Minireview

Acidocalcisomes in Apicomplexan parasites

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Abstract

Acidocalcisomes are acidic calcium stores found in diverse organisms, being conserved from bacteria to man. They possess an acidic matrix that contains several cations bound to phosphates, mainly present in the form of short and long polyphosphate chains. Their matrix is acidified through the action of proton pumps such as a vacuolar proton ATPase and a vacuolar proton pyrophosphatase. The calcium uptake occurs through a Ca2+/H+ counter transporting ATPase located in the membrane of the organelle. Acidocalcisomes have been identified in a variety of microorganisms, including Apicomplexan parasites such as Plasmodium and Eimeria species, and in Toxoplasma gondii. In this paper, we review the structural, biochemical and physiological aspects of acidocalcisomes in Apicomplexan parasites and discuss their functional roles in the maintenance of intracellular ion homeostasis.

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Index Descriptors and Abbreviations: Toxoplasma gondii; Plasmodium falciparum; Eimeria acervulina; Apicomplexan; Acidocalcisomes

1. Introduction

Ion homeostasis in protozoan parasites has been the subject of intense investigation in the last few years (Docampo and Moreno, 2001; Moreno and Docampo, 2003). Cell viability requires perfect functioning of these mechanisms since disruption of homeostasis of certain ions, such as Ca2+, can lead to cell death (Berridge et al., 2000). In addition, Ca2+ is involved in the invasion of host cells by different parasites, a process that is crucial for maintaining their life cycles (Moreno et al., 2007a,b; Lu et al., 1997; Vieira and Moreno, 2000; Lovett and Sibley, 2003; Moreno et al., 1994).

Different intracellular organelles are involved in the regulation of ion homeostasis in protozoan parasites, such as the endoplasmic reticulum, mitochondria, and acidocalcisomes. The acidocalcisomes were first described in Trypanosoma brucei (Vercesi et al., 1994) and T. cruzi (Docampo et al., 1995), and, since then, have been identified in a variety of microorganisms, including Apicomplexan parasites such as T. gondii and Plasmodium spp. (Docampo et al., 2005). In this review, we will examine the structural, chemical and physiological properties of acidocalcisomes from Apicomplexan parasites, as well as their functional role in the cell biology of these parasites.

2. Acidocalcisomes in Apicomplexan parasites

They are characterized by their acidic internal milieu, high electron density, and high content of phosphorus in the form of phosphate, pyrophosphate and polyphosphate (poly P) and cations such as calcium, magnesium, sodium, potassium, and zinc. Acidocalcisomes were first identified in Coccidian parasites in 1907 (Kunze, 1907), and in Toxoplasma gondii in 1966 (Mira Gutierrez and Del Rey Culero, 1966) when they were named metachromatic or...
volutin granules, for their ability to stain red when treated with toluidine blue (metachromasia). In *T. gondii* they were also named ‘black granules’ (Bonhomme et al., 1993). The name acidocalcisome was first used in *T. gondii* when it was shown that these organelles possess mechanisms for the transport of protons and calcium (Moreno and Zhong, 1996).

3. Acidocalcisomes in *Toxoplasma gondii*

Acidocalcisomes are the largest store of Ca\(^{2+}\) in *T. gondii* (Bouchot et al., 1999; Luo et al., 2001; Moreno and Zhong, 1996). Their acidity is easily demonstrated through the incubation of tachyzoites with the weak base Acidine Orange (AO) and subsequent observation by fluorescence microscopy. Cells incubated in the presence of AO show orange labeling of several acidocalcisomes, which are the most acidic compartments in the cell (Fig. 1). Other compartments that have been shown to be acidic, such as rhoptries (Shaw et al., 1998) and a vacuolar compartment involved in microneme protein maturation (Harper et al., 2006), are not as evident by acridine orange staining.

In thin sections, the acidocalcisomes of *T. gondii* appear as empty vesicles sometimes with an electron dense material adjacent to the inner face of their membrane (Fig. 2). By electron spectroscopic imaging of whole cells directly dried onto Formvar-coated grids it is possible to see several acidocalcisomes per cell (Fig. 3A and B). Acidocalcisomes appear as spherical electron dense organelles randomly spread throughout the cell body. Approximately 10 acidocalcisomes, with diameters varying between ~150 and ~400 nm, are observed per cell. X-ray microanalysis of intact cells (Luo et al., 2001) has revealed considerable amounts of oxygen, sodium, magnesium, phosphorus, chlorine, potassium, calcium and zinc concentrated in the acidocalcisomes (Fig. 3F), similarly to what has been reported previously in the acidocalcisomes of trypanosomatids (LeFurgey et al., 2001; Miranda et al., 2000, 2004; Rodrigues et al., 1999; Scott et al., 1997). The low sulfur content detected by elemental analysis suggests a low content of proteins within acidocalcisomes.

