Lysosomal phospholipase A₁ in *Trypanosoma cruzi*: an enzyme with a possible role in the pathogenesis of Chagas' disease

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We found that, as in African trypanosomes, endogenous phospholipase A₁ (Plase A₁) activity can catalyse extensive deacylation of phospholipids upon cell death in all life stages of *Trypanosoma cruzi*. A major lysosomal Plase A₁ was purified and characterized. The enzyme products can explain the lesions surrounding

degenerating *T. cruzi* cells in host tissues. Thus Plase A₁ emerges as a target to block pathogenesis in trypanosomal infections.

Key words: epimastigotes, phospholipids, phosphatidylcholine, trypanosomiasis.

INTRODUCTION

Phosphatidylcholine (PC)-degrading activities have been the subject of a number of studies in African trypanosomes [1–8]. In four different species, calcium-independent phospholipase A₁ (Plase A₁) was the predominant PC-degrading activity detected. The levels of Plase A₁ varied widely, with very high activity in the pathogenic *Trypanosoma brucei* and relatively low activity in the non-pathogenic *T. lewisi* species. Plase A₁ in *T. brucei* was also investigated by Oppendoerfer and van Roy [3]. These authors found high levels of activity in bloodstream forms, but not in procyclic culture forms, and suggested an important physiological role for the enzyme in the first. This aspect was clarified by Bowes et al. [8], who showed that *T. brucei* Plase A₁ appears to bear lysophospholipase 1 activity [9] and, as such, it participates in the supply of fatty-acyl and polar-head moieties for the synthesis of cellular phospholipids. In a recent study [10], incubation of live *T. brucei* with 1-palmitoyl-2-[¹⁴C]arachidonoyl-PC yielded free arachidonate, suggesting the involvement of Plase A₂ in this process. Arachidonic acid, as well as other unsaturated fatty acids, was shown to enhance calcium influx in this cell line [10]. Thus phospholipid deacylation appears to be a signalling event in *T. brucei*.

The presence of PC-degrading phospholipases has not been examined previously in the South American counterpart, *T. cruzi*, a parasite that causes Chagas' disease. In addition to their potential role in cell signalling, these enzymes are of interest in *T. cruzi* for a number of reasons. First, marked inflammatory responses are well known to appear surrounding degenerating, but not healthy, amastigote nests in various tissues of Chagas' disease patients. This strongly suggests that autolytic processes initiate factors which cause inflammation [11]. The identity of such factors is unknown but there are indications that they may be phospholipid-breakdown products, as it has been reported that free fatty acids and lysophospholipids released from killed *T. cruzi* trypomastigotes have toxic effects on cultured cells [12]. This is in agreement with the pathogenic mechanism proposed for African trypanosomiasis by Tizard et al. [13]. These authors also observed that dying trypanosomes accumulate non-esterified

fatty acids and that these may be directly implicated in pathogenesis [5,6,13]. Secondly, since *T. cruzi* is an intracellular parasite, membrane-disrupting mechanisms are necessary for invasion of, as well as escape from, host cells. Phospholipases may participate in both of these processes. Thirdly, we have found previously that when *T. cruzi* epimastigotes grown at 28 °C are transferred to 37 °C, a fatty acid exchange between phospholipids and neutral lipids appears to take place, since the ratios of saturated to unsaturated fatty acids in these two lipid pools are transposed [14]. This novel mechanism of membrane-lipid adaptation suggests the action of phospholipase type A activity as part of a deacylation–reacylation cycle. In view of their possible roles in various aspects of *T. cruzi* biology and pathogenic effects, the present study was undertaken to investigate autolytic degradation of phospholipids and to characterize the enzymic activities catalysing these reactions.

EXPERIMENTAL

Materials

Modified Grace medium, 199 medium and fetal-calf serum were purchased from Gibco BRL (Gaithersburg, MD, U.S.A.). Soybean PC (grade II-S), quinine, chloroquine, chlorpromazine, dibucaine, procaine, PMSF, leupeptin, aprotinin, benzamidine, tosyl-L-lysylchloromethane (Tos-Lys-CH₂Cl, 'TLCK'), Trizma, *p*-nitrophenyl α -mannopyranoside, *p*-nitrophenol, Triton X-100, digitonin, Kodak X O-AR film and concanavalin A (ConA)–Sephacrose 4B were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Amiodarone was from Roemmers Laboratories (Buenos Aires, Argentina). Silica Gel 60 TLC plates and solvents were from Merck (Darmstadt, Germany). DEAE–Trisacryl and CM–Sephacrose were purchased from Pharmacia (Uppsala, Sweden). Silver Stain kit was from Bio-Rad (Hercules, CA, U.S.A.). 1-Palmitoyl-2-[¹⁴C]oleoyl PC (54 μ Ci/ μ mol), 1,2-di[¹⁴C]oleoyl PC (110 μ Ci/ μ mol), [¹⁴C]sodium acetate (54 μ Ci/ μ mol) and [1-¹⁴C]oleate (58 μ Ci/ μ mol) were obtained from Amersham (Arlington Heights, IL, U.S.A.).

