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Acidocalcisomes of Eukaryotes

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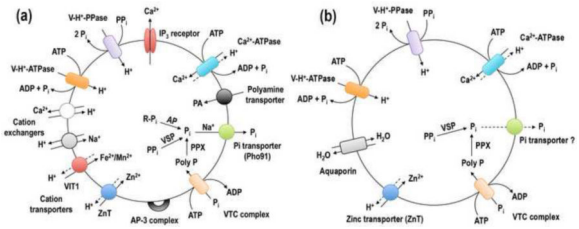
Abstract

Acidocalcisomes are organelles rich in polyphosphate and cations and acidified by proton pumps. Although they have also been described in prokaryotes they have been better characterized in unicellular and multicellular eukaryotes. Eukaryotic acidocalcisomes belong to the group of lysosome-related organelles. They have a variety of functions, from the storage of cations and phosphorus to calcium signaling, autophagy, osmoregulation, blood coagulation, and inflammation. Acidocalcisomes of several unicellular eukaryotes possess a variety of transporters, channels and pumps implying a large energetic requirement for their maintenance and suggesting other important functions waiting to be discovered.

Graphical Abstract



Introduction

Since their first description in the protist parasites *Trypanosoma brucei* [1] and *Trypanosoma cruzi* [2], the etiologic agents of African and American trypanosomiasis, respectively, acidocalcisomes have been reported in bacteria [3,4], as well as in many unicellular and multicellular eukaryotes, including humans [5-7]. Two organelles in mammals, the platelet dense granules [8] and the mast cell granules [9], have characteristics in common to acidocalcisomes of protists. The biogenesis of these organelles in several protists [10,11] and mammalian cells [12] involves the function of the adaptor protein 3 (AP-3) complex, as is typical of lysosome-related organelles. Ablation of β3 and δ subunits of the AP-3 complex

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in *T. brucei* resulted in disappearance of acidocalcisomes with no alterations in trafficking of different markers to lysosomes [11].

Acidocalcisomes are characterized by their high electron-density when observed by electron microscopy (Fig. 1), and by their acidity and high calcium concentration. These characteristics are the reasons for their name and their grouping as one of the acidic calcium stores of the cell [13]. Another peculiarity common to acidocalcisomes of different species is their high content of phosphorus in the form of orthophosphate, pyrophosphate, and short- and long-chain polyphosphate [14]. Polyphosphate is a polymer of three to hundreds of orthophosphate monomers linked by high-energy phosphoanhydride bonds similar to those present in ATP [15].

In protists the acidity of acidocalcisomes is maintained by a vacuolar H^+-pyrophosphatase (V-H^+-PPase), an enzyme also present in the vacuole of plants, and in some species acidocalcisomes have, like the plant vacuole, an additional proton pump, a vacuolar H^+-ATPase (V-H^+-ATPase). In multicellular organisms lacking a V-H^+-PPase, the V-H^+-ATPase maintains their acidity [5].

Recent reviews [5,16] have described the acidocalcisomes from prokaryotes. In this review we will limit our discussion to recent work on acidocalcisomes from eukaryotes.

Acidocalcisomes in protists

A large number of protists possess acidocalcisomes, among them trypanosomatids and Apicomplexan parasites, algae, slime molds, and fungi. We will discuss each of these organisms in the following sections.

Trypanosomatids

These have been the organisms in which acidocalcisomes were first described and that were studied in more detail. Early reports in *T. brucei* [1] and *T. cruzi* [2] described the presence of an energy-driven calcium transport mechanism (Ca^{2+}-ATPase) in a compartment that is acidified by an ATP-dependent proton pump sensitive to bafilomycin A_1 (V-H^+-ATPase) and that was named the *acidocalcisome*. Evidence was also provided of the presence of a Na^+/H^+ and Ca^{2+}/H^+ exchangers in the membrane of this compartment in the procyclic stages (forms present in the insect vector) of *T. brucei* [17] and in promastigotes of *Leishmania donovani* [18], one of the agents of visceral leishmaniasis. These compartments were later [19] found to correspond to what were known as polyphosphate bodies, which are characterized by their high electron density when examined by electron microscopy (Fig. 1), and by the presence of phosphorus in the form of polyphosphate, and several cations like Ca^{2+}, Mg^{2+}, Na^+, K^+, and Zn^{2+}, as detected by X-ray microanalysis [19,20]. These cations balance the negative charges of polyphosphate. The discovery of a V-H^+-PPase in these organelles [21,22] provided an important marker for their subcellular identification and characterization in other trypanosomatids such as *L. donovani* [23], *Phytomonas françai* [24], and monogenetic trypanosomatids [25], as well as in other protists (see below). This period of morphological characterization was followed by the molecular identification of different transporters, pumps and channels present in the membrane of the organelles. Genes encoding the acidocalcisome Ca^{2+}-ATPase (plasma membrane-type or PMCA) were reported in *T. cruzi*