*Toxoplasma gondii* acidocalcisomes have a plasma membrane type Ca\(^{2+}\)-ATPase (PMCA), involved in Ca\(^{2+}\) uptake, with similarity to vacuolar Ca\(^{2+}\)-ATPases of other unicellular eukaryotes (Bouchot et al., 2001; Luo et al., 2001). In addition, there occur two proton pumps, a vacuolar H\(^+\)-ATPase (V-H\(^+\)-ATPase) and a vacuolar H\(^+\)-pyrophosphatase (V-H\(^+\)-PPase), both contributing to the acidification of acidocalcisomes (Moreno et al., 1998; Rodrigues et al., 2000; Drozdowicz et al., 2003; Luo et al., 2001). Ca\(^{2+}\) release from acidocalcisomes of *T. gondii* has only been detected upon their alkalinization (Moreno and Zhong, 1996) or after poly P hydrolysis (Rodrigues et al., 2002a) (Fig. 4).

The gene encoding the acidocalcisomal Ca\(^{2+}\)-ATPase (*TgA1*) was able to complement yeasts deficient in the vacuolar Ca\(^{2+}\)-ATPase gene *PMC1*, providing genetic evidence for its function (Luo et al., 2001). This calcium pump is closely related to other acidocalcisomal Ca\(^{2+}\)-ATPases such as those present in *Trypanosoma cruzi*, *T. brucei*, and *Dictyostelium discoideum*, as well as to the vacuolar Ca\(^{2+}\)-ATPases of yeast, and *Entamoeba histolytica* (Luo et al., 2001). These pumps lack a calmodulin-binding domain, in contrast to other PMCA-type Ca\(^{2+}\)-ATPases. *T. gondii* mutants deficient in *TgA1* have decreased virulence in vitro and in vivo due to their deficient invasion of host cells (Luo et al., 2005). Poly P content in tachyzoites is drastically reduced in these mutants, and basal Ca\(^{2+}\) levels are increased and unstable. Microneme secretion is also affected. Complementation of *null* mutants with *TgA1* restores most of these defects in *T. gondii* (Luo et al., 2005).

The V-H\(^+\)-ATPase was first detected in *T. gondii* by its sensitivity to bafilomycin A\(_1\), a specific inhibitor of this pump when used at low concentrations (Bowman et al., 1988). Bafilomycin A\(_1\) is able to release calcium from acidocalcisomes of intact tachyzoites loaded with the fluorescent calcium indicator Fura 2 (Moreno and Zhong, 1996). The V-H\(^+\)-ATPase also localizes to the plasma membrane where it has a role in regulating intracellular pH homeostasis (Moreno et al., 1998).

A V-H\(^+\)-PPase activity is also detected in *T. gondii* (Rodrigues et al., 2000). This enzyme localizes in acidocal-
cisomes (Drozdowicz et al., 2003; Luo et al., 2001; Rodrigues et al., 2000), as well as in a vacuole involved in microneme protein maturation (Harper et al., 2006). This enzyme is also located in the plasma membrane and Golgi
complex of other cells (Docampo et al., 2005), although there is no evidence at present of communication or trafficking between acidocalcisomes and the plasma membrane. A truncated version of the enzyme (lacking the N-terminal region) was functionally expressed in yeast (Drozdowicz et al., 2003). Interestingly, the V-H+-PPase of *T. gondii* changes its intracellular localization during invasion of host cells as shown by specific staining using antibodies raised against a conserved domain of the *T. gondii* V-H+-PPase (Drozdowicz et al., 2003). A chimaera of the *T. gondii* V-H+-PPase with the N-terminal extension of *T. cruzi* V-H+-PPase at its N-terminus or the complete gene were recently found to complement yeast cells deficient in the soluble pyrophosphatase (Drake et al., 2004).

The acidocalcisomal enzyme belongs to the K+-stimulated group of V-H+-PPases (type I) (Drozdowicz et al., 2003; Rodrigues et al., 2000). It is concentrated and it could be used as a marker for acidocalcisome purification in cell fractionation experiments (Rodrigues et al., 2002a).

