Available online at www.sciencedirect.com
SCIENCE DIRECT®
Molecular & Biochemical Parasitology 140 (2005) 175–182
MOLECULAR & BIOCHEMICAL PARASITOLOGY

A proton pumping pyrophosphatase in acidocalcisomes of *Herpetomonas* sp.

Lia Carolina A. Soares Medeiros [a], Bernardo Luis Moraes Moreira [a], Kildare Miranda [b, c], Wanderley de Souza [b], Helmut Plattner [d], Joachim Hentschel [d], Hector Barrabin [a, *]

[a] *Departmento de Bioquímica Médica, ICB-CCS Universidade Federal do Rio de Janeiro, UFRJ Ilha do Fundão, 21941-590 Rio de Janeiro, Brazil*
[b] *Laboratório de Ultraestrutura Celular Hertha Meyer, Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do, Rio de Janeiro, Brazil*
[c] *Laboratório de Biologia Celular e Tecidual, Centro de Biociências e Biotecnologia, Universidade Estadual do Norte Fluminense Darcy Ribeiro, Brazil*
[d] *Lehrstuhl f Zellbiologie und Ultrastrukturforschung, Fachbereich Biology, Universität Konstanz, Konstanz, Germany*

Received 11 February 2004; received in revised form 27 December 2004; accepted 27 December 2004
Available online 19 January 2005

Abstract

Acidocalcisomes are acidic calcium storage organelles found in several microorganisms. They are characterized by their acidic nature, high electron density, high content of polyphosphates and several cations. Electron microscopy contrast tuned images of *Herpetomonas* sp. showed the presence of several electron dense organelles ranging from 100 to 300 nm in size. In addition, X-ray element mapping associated with energy-filtering transmission electron microscopy showed that most of the cations, namely Na, Mg, P, K, Fe and Zn, are located in their matrix. Using acridine orange as an indicator dye, a pyrophosphate-driven H^+ uptake was measured in cells permeabilized by digitonin. This uptake has an optimal pH of 6.5–6.7 and was inhibited by sodium fluoride (NaF) and imidodiphosphate (IDP), two H^+-pyrophosphatase inhibitors. H^+ uptake was not promoted by ATP. Addition of 50 μM Ca^{2+} induced the release of H^+, suggesting the presence of a Ca^{2+}/H^+ countertransport system in the membranes of the acidic compartments. Na^+ was unable to release protons from the organelles. The pyrophosphate-dependent H^+ uptake was dependent of ion K^+ and inhibited by Na^+ *Herpetomonas* sp. immunolabeled with monoclonal antibodies raised against a *Trypanosoma cruzi* V-H^+-pyrophosphatase shows intense fluorescence in cytoplasmatic organelles of size and distribution similar to the electron-dense vacuoles.

Together, these results suggest that the electron dense organelles found in *Herpetomonas* sp. are homologous to the acidocalcisomes described in other trypanosomatids. They possess a vacuolar H^+-pyrophosphatase and a Ca^{2+}/H^+ antiport. However, in contrast to the other trypanosomatids so far studied, we were not able to measure any ATP promoted H^+ transport in the acidocalcisomes of this parasite.

Keywords: Trypanosomatids; H^+-PPase; Herpetomonas; Ca^{2+}; Acidocalcisomes

1. Introduction

The flagellated trypanosomatids of the genus *Phytomonas* and some *Herpetomonas* are parasites of plants. In some cases they parasitize plants without apparent pathogenicity but they can also cause diseases of economic significance in plantations of coconut, oil palm, cassava and coffee [1–4]. These trypanosomatids have also been detected in various edible fruit, such as guavas, pomegranates, peaches and tangerines and in their insect vectors [4]. The parasites live mostly in the xylem and phloem of the infected plants and are transmitted through the bite of phytophagous insects [1–4]. In the biological cycle of these pathogens, several plant-sucking insects act as intermediate hosts and the plant acts as the main host [5]. These parasites have ultrastructural features typical of the family Trypanosomatidae containing kinetoplast,

Abbreviations: EDX, energy dispersive X-ray microanalysis; NEM, *N*-ethylmaleimide; PP_i, pyrophosphate; polyP, polyphosphates; V-H^+-PPase, vacuolar-proton-pyrophosphatase; IDP, imidodiphosphate; PBS, phosphate-buffered saline; AO, acridine orange

* Corresponding author. Tel.: +55 21 2590 4548; fax: +55 21 2270 8647.
E-mail address: barrabin@bioqmed.ufrj.br (H. Barrabin).

0166-6851/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.molbiopara.2004.12.012

glycosomes, endoplasmic reticulum and one single tubular mitochondrion [4,6,7].

In the last few years a singular intracellular acidic compartment named acidocalcisome was characterized in numerous organisms. These were first described in *Trypanosoma brucei* [8] and *Trypanosoma cruzi* [9], then in *Leishmania amazonensis* [10], *Leishmania donovani* [11], *Plasmodium berghei* [12], *Plasmodium falciparum* [13], *Toxoplasma gondii* [14] and in organisms such as *Chlamydomonas reinhardtii* [15], *Dictyostelium discoideum* [16] and more recently in the bacterium *Agrobacterium tumefaciens* [17]. These acidic organelles are electron dense, posses a surrounding membrane, have variable size, ranging from 200 ± S.E. 90 nm of diameter, and contain very high amounts of Mg, Ca, Na, Zn and short and long chain polyphosphates and low amounts of Cl, K and sulfur [for reviews see 18–21].

