Review Article

Phospholipases A in Trypanosomatids

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Received 23 December 2010; Accepted 7 February 2011

Academic Editor: Claudio Alejandro Pereira

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Phospholipases are a complex and important group of enzymes widespread in nature, that play crucial roles in diverse biochemical processes and are classified as A1, A2, C, and D. Phospholipases A1 and A2 activities have been linked to pathogenesis in various microorganisms, and particularly in pathogenic protozoa they have been implicated in cell invasion. Kinetoplastids are a group of flagellated protozoa, including extra- and intracellular parasites that cause severe disease in humans and animals. In the present paper, we will mainly focus on the three most important kinetoplastid human pathogens, Trypanosoma brucei, Trypanosoma cruzi, and Leishmania spp., giving a perspective of the research done up to now regarding biochemical, biological, and molecular characteristics of Phospholipases A1 and A2 and their contribution to pathogenesis.

1. Introduction

Kinetoplastids are a group of flagellated protozoans distinguished by the presence, in their single large mitochondrion, of a DNA-containing region known as kinetoplast. These unicellular organisms have a similar genomic organization and cellular structures and undergo morphological changes during their life cycles, being transmitted by different insect vectors. The members of this group include extra- and intracellular parasites that cause severe diseases in humans and animals, as well as various free-living forms. Herein, we will mainly focus on the three most important human pathogens Trypanosoma brucei, Trypanosoma cruzi, and Leishmania spp.

Trypanosoma brucei and its subspecies are extracellular parasites transmitted by tsetse flies and responsible of human African trypanosomiasis (HAT), also known as African sleeping sickness, and Nagana in cattle. The life cycle of African trypanosomes is complex and represented by extracellular stages found in blood, lymph, and spinal fluid in the mammal [1]. The disease threatens over 70 million people and uncounted numbers of cattle in 36 countries of sub-Saharan Africa, having a devastating impact in human health and economy in affected areas [2]. HAT symptoms occur in two stages; in the first, haemolympathic phase, parasite invasion of the circulatory and lymphatic systems is associated with severe swelling of lymph nodes. If untreated, the disease overcomes the host’s defences and can cause more extensive damage. The second stage, neurological phase, begins when the parasite invades the central nervous system by passing through the blood-brain barrier; without treatment, it is fatal and the damage caused can be irreversible [1].

Trypanosoma cruzi in contrast, is an intracellular parasite that invades all types of nucleated cells in the mammalian host. This protozoa, transmitted through the faeces of bloodsucking insects of the Triatominae family, enters the mammalian host via damage to the skin and causes Chagas disease in humans, a chronic inflammatory condition characterized by cardiomyopathy, megacolon, and mega-esophagus [3]. The disease is prevalent throughout America and according to WHO estimations, 25 million people are at risk and 10 million are infected worldwide [4]. Chemotherapy against Chagas disease is limited and unsatisfactory. The two available drugs, nifurtimox and beznidazole, are capable of curing at least 50% of recent infections and both produced side effects [5].

The genus Leishmania comprises 20 species of intracellular protozoan that are transmitted by phlebotomine sandflies and infect specifically cells of the mononuclear phagocyte system in mammals. These parasites cause various diseases
ranging from self-healing cutaneous leishmaniasis, mucocutaneous leishmaniasis, with partial or total destruction of the mucous membranes, to severe and lethal (if untreated) visceral leishmaniasis, also known as kala-azar. Leishmaniasis is widespread in Southern Europe, Africa, Asia, and America, threatening 350 million people in 88 countries around the world and represents an important global health problem that results in a significant economic burden [6].