Abbreviations used: LPC, lysophosphatidylcholine; PC, phosphatidylcholine; Plase A₁, phospholipase A₁; ConA, concanavalin A.

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Cells and cultures

T. cruzi epimastigotes, RA lethal strain [15], were axenically grown at 28 °C in a biphasic medium for 48 h, as previously described [16], and harvested by centrifugation at 1000 *g* for 15 min. Amastigotes and trypomastigotes were purified from Vero cultures following the protocol of Schmatz and Murray [17]. For homogenization, cells were washed and resuspended in 10 mM Tris/HCl, pH 7.4, in the presence of protease inhibitors [0.01 % (w/v) leupeptin, 2 mM PMSF, 25 units/ml aprotinin, 1 mM benzamide, 0.5 mM Tos-Lys-CH₂Cl] and subjected to three cycles of freezing and thawing. Homogenates were stored at -20 °C, and several frozen batches were pooled for purification, although partial loss of activity occurs under these storage conditions.

Autolytic lipid degradation in epimastigotes, trypomastigotes and amastigotes

To study the degradation of endogenous phospholipids, 10 μ Ci of [¹⁴C]sodium acetate (54 μ Ci/ μ mol) were added to an epimastigote culture in biphasic medium. After 48 h at 28 °C, the epimastigotes (3.6×10^9 cells) were harvested and resuspended in 25 mM sodium succinate (2 ml), pH 6.0, with added protease inhibitors. The parasites were killed by freezing and thawing, and aliquots of this suspension, corresponding to 2×10^8 cells each, were incubated at 37 °C for up to 24 h in the presence or absence of 1 mM CaCl₂ or 1 mM EDTA. Lipids were then extracted according to the method of Bligh and Dyer [18] and separated by TLC using a double solvent mixture system [19,20]. Radioactivity in the various lipid spots was detected by autoradiography. Lipids were identified by comparison with authentic standards run on the same plates. Portions of the plates containing the labelled non-esterified fatty acid spots were excised, and the radioactivity was quantified by liquid scintillation spectroscopy. In the case of trypomastigotes and amastigotes, 10 μ Ci of [1-¹⁴C]oleate was used as lipid precursor. The purified parasites were resuspended in PBS containing protein inhibitors and sonicated to complete disruption. Aliquots corresponding to 1.2×10^7 trypomastigotes or 7×10^6 amastigotes were then incubated at 37 °C for 0 or 12 h, in the presence of 0.1 % (w/v) sodium azide, and incubations were terminated by lipid extraction. The resulting lipid extracts were examined by TLC and autoradiography as described above.

Plase A₁ assays

Phospholipase activities were investigated analysing the products of the hydrolysis of radiolabelled PC, using methods similar to those previously employed in the characterization of the *Tetrahymena thermophila* phospholipase system [21]. The incubation mixtures consisted of 140 μ l of enzyme sample or PBS (control), 40 μ l of substrate mixture (0.01 μ Ci/assay of 1-palmitoyl-2-[1-¹⁴C]oleoyl PC and 0.6 mg/assay of soy-bean PC) and 20 μ l of buffer (1 M sodium acetate, pH 4.7), unless otherwise indicated. Water-saturated diethyl ether (200 μ l) was added to inhibit lysophospholipase activity [21]. Incubations were carried out for 60 min at 37 °C, the lipids were extracted and separated by TLC. Radioactivity was detected by autoradiography and estimated in excised portions of the plate, as described above. The amount of radioactivity present in the lysophosphatidylcholine (LPC) spot, together with the specific radioactivity of PC used in the assays, was used to calculate the amount of LPC produced, which is a direct measurement of Plase A₁ activity. In the indicated experiments, 1,2-di-[1-¹⁴C]oleoyl PC was used as the radiolabelled

substrate. Within 10 % of substrate hydrolysis, the assay was linear with respect to time and enzyme concentration.

