis* and amplification of HIV-1 transmission. Lancet **351**, 213–214.

Steinberg T. H. and Di Virgilio F. (1991), Cell-mediated cytotoxicity: ATP as an effector and the role of target cells. Curr. Opin. Immunol. **3**, 71–75.

Van Belle H. (1976), Alkaline phosphatase. I. Kinetics and inhibition by levamisole of purified isoenzymes from humans. Clin. Chem. **22**, 972–976.

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

Wang T.-F. and Guidotti G. (1996), CD39 is an ecto-(Ca^{2+}, Mg^{2+})-apyrase. J. Biol. Chem. **271**, 9898–9901.

Wang T.-F., Ou Y. and Guidotti G. (1998), The transmembrane domains of ectoapyrase CD39 affect its enzymatic activity and quaternary structure. J. Biol. Chem. **271**, 24814–24821.

Zhang Z. F. and Begg C. B. (1994), Is *Trichomonas vaginalis* a cause of cervical neoplasia? Results from a combined analysis of 24 studies. Int J Epidemiol **23**, 682–690.

Zimmermann H. (1999), Two novel families of ectonucleotidases: molecular structures, catalytic properties and a search for function. Tr. Pharmacol. Sci. **20**, 231–236.