One of the major components of biomembranes present in all living organisms are phospholipids (PL), which form the lipid bilayer and serve as hydrophobic anchors of membrane proteins. These compounds can be enzymatically modified by the action of phospholipases (PLAs), with generation of bioactive lipid molecules that can act as second messengers by the action of phospholipases (PLAs), with generation of proteins. These compounds can be enzymatically modified and also modulate the immune response [7–9]. Moreover, bioactive lipid molecules that can act as second messengers by the action of phospholipases (PLAs), with generation of proteins. These compounds can be enzymatically modified and also modulate the immune response [7–9].

PLAs are a complex and important group of enzymes, widespread in nature, that play crucial roles in diverse biochemical processes and are classified as A1, A2, C, and D, depending on the site of hydrolysis [15]. These enzymes cleave cell membrane and intracellular PL, releasing a variety of products such as lysophospholipids (LPL), free fatty acids (FFA), diacylglycerols (DG), choline phosphate, phospho-inositides and phosphatidic acid, among others (Figure 1).

Phospholipase A1 (PLA1) (EC 3.1.1.32) specifically hydrolyses acyl groups from PL at the sn-1 position, producing FFA and LPL (Figure 1) [15]. PLA1 activities have been detected in various cell types and tissues from a wide range of organisms by measuring hydrolysis of phosphatidylcholine (PC) to lysophosphatidylcholine (LPC) [15]. But despite their apparent ubiquity and diversity, up to now a limited number of PLA1s have been cloned and characterized [16–18]. Increasing evidence indicates that some PLA1s are capable of generating bioactive lipids, a role traditionally assigned to PLAs A2, C, and D, though the biological relevance of this particular PLA deserves to be deeply studied [16, 19, 20].

At present time, nine PLA1 molecules are known in mammals, being six extracellular and three intracellular enzymes, sharing no sequence homology between them and probably having distinct functions [7]. The extracellular PLA1s belong to the pancreatic lipase gene family, that is conserved in an extensive range of organisms from insects to mammals and have been biochemically characterized and classified, according to their substrate specificities, structures, expression patterns, and possible functions [7]. Some PLA1s have a broad substrate specificity and might also have triacylglycerol lipase activity (EC 3.1.1.3) [21]. Others, such as phosphatidylserine-specific PLA1s from rat platelets and membrane-associated phosphatidic acid PLA1α and PLA1β, show strict substrate specificity [20]. The latter PLA1s have specific roles in producing bioactive LPL, lysophosphatidylserine, and lysophosphatidic acid [7].

Phospholipases A2 (EC 3.1.1.4), in contrast to PLA1, are the most widely studied. Great advances in understanding the structure and function of the superfamily of Phospholipase A2 (PLA2) has occurred in the last decades [9, 22–25]. This superfamily includes fifteen groups, comprising four main types including secreted PLA2 (sPLA2), cytosolic PLA2 (cPLA2), Ca2+-independent PLA2 (iPLA2), and platelet activating factor acetyl hydrolyase/oxygenated lipoprotein associated PLA2 (LpPLA2) [9]. The classification is based upon the following characteristics: source, secreted, or cytosolic, availability of structural information, molecular weight, cofactors, and inhibitor specificity [9]. sPLA2s have a low molecular weight (~14 kDa) and contain a large number of disulphide bridges, consistent with their extracellular environment, and require millimolar concentrations of Ca2+ for optimum catalytic activity [9]. cPLA2s have a high molecular weight (~85 kDa) and preferentially hydrolyze PL containing arachidonic acid, therefore playing a key role in the biosynthesis of eicosanoids, precursors of prostaglandins and thromboxane [26]. Full activation of these enzymes requires Ca2+ binding to an N-terminal C2 domain and phosphorylation on serine residues [23]. iPLA2s have a high molecular weight (85–88 KDa), contain seven to eight ankyrin repeats, one of the most common sequence motif, and the consensus lipase motif GXSXG, being detected mainly in human tissues [27].

Considering the important and various roles that PLAs possess, in the present review, we will summarize the research done to date in Trypanosomes regarding biochemical, biological, and molecular characteristics of PLAs and their contribution to pathogenesis.