Kinetic studies have shown that acidocalcisomes maintain a low internal pH due to the presence of a V-H^+-ATPase [22–24] and a V-H^+-PPase [8,11,13,15,25–27] which pump H^+ into the lumen of the organelle. They also posses a Ca^{2+}–H^+ translocating ATPase, a Ca^{2+}–H^+ exchanger and a Na^+–H^+ exchanger which permit a complex regulation of these ions by the cell It was proposed that acidocalsisomes are involved in mechanisms of Ca^{2+} signaling, osmoregulation, pH homeostasis and energy storage [16,28].

The study of the mechanisms by which a plant parasite (*Herpetomonas* sp.) regulates intracellular H^+ and Ca^{2+} distribution, to maintain cell viability, could pro-

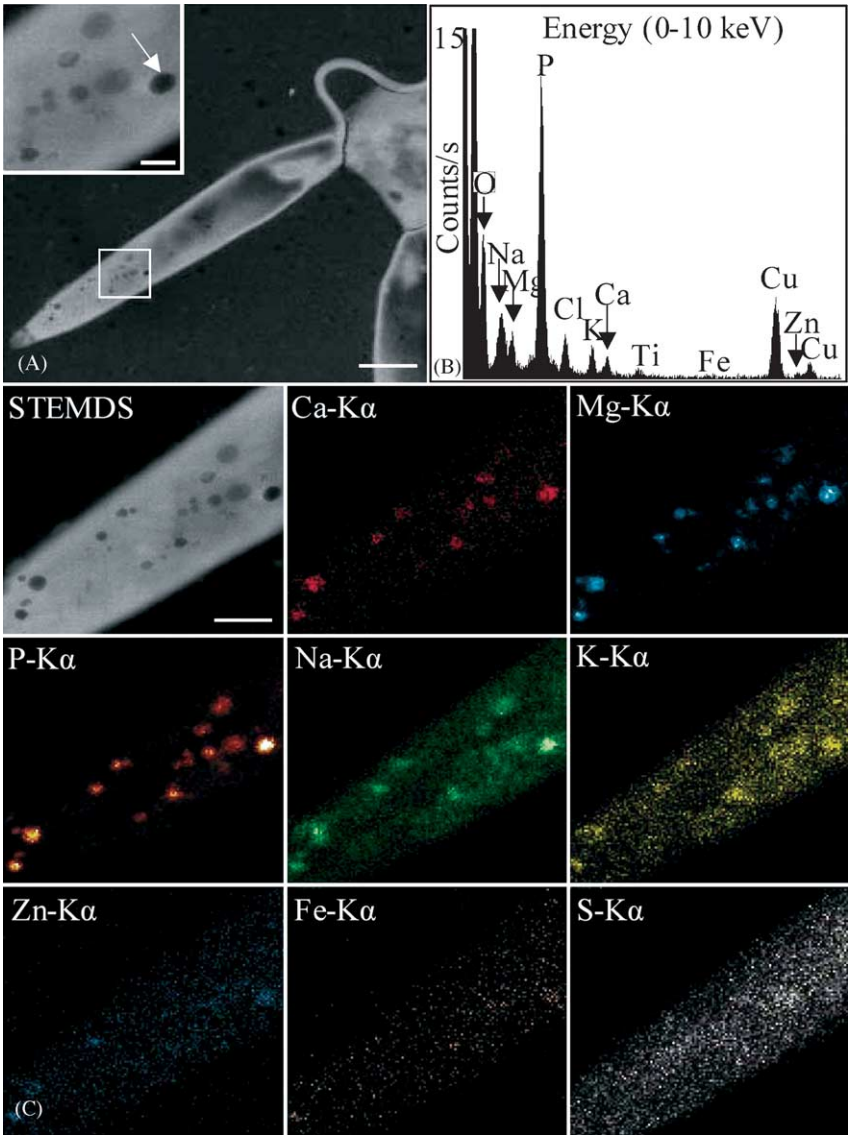

Fig. 1. Presence of acidocalcisomes in promastigotes of *Herpetomonas* sp. (A) Electron spectroscopic imaging of whole unfixed *Herpetomonas* sp. promastigote (Δ*E* between 60 and 80 eV). (B) corresponding X-ray spectrum of the acidocalcisome pointed out in (A). Copper peaks in the spectrum came from the support grid and titanium peaks from the specimen holder. Carbon and chlorine signals were similar in the acidocalcisomes and control regions (cytoplasm). (C) Electron spectroscopic image of a portion of a whole promastigote (Δ*E* between 60 and 80 eV). Elemental images of the cell displayed in (C) corresponding to: calcium; magnesium; phosphorus; sodium; potassium; zinc; iron; and sulfur. Scale bars: (A) 3.0 μm (inset 400 nm), (C) 800 nm.