Subcellular localization of Plase A₁

Washed cells were permeabilized by digitonin treatment, as described by Cazzulo et al. [22]. To determine the subcellular location of Plase A₁, the activities of the following marker enzymes were assayed, in addition to Plase A₁, in aliquots of the supernatants: citrate synthase (mitochondrial) [23], hexokinase [23] and phosphoenolpyruvate carboxykinase (glycosomal) [24], L-aspartate-activated malic enzyme (cytosolic) [23], cruzipain [23] and α -mannosidase (lysosomal) [25]. Since it was observed that digitonin stimulates Plase A₁, the results of the assays were corrected accordingly. To this end, a curve was prepared, which plotted the activity of Plase A₁ from crude extracts in the presence of different concentrations of digitonin.

Purification of Plase A₁

Homogenates of *T. cruzi* epimastigotes (1–6 g wet weight) were prepared by freezing and thawing and were centrifuged for 60 min at 105000 *g* at 4 °C. The supernatants were subjected to DEAE-Trisacryl ion-exchange chromatography in a 6 cm $\times$ 2.4 cm column equilibrated with 10 mM Tris/HCl, pH 7.4, and the products were eluted with a linear NaCl gradient (0–0.3 M). ConA–Sephacryl 4B chromatography was performed on a column with a 3.5 ml bed volume, equilibrated with 1 mM CaCl₂/1 mM MnCl₂/0.5 M NaCl/20 mM Tris/HCl (pH 7.4). Separation was effected with 10 mM α -methyl mannopyranoside. A final CM–Sephacryl ion-exchange step was carried out in a 30 ml column equilibrated with 50 mM sodium acetate (pH 4), and separation was effected with a sodium chloride gradient (0–0.3 M). After each step, samples containing Plase A₁ activity were pooled and dialysed against the buffer used for the following chromatographic procedure.

Electrophoretic analysis of the various pools

Aliquots of the preparations obtained at each stage of the purification procedure were analysed by SDS/PAGE [26]. Protein bands were visualized by Coomassie Blue or silver staining.

Molecular-mass estimation

An aliquot of pure Plase A₁ was applied on to a Sephadex G-75 column equilibrated with 0.15 M NaCl/10 mM Tris/HCl (pH 7.4). The column was calibrated with the following molecular-mass standards: BSA (67 kDa), ovalbumin (45 kDa) and carbonic anhydrase (29 kDa). Elution was carried out with the same buffer at a flow rate of 3 ml/h, 2 ml fractions were collected and Plase A₁ activity was assayed in each of them and the molecular mass of the enzyme was estimated.

Protein determinations

Protein concentrations were determined by the method of Lowry et al. [27].

RESULTS AND DISCUSSION

Rapid and extensive breakdown of endogenous phospholipids was found in autolysing parasites from all life stages of *T. cruzi*. Epimastigote, trypomastigote and amastigote suspensions were radiolabelled with lipid precursors, lysed and incubated at 37 °C. Incubations were carried out for up to 24 h, in the case of epimastigotes, and for 12 h in the case of trypomastigotes and amastigotes. Analysis of the radiolabelled lipids showed, in all