2. Biochemical and Biological Characteristics of the Trypanosomatids Phospholipases A

2.1. Phospholipase A1. In 1978, Tizard et al. described the presence of a haemolytic activity in T. congolense, due to the FFA generated by the action of PLA on endogenous PC, meanwhile in the nonpathogenic T. lewisi, no FFA generation
was observed and therefore, nonhaemolytic activity was detected [28]. Further, it was determined in four different species of African trypanosomes that Ca²⁺-independent PLA₁ was the predominant PC-degrading activity. The levels of PLA₁ varied widely, with very high activity in the pathogenic *T. brucei* and relatively low activity in the nonpathogenic *T. lewisi* species [29]. Other authors found that *T. brucei* bloodstream forms possess high levels of PLA₁ activity, whereas in the procyclic culture forms PLA₁ specific activity was only 15% of that of bloodstream forms, suggesting an important physiological role for the enzyme in the mammalian stage [30]. Bloodstream trypanosomes are covered with a dense layer of Variant Surface Glycoprotein (VSG), which protects the parasite from lysis by host complement via the alternative pathway [31]. It has been suggested that the high activity of PLA₁ in these forms, may play a role in the acquisition of fatty acids for synthesis of the VSG and also provide a source of myristate that can be employed for remodelling the lipid anchor of the VSG [32]. PLA₁ was purified from *T. brucei* bloodstream forms, where the major portion was found as a soluble activity in the cytosol and the minor as a particle-bound activity associated with lysosomal markers. Both enzymes had optimal activity at acid pH and were activated by Triton X-100 [30]. Although cultured procyclic trypanomastigotes also possess PLA₁ activity, the levels were significantly reduced compared to bloodstream forms, due to a decrease in soluble PLA₁ levels of lysosomal activity were present in both stages [30]. Other authors reported that PLA₁ activity eluted together with a lysophospholipase activity (LPLA)
promastigotes a PLA 1 activity hydrolyzing PC. Moreover, we
our laboratory have also demonstrated in L. braziliensis
independent of the bivalent cations Ca\(^{2+}\), Mn\(^{2+}\) and Mg\(^{2+}\),
part of a deacylation-reacylation cycle in [35]. This mechanism of
membrane lipid adaptation suggested for the first time the action of PLA activity as
lipids [35]. This mechanism of membrane lipid adaptation
was similar to those reported for TbPLA 1 and other PLA 1s
in mammalians [43, 44]. As previously demonstrated in
T. brucei, where the mammalian stages possess the highest levels of PLA 1 activity
[30, 37]. Interestingly, in both infective stages membrane-bound PLA 1 activity was remarkably higher than those
detected in organelle bound or soluble fractions [38]. This localization does not appear to have a similar counterpart in T. brucei, where the major proportion of activity (more than 90% of the total) corresponds to a soluble cytosolic fraction [30]. In T. cruzi epimastigotes, in contrast, the enzyme was only detected in lysosomes [37]. It is remarkable that only infective stages secreted PLA 1 to the extracellular media [38], similarly to other enzymes that participate in T. cruzi endocytic pathway, such as cruzipain and trans-sialidase [39–42]. We purified T. cruzi PLA 1 (TcPLA 1) from epimastigote and amastigotes, obtaining in both cases a unique band of \(\sim 38\) kDa. These enzymes proved to be independent of the bivalent cations Ca\(^{2+}\), Mn\(^{2+}\) and Mg\(^{2+}\), had an optimum acidic pH, and were activated by Triton X-100. The biochemical characteristics of TcPLA 1 activities were similar to those reported for TbPLA 1 and other PLA 1s from mammalians [43, 44]. As previously demonstrated in T. brucei we also determined the presence of LPLA activity in autolysing parasites [17, 33, 45].