[26], and *T. brucei* [27]. The gene encoding the V-H^+-PPase of acidocalcisomes was cloned from *T. cruzi* and functionally expressed in yeasts [28], and knocked down in *T. brucei*, showing its essential role for growth in the insect and mammalian forms of the parasite [29]. A water channel or aquaporin was found in acidocalcisomes of *T. cruzi* and shown to translocate to the contractile vacuole complex of these parasites in a cyclic AMP- and microtubule-dependent process during hyposmotic stress [30,31]. The identification of the nature of the abundant phosphorus content of acidocalcisomes as orthophosphate, pyrophosphate and short- and long-chain polyphosphate, was done first by ^{31}P-NMR spectroscopy [32-34] and later by biochemical techniques [14]. The mechanism for synthesis and translocation of the polymer, was first discovered in the acidocalcisome-like organelle (vacuole) of yeasts [35,36] and later in trypanosomatids [37,38]. The mechanism involves the function of a vacuolar transporter chaperone (VTC) complex that in yeast consists of four proteins (Vtc1-4) that form heterotrimeric complexes. Two of these subunits (Vtc1 and Vtc4) were identified in trypanosomatids, where Vtc4 is the catalytic subunit. The complex is essential in both insect and mammalian stages of *T. brucei* [37]. A Ca^{2+} channel, the inositol 1,4,5-trisphosphate receptor (IP_3R), was also localized to the acidocalcisomes of *T. brucei* and demonstrated to be essential for growth and infection [39]. Its presence suggests a role for acidocalcisomes in Ca^{2+} signaling.

Proteomic analyses of acidocalcisomes of trypanosomatids [40,41] identified several cation transporters, such as putative Zn^{2+} transporters, an orthologue to the vacuolar iron transporter (VIT) of plants and the Ca^{2+}-sensitive cross-complementer 1 (CCC1) of the yeast vacuole, which are involved in iron and manganese sequestration, an orthologue to the phosphate-sodium symporter Pho91 of the yeast vacuole, involved in Na^+ and phosphate release, and an orthologue to a polyamine transporter, suggesting roles in inorganic and organic cation uptake, and phosphorus release [41]. The presence of a V-H^+-ATPase, a V-H^+-PPase, Vtc1, and Vtc4, and the IP_3R was also confirmed in these studies, as well as of an enzyme involved in pyrophosphate and polyphosphate metabolism, the vacuolar soluble pyrophosphatase (VSP), and an acid phosphatase [41].

More recent studies have been oriented towards the analysis of the functional role of acidocalcisomes. A role as a cation, phosphorus, and basic amino acid [42] store is evident. It was shown that in *T. cruzi* the polyphosphate content of the cells is significantly reduced after the lag phase of growth suggesting a requirement for phosphorus to resume growth [14]. As indicated above the presence of mechanisms for Ca^{2+} uptake (Ca^{2+}-ATPase) and release (IP_3R) suggest a role in Ca^{2+} signaling [6]. A role in autophagy in *T. brucei* was recently revealed [43]. Downregulation of components of the AP-3 complex, involved in acidocalcisome biogenesis, inhibits starvation-induced autophagosome formation [43]. In *T. cruzi,* several studies reported the role of acidocalcisomes in osmoregulation. Upon hyposmotic stress there is swelling and fusion of acidocalcisomes with the contractile vacuole complex and translocation of an aquaporin that favors contractile vacuole swelling and water expulsion [30,44]. Changes in acidocalcisome polyphosphate content accompany the changes in osmolarity [30]. Changes in acidocalcisome ions also occur in *L. major* submitted to hyposmotic stress [45]. The results suggest that hydrolysis of polyphosphate upon hyposmotic stress is followed by translocation of phosphorus and ions to the contractile vacuole enhancing their osmolarity and favoring water intake through the