Figure 1 Detection of phospholipase A activity in *T. cruzi*

(A) Degradation of endogenous phospholipids. Suspensions of *T. cruzi* epimastigotes, trypomastigotes or amastigotes radiolabelled with lipid precursors were lysed and incubated at 37 °C. In the case of epimastigotes, incubations were extended for up to 24 h and in the case of trypomastigotes and amastigotes, for 12 h. No diethyl ether was added in these incubations. Lipids were extracted and chromatographed on TLC plates. Radioactivity in the lipid spots was detected by autoradiography. Lanes 1–4, epimastigotes metabolically labelled with [¹⁴C]sodium acetate after 0 h (lane 1) and 24 h with no additions (lane 2), or in the presence of 1 mM CaCl₂ (lane 3) or 1 mM Na₂EDTA (lane 4). Lanes 5 and 6: trypomastigotes pre-labelled with [¹⁴C]oleate after 0 h (lane 5) or 12 h (lane 6). Lanes 7 and 8: amastigotes pre-labelled with [¹⁴C]oleate after 0 h (lane 7) and 12 h (lane 8). O, origin; PI, phosphatidylinositol; LPE, lysophosphatidylethanolamine; PE, phosphatidylethanolamine; PA, phosphatidic acid; MG, monoacylglycerol; St, sterols; DG, diacylglycerol; FFA, non-esterified fatty acids; TG, triacylglycerol; SE, steryl esters. It should be noted that, as these incubations were carried out in the absence of diethyl ether, no inhibition of lysophospholipase activities was expected. (B) Accumulation of non-esterified fatty acids in autolysing epimastigotes as a function of time. Incubation of epimastigotes with [¹⁴C]sodium acetate was carried out as in (A), without the addition of CaCl₂ or Na₂EDTA, for the indicated time periods. Lipids were extracted and separated by TLC and the radioactivity present in the free fatty acid spots was quantified by liquid scintillation spectroscopy. (C) Phospholipase activities in *T. cruzi* epimastigotes. Aliquots of an epimastigote homogenate (corresponding to 2×10^8 cells each) were incubated for 1 h with 1-palmitoyl-2-[1-¹⁴C]oleoyl PC, at pH 4.7 or pH 7.4, in the presence of diethyl ether, after which lipids were extracted as described in the Experimental section. The Figure shows an autoradiogram of the products separated by TLC. Lane 1, substrate blank at pH 4.7; lane 2, epimastigote homogenate at pH 4.7; lane 3, substrate blank at pH 7.4; lane 4, epimastigote homogenate at pH 7.4.

cases, an extensive decrease in PC, together with an increase in non-esterified fatty acids (FFA) (Figure 1A), suggesting the activity of a phospholipase A. The accumulation of non-esterified fatty acids was markedly greater than the enhancement in LPC, indicating lysophospholipase A activity. It is important to point out that no diethyl ether was used in these experiments in order to mimic natural conditions. Therefore no inhibition of lysophospholipase A activity was expected in these samples. Maximal accumulation of non-esterified fatty acids in autolysing epi-

mastigotes was reached after 15 h of incubation (Figure 1B). The production of non-esterified fatty acids from endogenous phospholipids in epimastigotes was not markedly affected by the addition of 1 mM CaCl₂ or 1 mM Na₂EDTA (Figure 1A, lanes 3 and 4).

We further examined the presence of PC phospholipase activities in *T. cruzi* epimastigote homogenates using 1-palmitoyl-2-[1-¹⁴C]oleoyl PC as substrate, either at pH 4.7 or pH 7.4 (Figure 1C). The only radiolabelled product detected was LPC. At pH 4.7 the activity was markedly higher than at pH 7.4. These incubations were carried out in the presence of diethyl ether, which inhibits lysophospholipase A activity, therefore allowing the accumulation of LPC. No increases in non-esterified fatty acids, diacylglycerol or phosphatidic acid were found at either pH under these conditions. Similar results were obtained when 2 mM CaCl₂ or 2 mM Na₂EDTA were added to the incubation mixtures (results not shown). These results demonstrate the presence of Plase A₁ in these samples. They also indicate that PC was not degraded by Plase A₂, C or D under these experimental conditions. Thus *T. cruzi* closely resembles African trypanosomes, in which Plase A₁ was also found to be the predominant PC-degrading enzyme and no phospholipases C or D acting on PC were detectable [2].

T. cruzi Plase A₁ was purified from epimastigote homogenate supernatants by sequential DEAE–Trisacryl (Figure 2A), ConA–Sephacrose 4B (Figure 2B) and CM–Sephacrose chromatography (Figure 2C). The results of this procedure are summarized in Table 1. A 1900-fold increase in enzyme specific activity and a recovery of 7.8 % were achieved. Figures 2(D) and 2(E) show an electrophoretic analysis of the various fractions. The final preparation showed a single band with a molecular mass of approx. 38 kDa. Chromatography on Sephadex G-75 of the purified Plase A₁ showed that the activity eluted as a single symmetrical peak with an apparent molecular mass of 40 kDa (results not shown). Thus the enzyme appears to behave as a monomer in solution.