*Toxoplasma gondii* acidocalcisomes have high levels of phosphorus in the form of inorganic pyrophosphate (PPi) and polyphosphate (poly P). It is not known why PPi and poly P are concentrated in acidocalcisomes. Only three reactions use PPi in *T. gondii*, one is catalyzed by the phosphofructokinase (Feng, J. and Moreno, S., unpublished results). All these enzymes are apparently cytosolic, and a transporter would be required to transfer PPi from the acidocalcisomes to the cytosol. *T. gondii* is sensitive *in vitro* and *in vivo* to PPi analogs. Two main targets have been described for these drugs. One is the acidocalcisomal vacuolar proton translocating pyrophosphatase (V-H+-PPase), which is sensitive to aminomethyleneediphosphonate (AMDP) and imidodiphosphate (IDP) (Rodrigues et al., 2000). The second is an enzyme of the isoprenoid synthesis pathway, i.e., farnesyl diphosphate synthase, which is sensitive to inhibition by aminobisphosphonates (Ling et al., 2005).

Poly P is a polymer of a few to many hundreds of inorganic phosphate (Pi) residues, which are linked by high-energy phosphoanhydride bonds. They have been found in every category of organism examined, from bacteria to humans (Kornberg, 1999). In different organisms poly P is accumulated in very large amounts in acidocalcisomes (Docampo and Moreno, 2001). Although poly P was thought to have a role as an energy source, this has been disputed on the basis of its low metabolic turnover as compared to that of ATP (Chapman and Atkinson, 1977). An important regulatory role has been suggested instead (Rao and Kornberg, 1996). However, it has been shown that poly P kinase can transfer in a reverse reaction the terminal phosphate residue of poly P to ADP to yield ATP, so that poly P could participate in the global maintenance of the intracellular nucleotide pool (Ishige and Noguchi, 2000). In addition, long-chain poly P has been shown to be essential for adaptation to various types of stress and for survival of bacteria in stationary phase (Rao and Kornberg, 1996). Similar studies have been reported in yeast (Castro et al., 1995). It has been demonstrated that in bacteria poly P can also function as a phosphate reserve under conditions of phosphate starvation, in cation sequestration and storage, in cell membrane formation and function, in gene}

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**Fig. 4.** Schematic representation of a typical acidocalcisome. Ca\(^{2+}\) uptake occurs in exchange for H\(^{+}\) by a reaction catalyzed by a vacuolar Ca\(^{2+}\)-ATPase. A H\(^{+}\) gradient is established by a vacuolar H\(^{+}\)-ATPase and a vacuolar H\(^{+}\)-pyrophosphatase (V-H+-PPase). Ca\(^{2+}\) release could occur in exchange for H\(^{+}\) and is favored by sodium-proton exchange. An aquaporin allows water transport. Other transporters (for example, for Mg, Zn, inorganic phosphate (Pi) and pyrophosphate (PPi), basic amino acids) are probably present. The acidocalcisome is rich in pyrophosphate, short- and long-chain polyphosphate (poly P), magnesium, calcium, sodium and zinc. An exopolyphosphatase (PPX), a pyrophosphatase (PPase) and a polyphosphate kinase (PPK) may also be present. A question mark was added to indicate the lack of biochemical evidence for their presence. Adapted from Moreno et al., 2007b, with permission.

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**Fig. 5.** Acidocalcisomes in *Plasmodium falciparum* merozoites. Transmission electron microscopic image of whole unstained and unfixed merozoites showing numerous acidocalcisomes spread throughout the cells. Scale bar: 0.5 \(\mu\)m. From Ruiz et al. (2004), with permission.
activity control, in regulation of enzyme activities, and as a component of channels and pumps (Kornberg, 1999; Kulaev and Kulakovskaya, 2000). Most of these studies have been done with bacteria, and little is known about the functions of poly P in eukaryotes. The enzymes involved in poly P metabolism are the polyphosphate kinase (synthesis) and the endo- and exo-polyphosphatases (degradation) (Kornberg, 1999; Kulaev and Kulakovskaya, 2000). Genes encoding an exopolyphosphatase (Wurst et al., 1995) and an endopolyphosphatase (Sethuraman et al., 2001) from Saccharomyces cerevisiae, together with a gene encoding an acidocalcisomal exopolyphosphatase from Leishmania major (Rodrigues et al., 2002b) and an exopolyphosphatase from Trypanosoma brucei (Lemercier et al., 2003) are the only ones from eukaryotic organisms that have been cloned and expressed, while two poly P kinase genes have been found in the genome of D. discoideum (Gomez-Garcia and Kornberg, 2004; Kornberg, 1999). Acidocalcisomes from T. gondii also contain a polyphosphatase activity (Rodrigues et al., 2002a).