translocated aquaporin and then water elimination by the contractile vacuole to the extracellular medium [46,47]. A role in osmoregulation was also suggested by experiments that down-regulated [37] the expression of enzymes involved in the synthesis of acidocalcisome polyphosphate. On the other hand, overexpression of the acidocalcisome vacuolar soluble pyrophosphatase (VSP) resulted in decreased acidocalcisome polyphosphate levels and parasite persistence in tissues, suggesting a role for polyphosphate in infection [48]. Fig. 2 shows schemes of the pumps, channels, transporters, and enzymes known to the present in *T. cruzi* and *T. brucei* acidocalcisomes.

Apicomplexan parasites

The presence of acidocalcisomes in *Toxoplasma gondii*, the agent of toxoplasmosis, was reported soon after their discovery in trypanosomatids [49]. Since then several Apicomplexan parasites like *Plasmodium falciparum* [50], agent of malaria, *Eimeria* spp., agents of coccidiosis [51], and *Garnia gonadati*, a parasite of an Amazonian reptile [52], were found to have acidocalcisomes. A Ca^{2+}-ATPase [53,54] and Na^{+}/H^{+} and Ca^{+}/H^{+} exchangers [55] were found in acidocalcisomes of the tachyzoite forms of *T. gondii*, together with a V-H^{+}-PPase, although this last enzyme also localizes in other compartments, like the plant-like vacuole [56]. Two subunits of the VTC complex were found in *T. gondii*, Vtc2 and Vtc4, although Vtc2 does not appear to co-localize with the V-H^{+}-PPase [57]. Both the acidocalcisome Ca^{2+}-ATPase [54] and the V-H^{+}-PPase [58] are essential for growth and virulence of *T. gondii*.

Algae

Chlamydomonas reinhardtii has typical electron-dense acidocalcisomes rich in polyphosphate and cations (Ca^{2+}, Mg^{2+}, Zn^{2+}), which are acidified by both a V-H^{+}-PPase and a V-H^{+}-ATPase [59]. Acidocalcisomes are also in close association with the contractile vacuole complex of these organisms [59]. They were recently found to contain copper, and suggested to have a role in preventing mismetallation during zinc deficiency [60]. Copper was also found in acidocalcisome-like organelles of *Euglena gracilis* [61]. *C. reinhardtii* acidocalcisomes also possess an orthologue of Vtc1, and mutants deficient in this protein have less acidocalcisomes and polyphosphate [62]. A Vtc4 orthologue is also present in the genome of this alga. A proteomic analysis of acidocalcisomes (polyphosphate vacuoles) of the red alga *Cyanidioschyzon merolae* detected evidence of several pumps and transporters in common with those of acidocalcisomes of *T. brucei,* such as V-H^{+}-PPase, V-H^{+}-ATPase, Zn^{2+} transporter, acid phosphatase, Vtc1 and VIT, as well as of other proteins (putative metallopeptidase, prenylated Rab receptor, ABC transporter and o-methyltransferase) identified by epitope tagging and specific antibodies [63].

Slime molds and fungi

The slime mold *Dictyostellium discoideun* possesses mass-dense granules rich in polyphosphate and cations (Ca^{2+}, Mg^{2+}) with similar characteristics to the acidocalcisomes of trypanosomatids and algae [64]. Antibodies against a Ca^{2+}-ATPase (PAT1) and a V-H^{+}-ATPase were found to co-localize in acidocalcisomes with antibodies against the plant V-H^{+}-PPase and with pyrophosphatase activity [64]. However, despite the detection of

membrane-bound pyrophosphatase activity [65], a gene orthologue to V-H^+-PPases has not been reported in the genome of the slime mold. As occurs with *C. reinhardtii* and *T. cruzi, D. discoideum* acidocalcisomes are also in close contact with the contractile vacuole complex of the organism [64]. Two polyphosphate kinases were identified in *D. discoideum* and their vacuolar (potentially acidocalcisome) localization was proposed [66,67].