A number of studies were carried out to characterize Plase A₁ purified from *T. cruzi*. Preliminary observations indicated that the enzyme had an acidic pH optimum. To further analyse the variations of activity with pH, we incubated aliquots of purified enzyme with radiolabelled PC as described in the Experimental section, except that a universal buffer (0.1 M Tris/HCl/0.1 M sodium citrate at pK_as of 3.1, 4.8, 6.4 and 8.1), adjusted to various pH values, was used. The results showed that *T. cruzi* Plase A₁ had a pH optimum between 4 and 5 (Figure 3). The activity was stimulated by the addition of Triton X-100; final concentrations of 0.1, 0.3, 1 or 10 % of this detergent yielded increases in activity (means $\pm$ S.D.) of 2.1 ± 0.8 , 9.2 ± 2.3 , 12.4 ± 3.5 and 11.8 ± 2.9 -fold respectively. Additional studies with the purified preparation, performed at pH 4.7, showed that addition of 2 mM EDTA, 2 mM CaCl₂ or 2 mM MgCl₂ had no effect on Plase A₁ activity and activities measured under these conditions were 97 ± 3 , 103 ± 7 and 96 ± 7 % respectively, compared with control samples that received no additions. These results are in agreement with those obtained using whole homogenates and show that Plase A₁ activity is independent of divalent cations.

The effect of a number of compounds with potential inhibitory activity on *T. cruzi* Plase A₁ were investigated. These included the antimalarial drugs quinine and chloroquine, which are inhibitors of rat liver lysosomal phospholipases [28], amiodarone, a rat macrophage lysosomal Plase A₁ inhibitor [29], chlorpromazine, an inhibitor of lysosomal lipases [30], and the local anaesthetics dibucaine [31], procaine [32] and xylocaine [33]. All inhibitors were tested in concentrations ranging from 0.01 mM to 1 mM. Only chlorpromazine had an inhibitory effect, with an

Figure 2 Purification and electrophoretic analysis of *T. cruzi* Plase A₁.

(A) LPC release after chromatography on DEAE-Trisacryl of an aliquot of the soluble fraction of *T. cruzi* epimastigote homogenate at pH 7.4. Elution of Plase A₁ was effected with a NaCl gradient (0–0.3 M). (B) LPC release after concanavalin A–Sepharose chromatography. Elution was achieved using 10 mM α -methylmannopyranoside (α -mM). (C) LPC release after carboxymethyl-Sepharose chromatography at pH 4.0. Elution was carried out with a NaCl gradient (0–0.3 M). Absorbance at 280 nm and Plase A₁ activity (nmol of LPC released/min per ml) were determined in each fraction). (D) Coomassie-Blue stained gel, after SDS/PAGE, showing the products of the various purification steps. Lane 1, homogenate; lane 2, homogenate supernatant; lane 3, DEAE-Trisacryl eluate; lane 4, ConA-Sepharose 4B eluate. (E) Silver-stained gel, after SDS/PAGE. Lane 5, ConA-Sepharose 4B eluate; lane 6, CM-Sepharose eluate. The ConA-Sepharose preparation produced a protein smear below the main band, which was removed by CM–Sepharose chromatography.

I_{50} of 1 mM. The remainder of the compounds tested were without effect. As in the case of rat liver lysosomal phospholipases, *T. cruzi* Plase A₁ was not inhibited by 1 mM HgCl₂. As this salt is an effective inhibitor of thiol-dependent proteases, it

may thus be used to preserve Plase A₁ activity in crude preparations.

The data obtained emphasize similarities with rat liver lysosomal Plase A₁ [34]. Similarly to the latter, *T. cruzi* Plase A₁ is a

Table 1 Purification of *T. cruzi* Plase A₁

Purification step	Total protein (mg)	Total activity (nmol LPC · min)	Specific activity (nmol LPC · min · mg of protein)	Purification (-fold)	Yield (%)
Homogenate	160	29.6	0.18	1	100
Supernatant (105 000 g)	34	18.6	0.54	3	63
DEAE-Trisacryl eluate	0.405	8.4	20.74	115	28
ConA-Sepharose eluate	1.6×10^{-2}	3.1	187.50	1042	10
CM-Sepharose eluate	6.5×10^{-3}	2.3	353.84	1966	8

Figure 3 Characterization of *T. cruzi* Plase A₁

(A) pH optimum. Plase A₁, purified by DEAE-Trisacryl and Con-A-Sepharose, was assayed as described in the Experimental section (1 μ g of protein per assay), using a universal buffer composed of 0.1 M Tris/HCl, 0.1 M sodium citrate, 1% (v/v) Triton X-100 (final concentrations), adjusted to different pH values with either HCl or NaOH. Activity is expressed as a percentage of the maximal activity, which was obtained at pH 4.0 (0.16 nmol LPC/min per mg of protein). (B) Subcellular localization. Selective release of Plase A₁ ($\blacktriangle$) and cytoplasmic (Δ , malic enzyme, ME), mitochondrial (*, citrate synthase, CS), lysosomal ($\bullet$, α -mannosidase, α -MAN; $\square$, cruzipain, CRUZIP) and glycosomal ($\blacksquare$, hexokinase, HK; $\circ$, phosphoenolpyruvate carboxykinase, PEPCK) marker enzymes upon exposure of washed epimastigotes to various concentrations of digitonin is shown.