Short and long chain poly P levels rapidly decreased upon exposure of tachyzoites to agents that mobilize

Fig. 6. Acidocalcisomes in Eimeria acervulina. (A) Transmission electron microscopy of sporozoites of Eimeria acervulina, showing the fine structure of acidocalcisomes (arrows). Acidocalcisomes partially (white arrow) of totally (black arrow) filled by the electron dense content, mainly present in the form of polyphosphate-bound cations (not shown), are observed. RB, refractile body, N, Nucleus. Scale bar, 500 nm. (B) Acridine orange of a sporozoite of E. acervulina. Several acidic organelles (stained in orange) are observed in the cytoplasm. Note binding of acridine orange to the nucleus and the refractile body (green). Scale bar: 3 μm. (C) and (D) Immunofluorescence of E. acervulina using antibodies raised against a conserved sequence of Arabidopsis thaliana V-H^+PPase. Staining of small intracellular structures (presumably the acidocalcisomes) can be seen in the anterior region of the parasite (D). (C) Differential interferential contrast microscopy corresponding to the fluorescence image shown in (D). Scale bar: 3 μm.
Concentration induced by bafilomycin A1, nigericin (a Ca2+ such as calcium ionophores (ionomycin), alkalinizing agents (NH4Cl) or inhibitors of the V-H+-ATPase (bafilomycin A1) (Rodrigues et al., 2002a). This would be compatible with a role for poly P in the adaptation of the parasites to environmental stress.

4. Acidocalcisomes in malaria parasites

The presence of calcium-containing acidic compartments similar to acidocalcisomes in different species of Plasmodium spp. is well established (Garcia et al., 1998; Marchesini et al., 2000; Alleva and Kirk, 2001). A significant amount of Ca2+ is stored in these acidic compartments, as indicated by the increase in intracellular Ca2+ concentration induced by bafilomycin A1, nigericin (a K+ /H+ exchanger), monensin (a Na+/H+ exchanger), aminomethylenediphosphonate (AMDP; a specific inhibitor or the vacuolar H+-pyrophosphatase), or the weak base NH4Cl, in the nominal absence of extracellular Ca2+. This assumption is supported by the effect of the ionophore ionomycin, which becomes effective only after alkalinization of acidic compartment by addition of bafilomycin A1, AMDP, nigericin, monensin, or NH4Cl (Marchesini et al., 2000).

Acidocalcisomes of similar fine structure and chemical composition to those of other unicellular eukaryotes have been found, especially in merozoites of P. falciparum (Ruiz et al., 2004) (Fig. 5). In addition, large amounts of short and long chain poly P are detected in different blood stages of P. falciparum. Concentrations for short-chain poly P are 10 times higher than those of long chain poly P. Both short and long chain poly P increase during differentiation of the blood stages, reaching maximal concentrations in the trophozoite stages and then decreasing in the schizonts (Ruiz et al., 2004). Incubation of these cells with chloroquine, which has an alkalinizing effect, was shown to decrease the poly P content of their acidocalcisomes (Ruiz et al., 2004).

Plasmodium falciparum merozoites show several acidocalcisomes per cell. The acidocalcisomes have a diameter of about 50 ± 11 nm, and some of them have an irregular form. X-ray microanalysis of these acidocalcisomes showed peaks of phosphorus, calcium, carbon and oxygen (Ruiz et al., 2004). While some attempts to identify acidocalcisomes in intact forms of other P. falciparum stages were unsuccessful due to the high electron-density of the parasites (Ruiz et al., 2004), a study using soft X-ray microscopy reported the presence of "dense spheres" of similar size and shape in the cytoplasm of trophozoites (Magowan et al., 1997) that are morphologically identical to acidocalcisomes. In addition, another recent study revealed the presence of round organelles rich in phosphorus in trophozoites of P. falciparum (Rohrbach et al., 2005).

Taken together, these results demonstrate that, though with minor morphological differences, acidocalcisomes of similar characteristics to those present in other unicellular eukaryotes are present in P. falciparum (Ruiz et al., 2004).

5. Acidocalcisomes in other Apicomplexan parasites

Little is known about the presence of acidocalcisomes in other Apicomplexan parasites. The V-H+-PPase, a marker for acidocalcisomes, has not been found in the genome of Cryptosporidium parvum for which also no morphological evidence of acidocalcisomes has been presented. However, other Apicomplexan parasites, such as Sarcosystis neurona, Eimeria tenella and Eimeria acervulina (Miranda, K., unpublished) have acidic organelles that morphologically and physiologically resemble the acidocalcisomes of T. gondii (Fig. 6). The apparently different staining observed after acridine orange or antibody labeling in Fig. 6 could be due to the labeling of the nucleus and refractile body with acridine orange which makes difficult the visualization of acidocalcisomes present in different focal planes. This does not occur when antibodies against the V-H+-PPase are used since they specifically label the acidocalcisomes.

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