As concerns secreted TcPLA 1, we determined during metacyclogenesis, process in which epimastigotes differentiate into the infective metacyclic trypomastigotes, an increase in secreted enzyme activity simultaneously with the appearance of metacytic forms, as expected [38]. Accordingly, it has been reported that membrane PLA 1, A2, and C, may act in remodelling reactions needed for plasma membrane transformation during T. cruzi differentiation; these enzyme activities may be acting in remodelling reactions leading to the anchor of the mature glycoproteins of T. cruzi [46].

In the case of Leishmania spp., preliminary results of our laboratory have also demonstrated in L. braziliensis promastigotes a PLA 1 activity hydrolyzing PC. Moreover, we detected by Immunoblot two bands of \(\sim 37\) and 41 kDa, using polyclonal antibodies against both TcPLA 1 and TbPLA 1 [47]. These antibodies were obtained in our laboratory, since no commercial antibodies against any PLA 1 were available until this year [48].

2.2. Phospholipase A 2. The eukaryotic PLA 2 were the first of the PLAs to be recognized. The pancreatic PLA 2 has been known to degrade PC since 1878, and at the turn of the century cobra venom was shown by Keyes in 1902 to have haemolytic activity directed towards the membranes of erythrocytes [49]. Secreted and membrane-bound PLA 2 activity has been described in Bacteria, Fungi and Protozoa [50–54], but in the case of Trypanosomes just a few reports are available.

PLA 2 was isolated from T. congolense; the enzyme appeared to exist in a dimeric form with subunit molecular weights of 16.5 and 18 kDa, had optimum pH of 6.8, and showed specificity for 1,2-dimyristoyl-sn-PC and 1,2, dioleoyl-sn-PC [55]. Inhibition studies implicated a thiol group at the catalytic site of the enzyme, which was stable to heat treatment and possessed haemolytic and anticoagulating properties [55].

In T. brucei bloodstream forms, other authors reported a PLA 2 activity that could be stimulated by Ca\(^{2+}\) or by the amphiphilic peptide melittin, and that was responsible of the release of arachidonic acid, a prostaglandins precursor, being a pivotal enzyme in the control of Ca\(^{2+}\) influx [56, 57]. In addition, it was demonstrated in T. brucei procyclic forms, that the arachidonic acid generated endogenously could induce both Ca\(^{2+}\) entry and Ca\(^{2+}\) release from the intracellular compartments acidocalcisomes, suggesting that PLA 2 activity participates in T. brucei signalling events [58].

On the other hand, in L. donovani promastigotes and T. cruzi amastigotes, these authors found that arachidonic acid only induced Ca\(^{2+}\) entry, possibly due to low generation of arachidonic acid or to the low amount of releasable Ca\(^{2+}\) in the acidocalcisomes of these cells [58].

Previous reports in T. cruzi suggested that PLA 2 could mediate the association between the parasite and macrophages, but the authors did not clearly establish the source of the enzyme [59]. In epimastigotes, it has been described a haemolytic activity that destabilize in vitro red blood cells membranes and that could be attributed to PLA 2 activity [60]. However under our experimental conditions using zwitterionic PL such as PC or PE as substrates, no secreted PLA 2 was detected in the supernatants of living epimastigotes [38]. In this concern, other authors have described in this protozoa a membrane-bound PLA 2 activity acting only on anionic PL such as inositolphospholipids and inositolphosphoceramides [36].

PLA 2 degrading activities have been also reported in L. major, and they could be involved in the biosynthesis of lipophosphoglycan, the main macromolecule on the surface of the procyclic promastigote [61]. Other authors showed that parasite pretreatment with a low dose of pachymatismin, a glycoprotein extracted from a marine sponge, increased PLA 2 activity, however macrophage invasion was partially inhibited [62].
On the other hand, in *L. amazonensis* the modification of PL composition of infected macrophages has been described, with increasing levels of LPC, an effect that may reflect indirectly, the action of an endogenous/parasite PLA2 on the macrophage [63]. Furthermore, other studies showed PLA2 activity in supernatants and lysates of *L. (L.) amazonensis* promastigotes and suggested that this enzyme may be a progression factor for cutaneous leishmaniasis [64].