glycoprotein, as indicated by its binding to ConA-Sepharose, has acidic pH optimum, is independent of divalent cations and shows no sensitivity to Hg²⁺, suggesting that thiol groups are not involved in catalysis. To determine if *T. cruzi* Plase A₁ is also of lysosomal origin, we studied its subcellular localization. To this end, we analysed the selective release of this, and other enzymes, from various cellular compartments on exposure to digitonin. As shown in Figure 3(B), Plase A₁ clustered with the lysosomal markers cruzipain and α -mannosidase. These results indicate a lysosomal origin for *T. cruzi* Plase A₁.

In another set of experiments, we investigated the hydrolysis of 1-palmitoyl-2-[1-¹⁴C]oleoyl PC, at pH 4.7, by phospholipases in other life stages of *T. cruzi*, namely, amastigotes, culture trypomastigotes and metacyclic trypomastigotes. In these experiments, which were carried out in the presence of diethyl ether, Plase A₁ was the only PC-degrading activity detected in all life stages. The specific activities of this enzyme were 3.16 ± 0.02 , 30.8 ± 0.11 , 41.8 ± 0.10 and 64.1 ± 0.41 (nmol of LPC · min⁻¹ · mg⁻¹ of protein; $n = 3$) for epimastigotes, metacyclic trypomastigotes, amastigotes and cultured trypomastigotes respectively. The values for metacyclic trypomastigotes, amastigotes and cultured trypomastigotes were significantly higher ($P < 0.001$) than the value for epimastigotes. Epimastigotes are extracellular forms, whereas amastigotes and trypomastigotes are either intracellular or cell-invading forms. The higher Plase A₁ activity in the latter suggests that this activity may be involved in cell invasion or other processes, such as the phospholipid remodelling events that accompany passage from the poikilothermic insect to the homoiothermic mammal [14]. Among African trypanosomes, *T. brucei* has been shown to possess extremely high activities of Plase A₁ [2], much higher than those observed in *T. cruzi* in the present study or those found in other trypanosomal species. Opperdoes and van Roy [3] further analysed this point and concluded that, in *T. brucei*, the enzyme occurs in lysosomal and cytoplasmic compartments. The lysosomal form closely resembles the enzyme characterized in the present work. The cytoplasmic form, on the other hand, which accounts for most of the activity measured in bloodstream forms, does not appear to have a counterpart in *T. cruzi* epimastigotes.

The involvement of Plase A₁ activity in the breakdown of endogenous phospholipids in *T. cruzi* is supported by other studies. Thus the LPC generated in autolysing epimastigotes was shown to be predominantly of the 2-acyl type by a novel method involving chemical reacylation with a radiolabelled fatty acid, followed by bee venom Plase A₂ analysis of the resulting PC [35].

In other unicellular eukaryotes, diverse phospholipase activities are present in considerable amounts [36]. We found previously that *Te. thermophila* secretes phospholipase C and A₁ with acidic pH optima [21,37–39]. *Tetrahymena* Plase A₁ is similar to the enzyme found in *T. cruzi*, despite the phylogenetic distance between these two organisms. This suggests that the basic features of lysosomal Plase A₁ might have been defined early in the evolution of eukaryotes. Alternatively, these traits

may represent an optimal pattern at which different organisms arrived by convergence. We have shown previously that *Tetrahymena* phospholipases have important biological roles, and that their secretion appears to be closely interlinked with the particular membrane composition of these organisms [40]. This indicates the various functions that phospholipases may play in lower eukaryotes.

Release of fatty acids in autolysing parasites remains a plausible pathogenic mechanism in African trypanosomes. We show that, upon cell death, *T. cruzi* also rapidly accumulates fatty acids, which are likely to provoke disrupting effects in host cells. The production of these compounds can be accounted for by the lysosomal Plase A₁, the major PC phospholipase detected in all *T. cruzi* life stages. Thus Plase A₁-catalysed phospholipid deacylation could be a common mechanism underlying the pathogenic effects in both African and South American trypanosomiasis. This emphasizes the interest of this enzyme as a potential target to fight these diseases.

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