The yeast vacuole has been considered an acidocalcisome-like organelle [36] since it possesses large amounts of polyphosphate and cations, and pumps and transporters such as V-H^+-ATPase, Ca^{2+}-ATPase (PMC1), Ca^{2+}/H^+ exchanger (CAX), VTC complex (Vtc1p-4p), transporters for Zn^{2+} (ZnT), Mn^{2+} and Fe^{2+} (CCC1), polyamines, basic amino acids, and phosphorus (Pho91) [68]. However, the yeast vacuole possesses hydrolytic enzymes and do not possess a V-H^+-PPase, although this could be the result of their divergent evolution. Recent studies have shown that the polyphosphate metabolism in yeast is connected to that of highly phosphorylated inositol species and that mutants unable to produce inositol pyrophosphates have undetectable levels of polyphosphate, suggesting the presence of another potential pathway for their synthesis [69]. Vacuoles containing transporters similar to those in yeast acidocalcisome-like vacuoles are also found in other fungi [70,71].

Insect, echinoderm and bird eggs

Acidocalcisomes have been found in the yolk of insect [72], echinoderms [73], and bird [74] eggs. They are electron-dense and rich in short chain polyphosphate (chains of less than 100 orthophosphate residues) and cations. In chicken eggs acidocalcisomes are within larger acidic vacuoles forming compound organelles [74]. Acidification is through a V-H^+-ATPase in sea urchin and chicken eggs [73,74]. Although a V-H^+-PPase activity was detected in insect eggs [75] there is no genomic information of the presence of orthologues to the V-H^+-PPases present in acidocalcisomes of insect species.

Mammalian cells

Several lysosome-related organelles of mammalian cells have similarities with acidocalcisomes. The best studied are the platelet dense granules [8] and the mast cell granules [9]. Human platelet dense granules are electron-dense, possess polyphosphate of about 80 orthophosphate monomers, and cations (Ca^{2+}, K^+), and are also known to contain pyrophosphate, ATP, ADP and serotonin. Their acidification is through a V-H^+-ATPase [8]. Polyphosphate is also present in mast cell granules (RBL-2H3 cells) and human basophils although their size is smaller (~60 phosphate units). In mast cells polyphosphate is present in serotonin-containing granules but it does not co-localize with histamine-containing granules. These granules are also acidic, and presumably contain a V-H^+-ATPase [9]. Polyphosphate release from human dense granules is associated with promotion of blood clotting, and anti-fibrinolysis [76], and has a pro-inflammatory role [77]. Interestingly, human platelets appear to depend on the inositol pyrophosphate pathway for the synthesis of polyphosphate [78].

Conclusions

The common properties of acidocalcisomes of different eukaryotes are their high electron-density, their acidic nature, and their high calcium and polyphosphate content. Acidocalcisome acidification is by a V-H^+-PPase, by a V-H^+-ATPase, or by both. Ca^{2+} uptake is, at least in protists, by a Ca^{2+}-ATPase, while polyphosphate synthesis and translocation in several protists is by a VTC complex, and by unknown mechanisms in mammalian cells, possibly involving the inositol pyrophosphate pathway. The best-studied acidocalcisomes are those of trypanosomatids, in which several functions have been demonstrated or suggested such as their roles in autophagy, osmoregulation, calcium signaling, and phosphorus and cation storage. The discovery of the presence of polyphosphate in human platelets and mast cells and that this polymer can be released when cells are stimulated led to the discovery of the potent pro-coagulant, anti-fibrinolytic, and pro-inflammatory actions of polyphosphate, suggesting that this role could also be important for the pathogenesis of bacterial and parasitic infections since microorganisms are also rich in polyphosphate. Future studies will need to identify the enzymes involved in polyphosphate synthesis in mammalian cells, and demonstrate the role of acidocalcisomes in Ca^{2+} signaling. Other major challenges for the future are the determination of the structural organization of the acidocalcisome matrix and the mechanisms involved in their replication and fusion to other organelles, like the contractile vacuole complex, and its contact with the mitochondria, which could be very important for Ca^{2+} signaling.