In summary, there are increasing evidences of the presence and possible roles of PLA2 in the pathogenic Trypanosomes, so far however, these enzymes have not been purified or characterized in deep.

### 3. Phospholipases A of Trypanosomatids and Pathogenesis

PLA activity has been linked to pathogenesis in various microorganisms such as *Escherichia coli*, *Yersinia spp.*, *Helicobacter pylori*, *Neisseria spp.*, *Legionella spp.*, and *Campylobacter spp.*, which cause different disease syndromes; however, the exact mechanism of the PLA action has not been definitively determined [10–14, 65, 66]. PLA toxicity has been associated to cytolytic activity resulting from the accumulation of membrane destabilizing products or by the extensive destruction of membrane phospholipids [10]. In pathogenic protozoa PLAs have been implicated in cytopathic activity [33], in *Toxoplasma gondii* it has been described that PLA2 inhibition protected human monocyteic cells from parasite invasion [53]; in *Entamoeba histolytica*, PLA2 is one of the several factors related to virulence [54] and in *Cryptosporidium parvum* the use of PLA2 inhibitors as well as specific anti PLA2 antibodies significantly reduced invasion of human enterocytes [68].

The role of PLA2 in the pathogenesis of African trypanosomiasis has been intensively studied [29, 45, 69, 70]. Hambrey et al. described in the tissue fluids of *T. brucei* infected rabbits large amounts of PLA2 activity that increased with parasite burden, whereas in blood plasma this activity was also detected, but at a considerably lower level [70]. The enzyme seemed to be of trypanosomal origin, being either secreted by living parasites or released from dying organisms [70]. In intravascular locations PLA2 could contribute to the pathology of trypanosomiasis by causing cell membrane damage and could account for some or all of the connective tissue cell destruction, which is a prominent feature of infections with *T. brucei* [71].

The high level of PLA1 found in *T. congolense* and *T. brucei*, in comparison to other pathogens like *Escherichia coli* (1000 times fold higher) [33], and its relatively low level in the nonpathogenic rat trypanosome *T. lewisi*, suggested the importance of the enzyme in the pathology of African trypanosomiasis [28, 29, 45, 69, 72]. Given that *T. lewisi* and *T. congolense* are restricted almost entirely to the blood stream of the host, whereas *T. brucei* develops mainly in the connective tissues [71], it was suggested that PLA1 could help the latter to penetrate blood vessels endothelium and other barriers hindering and contributing to tissue damage [29].

In the pathogenic *T. brucei* and *T. congolense* it has been determined that PLA1 activity increased greatly during the autolytic process and large quantities of FFA were accumulated, whereas the non pathogenic *T. lewisi* failed to increase the enzyme activity even on prolonged autolysis [69]. PLA1 yields FFA and LPC, which is then further degraded by the LPLA to yield more FFA and glycerophosphorylcholine. FFA are cytotoxic and haemolytic as a result of their detergent-like properties [73], and they could account for the immunosuppression and the structural disturbances in lymphoid organs observed in African trypanosomiasis [69]. These observations deserve to be updated and deeply studied.

The first evidences related to phospholipid degrading enzymes in *T. cruzi*, was associated to the inflammatory responses that appear surrounding degenerating amastigote nests in various tissues of Chagas’ disease patients [74]. This finding strongly suggested that autolytic processes generate factors, possibly PL-breakdown products, which

