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Minireview

Acidocalcisomes in Apicomplexan parasites

Kildare Miranda [a,b], Wanderley de Souza [b], Helmut Plattner [c], Joachim Hentschel [c], Urara Kawazoe [d], Jianmin Fang [a], Silvia N.J. Moreno [a,*]

[a] Center for Tropical and Emerging Global Diseases and Department of Cellular Biology, 350 Paul D. Coverdell Center, University of Georgia, Athens, GA 30602, USA
[b] Laboratório de Ultraestrutura Celular Hertha Meyer, IBCCF, CCS, UFRJ, Rio de Janeiro, RJ, Brazil
[c] Fachbereich Biology, Universität Konstanz, 78457 Konstanz, Germany
[d] Departamento de Parasitologia, IB, UNICAMP, Campinas, SP, Brazil

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Abstract

Acidocalcisomes are acidic calcium stores found in diverse organisms, being conserved from bacteria to man. They posses 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 Ca^{2+}/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.

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 Ca^{2+}, can lead to cell death (Berridge et al., 2000). In addition, Ca^{2+} 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 Calero, 1966) when they were named metachromatic or

* Corresponding author. Fax: +1 706 583 0181.
E-mail address: smoreno@cb.uga.edu (S.N.J. Moreno).

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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 Acridine 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-

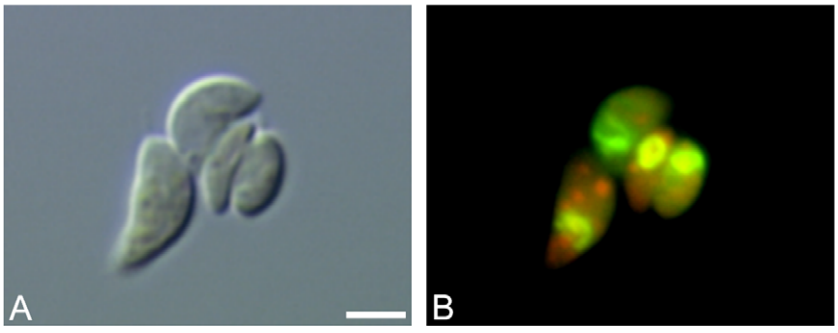

Fig. 1. Fluorescence detection of acidic compartments in isolated tachyzoites of *Toxoplasma gondii*. (A and B): Cells were incubated in the presence of 25 μg/ml Acridine Orange in Dulbecco's phosphate buffer saline (PBS) for 10 min, washed twice, mounted on a microscope slide and observed in a Zeiss Axioplan fluorescence microscope equipped with a 488 nm excitation filter set. Emission signal (above 500 nm) was detected with a Hamamatzu digital CCD camera (Model C5810) and an image analysis system attached to the microscope. (A) Differential interferential contrast image corresponding to the fluorescence image shown in (B). Scale bar for (A) and (B): 3 μm.