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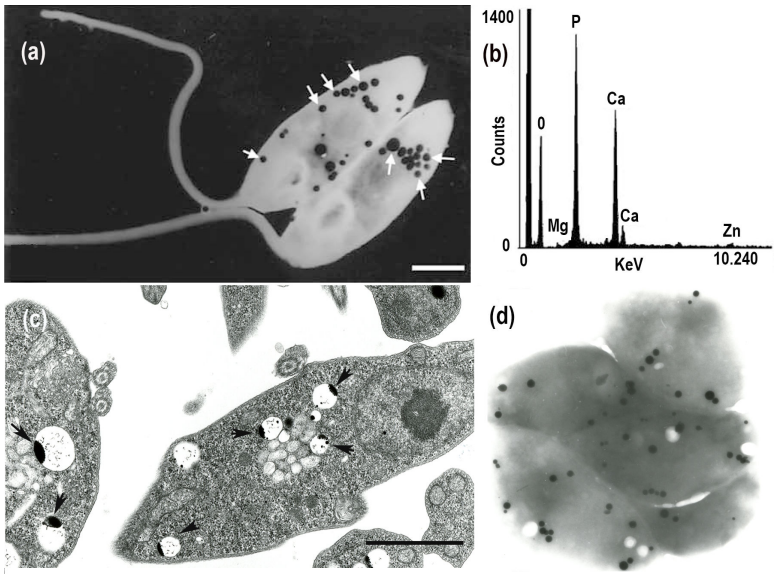

Figure 1.

Morphological and elemental composition of acidocalcisomes. **(a)** Electron spectroscopic image of the monogenetic trypanosomatid *Herpetomonas muscarum* adhered to a formvar/carbon coated grid showing the electron-dense acidocalcisomes (*white arrows*). Bar = 2 μm. **(b)** Semi-quantitative analysis of acidocalcisomes by X-ray microanalysis indicating the presence of oxygen (O), magnesium (Mg), phosphorus (P), potassium (K), calcium (Ca), iron (Fe), and zinc (Zn). **(c)** Conventional transmission electron microscopy of procyclic stages of *T. brucei* showing the acidocalcisomes as "empty" vacuoles containing electron dense inclusions (*black arrows*). Bar = 1 μm. (d) Direct transmission electron microscopy of several *Toxoplasma gondii* tachyzoites showing electron-dense acidocalcisomes (*black granules*). Bar = 1 μm. **(a)** and **(b)** are from Ref. 25 with permission. © *Elsevier*. **(c)** is from Ref. 22 with permission. © *American Association for Microbiology*.

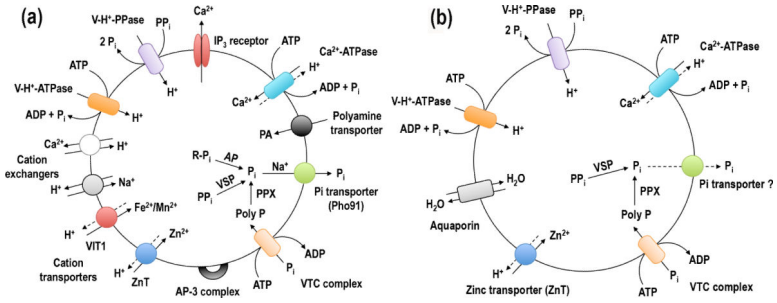

Figure 2.

Schematic representation of the acidocalcisomes of *T. brucei* and *T. cruzi*. **(a)** In the *T. brucei* acidocalcisomes, Ca^{2+} is taken up by a H^+-countertransporting Ca^{2+}-ATPase and released by the inositol 1,4,5,trisphosphate (IP_3) receptor. H^+ is pumped in electrogenically by either the V-H^+-PPase or the multisubunit V-H^+-ATPase. Ca^{2+}/H^+ and Na^+/H^+ exchangers could be used for Ca^{2+} release in exchange for Na^+ uptake. A vacuolar iron transporter (VIT1) can be used for either Mn^{2+} or Fe^{2+} uptake and a Zn^{2+} transporter (ZnT) for Zn^{2+} uptake. There is also a polyamine (PA) transporter. A VTC with at least two subunits (Vtc1 and Vtc4) synthesizes polyphosphate using ATP and translocates it into the organelle. A Na^+/P_i symporter (Pho91) releases Na^+ and Pi from acidocalcisomes. Within acidocalcisomes there is a vacuolar soluble pyrophosphatase (VSP), an exopolyphosphatase (PPX) and an acid phosphatase (AP). Several adaptor protein 3 (AP-3) complex subunits also localize to the acidocalcisome. **(b)** In the acidocalcisomes of *T. cruzi*, there is also an aquaporin that allows water transport, but there are fewer channels, transporters, exchangers or enzymes identified thus far.