### Table 1: Phospholipase A2 putative genes found in TriTrypDB.

| Gene | Organism          | Product                               | Syntenic | Comments |
|------|-------------------|---------------------------------------|----------|----------|
| LbrM34_V2.2930 | *L. braziliensis* | phospholipase A2-like protein, putative | yes      | no       |
| LinJ35_V3.3070 | *L. infantum*    | phospholipase A2-like protein, putative | yes      | no       |
| LmjF35.3020   | *L. major*       | phospholipase A2-like protein, putative | yes      | no       |
| LmxM34.3020   | *L. mexicana*    | phospholipase A2-like protein, putative | yes      | no       |
| Tbg972.9.7760 | *T. brucei gambiense* | phospholipase A2-like protein, putative | yes      | no       |
| TclL3000.0.00740 | *T. congolense* | product unspecified                     | no       | no       |
| Tc00.1047053510743.60 | *T. cruzi CL* | phospholipase A2-like protein, putative | yes      | no       |
| Tc00.1047053510659.250 | *T. brucei* Non-Esmeraldo-like | phospholipase A2-like protein, putative | yes      | no       |
| TclL3000.0.00740 | *T. vivax*       | phospholipase A2-like protein, putative | yes      | no       |
cause inflammation [11]. In this regard, it was demonstrated that FFA and LPL released from killed trypanomastigotes have toxic effects on culture cells [12]. These facts are in agreement with the pathogenic mechanism proposed for African trypanosomiasis [29, 69]. Accordingly, we determined in all T. cruzi stages the rapid and extensive breakdown of endogenous PL in autolyzing parasites [37]. A major increase in FFA was observed, significantly higher than the generation of LPC, indicating not only the presence of PLA activity but also LPLA activity [37]. We also found that living T. cruzi infective stages were able to hydrolyze LPC, confirming the presence of a LPLA activity (Belaunzarán et al. unpublished observations). It is well known that LPC is potentially toxic for the cells [75, 76] though this activity would thereby contribute significantly to the parasite self-protection against lysocompounds. Similarly, in living T. brucei it has been demonstrated that PLA1 is active against LPL [77]. Other authors reported that bloodstream forms can acquire substantial amounts of exogenous LPL through a pathway consisting of three enzymes associated with the plasma membrane: PLA1, acyl-COA ligase, and LPL acylCOA-acyl transferase [32]. These cytotoxic compounds can change the ionic permeability of the plasma membrane, though they are rapidly metabolized to ensure tolerable levels in the cell. Thus a membrane-bound PLA1 would protect T. brucei against the high levels of plasma LPC [32].

In T. cruzi, we already showed the involvement of PLA1 in the early events of parasite-host cell interaction preceding parasite invasion. We demonstrated that either intact infective parasites or purified PLA1 significantly modified the host cell lipid profile with generation of second lipid messengers (DG, FFA, and LPC) and concomitant protein kinase C activation [38], an enzyme that has been implicated in the upregulation of T. cruzi invasion [78].

With respect to Leishmania spp., it has been observed that LPC, which is scarce in the macrophage, increased significantly after infection with L. amazonensis [63]. As LPC and arachidonic acid are the products of PC cleavage by PLA1, the increase in the levels of LPC may suggest the action of the enzyme on the macrophage PC, producing prostaglandin E2 [63]. In this regard, it has been shown that this lipid mediator is increased after 1-2 hours of infection with L. donovani and can exacerbate the infection [79]. Nevertheless, whether the LPC generation was due to parasite PLA2 or to the activation of macrophage PLA2 remains unclear [63]. It is possible that the LPC could also be generated by a PLA1 activity, similarly to that we detected in L. braziliensis [47].

4. Bioinformatic Analysis of the Trypanosomatid Genomes for Phospholipases A

The publication of the genomes of the kinetoplastid parasites T. brucei [80], T. cruzi [81], Leishmania spp. [82], and other related organisms, allowed the scientific community to perform comparative analyses giving insight into the evolutionary similarities/differences among trypanosomatids. T. brucei PLA1 (Tb.927.1.4830) has been cloned and characterized and the analyses of its protein sequence indicated that this enzyme is not homologous to neutral lipases [34]. The only similarity to them was in the amino acid sequence that contains a lipase consensus pattern harbouring a conserved GXSXG motif, a marker of the serine hydrolase superfamily [34]. In these enzymes, the catalytic triad is typically constituted by a base residue (Histidine), an acid (Aspartic), and a nucleophile (Serine), belonging to the latter to the GXSXG motif. No eukaryotic homologues of TbPLA1 were found in T. cruzi and Leishmania spp., but orthologues of this enzyme were identified in T. congolense (TcIL3000.1.2010) and T. vivax (TvY486_0102170) [34]. Interestingly, TbPLA1 resembled a putative PLA1 homologue from Sodalis glossinidius, a proteobacterium endosymbiont of tsetse flies. These findings suggested that a T. brucei ancestor acquired the PLA1 gene through horizontal gene transfer after/during its adaptation to a parasitic lifestyle in the insect vector [34].

Regarding T. cruzi, we previously reported the presence in the T. cruzi data base (http://www.tcruzidb.org/) of at least sixteen different genes encoding putative lipases and the identified sequences presented a high degree of similarity among them (70–80%), may be haplotype variants [38]. When we further performed a search in the Kinetoplastid Genomic Resource TriTryDB (http://tritrypdb.org/tritrypdb/) using only the lipase consensus pattern of TbPLA1 and considering the biochemical characteristics of T. cruzi PLA1, the number of putative genes was reduced to eight [38]. One of them (Tc00.1047053510679.100) was cloned and expressed in E. coli, being the recombinant enzyme recognized by both anti-TcPLA1 and anti-TbPLA1 antibodies [48]. The eight sequences are currently under study in our laboratory, to elucidate the identity of each of these genes that codify for T. cruzi PLA1 and to obtain the active recombinant enzyme.

We extended these analyses to Leishmania spp., searching in the TriTryDB database for homologues of T. cruzi PLA1 putative genes and have identified in L. braziliensis, L. infantum, and L. major, three, nine and eight putative genes with the conserved lipase motif, respectively. One of the putative genes from L. braziliensis, LbrM31_V2.2750, was cloned and expressed in E. coli, being the recombinant protein recognized by both anti-TcPLA1 and anti-TbPLA1 antibodies [47]. At present, we are running assays with the aim of obtaining and characterizing the active recombinant enzyme.

The alignment of the protein sequences corresponding to the cloned T. brucei, T. cruzi, and L. braziliensis PLA1 (Tb.927.1.4830, Tc00.1047053510679.100, and LbrM31_V2.2750, resp.), with the putative PLA15 proteins of T. congolense and T. vivax, (TcIL3000.1.2010 and TvY486_0102170), shows that the sequences of T. brucei, T. congolense, and T. vivax are closely related, whereas T. cruzi and L. braziliensis only share with all of them the lipase motif (Figure 2). The fact that T. cruzi and L. braziliensis PLA1 protein sequences do not share significant homologies with African trypanosomes, particularly with TbPLA1, is in
agreement with that previously observed by Richmond and Smith [34].

Although PLA2 activity was detected in T. brucei, T. congolense, T. cruzi, L. major, and L. amazonensis years ago, still little is known about the identity of the genes that codify for them [36, 55, 56, 61, 63, 83]. We have performed a search in the TriTrypDB database and identified at least 9 putative PLA2-like proteins in the different Trypanosomas species (Table 1), but at the moment no PLA2 of trypansomal origin has been identified in the genomes or cloned.

5. Inhibitors of Trypanosomatid Phospholipases A

As described above, parasite PLAs participate in diverse and relevant cellular processes such as membrane remodelling, modification of membrane permeability, generation of lipid second messengers and parasite invasion. All these facts emphasize the interest of these enzymes as potential chemotherapeutic targets that could contribute to the control of parasite proliferation and survival.

A number of compounds with potential inhibitory activity on parasite PLA1 have been investigated. TbPLA1 activity was inhibited by several heavy metals through an undefined mechanism, being the most potent at lower concentrations cadmium and copper. Iron produced partial to total inhibition depending on the concentrations employed, whereas moderate inhibition was detected in the presence of relatively high concentrations of nickel and zinc [34]. As the active-site residue for TbPLA1 is Serine 131, the active-site serine modifiers iPr2P-F (di-isopropyl fluorophosphate), PMSF (phenylmethylsulfonyl fluoride), and E-600 (diethyl-p-nitrophenyl phosphate) were also assayed. Relatively little inhibition of the enzyme activity was observed but at very high concentration of inhibitors, suggesting that the catalytic triad active site of TbPLA1 is buried inside the enzyme and sheltered by a lid domain, a property shared with other lipases [34].

Other compounds with potential inhibitory activity on TcPLA1 were investigated in our laboratory, including the antimalarial drugs quinine and chloroquine, the antiarrhythmic drugs amiodarone and chlorpromazine, and the local antianesthetics dibucaine, procaine, and xylocaine. Among all of them, only chlorpromazine had an inhibitory effect, but at concentrations that induce cell toxicity [37].

As previously mentioned, in T. brucei Ca2+ influx can be regulated by PLA2 [56, 58]. Various inhibitors of this enzyme such as thioretheramide-PC, manoolide, arachidonyl trifluoromethyl ketone, and arachidonoyl benzoylacrylic acid (OBAA), a potent inhibitor of secreted PLA2 [56]. On the other hand, T. brucei gambiense and T. brucei brucei PLA2s were inhibited in a noncompetitive fashion when using organotin compounds like fatty acid derivatives of dibutyltin dichloride [83]. In the case of T. cruzi, it has been suggested that quinacrine, which inhibited erythrocyte lyases, blocked PLA2 activity [60].

Concerning Leishmania spp., up to now, there are no reports about the use of specific PLA inhibitors. However, it has been reported that the lysophospholipid analog (LPA) miltefosine, affects lipid metabolism in Leishmania donovani promastigotes, with reduction in PC and enhancement in PE and LPC, a process in which PLAs could participate among other enzymes [84]. The usefulness of lipid biosynthesis inhibitors has gained great interest in the last years to fight parasitic Trypanosomes [5, 85–88]. These compounds, initially developed to be antitumor agents, have proved to be highly effective in the treatment of visceral leishmaniasis [87, 89, 90]. Although their effectiveness is known, the mode of action against this parasite is not completely understood [91]. In T. cruzi, the synergy of the LPAs edelfosine, ilmozofine, and miltefosine with the ergosterol biosynthesis inhibitor, ketoconazole, induced alterations in the plasma membrane, reservosomes, and mitochondrion, indicating that these organelles are potential targets of these drugs, probably through interference with lipid metabolism [92].

Considering that PLAs are present in both, trypanosomes and mammalian host, it will be of relevance to achieve the knowledge of their three-dimensional structures to determine the differences/similarities among them. This would allow the rational design of specific inhibitors that could be employed as potential chemotherapeutic agents in the diseases caused by kinetoplastid pathogens.

6. Concluding Remarks

In summary, as presented in this paper PLAs of pathogen trypanosomes mediate a variety of processes in both protozoan and host cell lipid metabolism, being also considered virulence factors. However, the knowledge of these enzymes is far from complete, though in the future, continued biochemical, biological, and structural research are needed to obtain a full understanding of the molecular mechanism in which these enzymes participate. Unravelling the differences between parasite and host PLAs may contribute, besides, to the design of specific enzyme inhibitors that could be used in the treatment of the neglected diseases that trypanosomes cause.

Acknowledgments

The authors thank Guadalupe Giménez for critical comments on the paper. This work was supported by Universidad de Buenos Aires (UBA), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), and Agencia Nacional de Promoción Científica y Tecnológica (FONCYT).

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