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The hydrogenosome peripheral vesicle: Similarities with the endoplasmic reticulum

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Abstract

The hydrogenosome, an organelle that produces molecular hydrogen and ATP from the oxidation of pyruvate or malate under anaerobic conditions, presents some characteristics common to mitochondria. The hydrogenosome of *Tritrichomonas foetus*, a cattle parasite, is a spherical organelle that presents a peripheral vesicle the origin and behavior of which is poorly known. In this article it is reported an ultrastructural and microanalytical study using energy dispersive X-ray analysis, 3D reconstruction and cytochemistry of the hydrogenosome peripheral vesicle and then compare the results with the endoplasmic reticulum and the nuclear envelope of *T. foetus*. Similarities between the hydrogenosome peripheral vesicle and the ER are presented. This study included: (1) the detection of ER enzymes by cytochemistry, such as glucose-6-phosphatase, IDPase, acid phosphatase and Ca^{2+}-ATPase; (2) elemental composition by X-ray microanalysis and the mapping of calcium, phosphorus and oxygen in both ER and hydrogenosome peripheral vesicle; (3) freeze-fracture; (4) TEM of routine and cryofixed cells by high-pressure freezing and freeze-substitution; (5) 3D reconstruction, (6) monoclonal antibody anti-trichomonads ER; and (6) other cytochemical techniques that detects ER, such as the ZIO and lectins. We found a similar composition of the tested enzymes and other elements present in the ER when compared with the hydrogenosome's peripheral vesicle. It was concluded that, like mitochondria, hydrogenosome presents relationships with the ER, especially the peripheral vesicle.

Keywords: Hydrogenosome; Peripheral vesicle; *Tritrichomonas foetus*; X-ray microanalysis; Cytochemistry

1. Introduction

Tritrichomonas foetus is a flagellated parasitic protist that inhabits the urogenital tract of cattle and often results in reproductive failure, and is a cause of significant economic losses throughout the cattle-raising areas of the world. Recently, this organism has been subject to extensive investigation due to the presence of hydrogenosomes, an anaerobic energy producing organelle that raises several questions concerning mitochondria evolution and the origin of the eukaryotes. The hydrogenosome produces molecular hydrogen and ATP by oxidizing pyruvate or malate under anaerobic conditions (Müller, 1993). The organelle is present in some unicellular organisms that inhabit environments with low levels of oxygen. Examples include protists such as trichomonads as well as free-living ciliates and rumen fungi. Even though the organelle morphology may vary, some hydrogenosomes possess a peripheral vesicle that is a distinct compartment within the organelle (Díaz and De Souza, 1997; Benchimol et al., 1996a).

The origin of the hydrogenosome has been the subject of intense discussion and comparison with mitochondria, since it is enveloped by two membranes, (Benchimol and De Souza, 1983), divides autonomously by fission (Benchimol et al., 1996b), imports proteins post-translationally (Johnson et al., 1993), produces ATP (Lindmark and Müller, 1973), and presents cardiolipin (Rosa et al., 2006). However, it differs from mitochondria in that it seems to lack a genome, with a possible exception of hydrogenosomes from *Nyctotherus ovalis* (Akhmanova et al., 1998) they lack a respiratory chain, cytochromes, the F_0–F_1 ATPase, the tricarboxylic acid cycle

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and oxidative phosphorylation (Müller, 1993; Clemens and Johnson, 2000).

In many cell types, mitochondria are located very close to the cisternae of endoplasmic reticulum, and to the nuclear envelope (Franke and Kartenbeck, 1971; Bracker and Grove, 1971). A similar proximity was previously observed in hydrogenosomes (Benchimol, 1999). This structural relationship has brought a number of speculations on how ER membranes, especially the nuclear envelope, could take part in mitochondrial, and here, in hydrogenosome formation.

Hydrogenosome is a spherical or slightly elongated structure (when in process of division), presenting 0.3–0.5 μm in diameter, usually associated with cytoskeletal structures such as the axostyle and costa and in trichomonads contains a peripheral, flattened, membrane-bounded vesicle, which contains high levels of Mg^{++}, Ca^{++} and P and possibly functions in intracellular calcium regulation (Benchimol and De Souza, 1983; Chapman et al., 1985; Humphreys et al., 1994). The origin and function of this peripheral hydrogenosome vesicle has been the subject of previous studies (Benchimol, 2000; Díaz and De Souza, 1997), and is still under debate. Thus, it was decided to investigate more deeply the relationship between the endoplasmic reticulum and the hydrogenosome, using different methods. We show evidence of a striking similarity between the endoplasmic reticulum and the hydrogenosome peripheral flat vesicle in *T. foetus*.

2. Materials and methods

2.1. Cell culture

The K strain of *T. foetus* was isolated by Dr. H. Guida (Embrapa, Rio de Janeiro, Brazil) from the urogenital tract of a bull from the state of Rio de Janeiro, Brazil, and has been maintained in TYM Diamond's medium (Diamond, 1957). The cells were cultivated for 24 h at 36.5 °C, which corresponds to the end of the logarithmic growth phase.

2.2. Transmission electron microscopy

2.2.1. Routine preparation

Cells were washed three times in PBS at 37 °C, and fixed overnight at room temperature in 2.5% glutaraldehyde in 0.1 M cacodylate buffer. Afterwards, the cells were washed in PBS and post-fixed for 15 min in 1% OsO_4 in 0.1 M cacodylate buffer containing 5 mM $CaCl_2$ and 0.8% of potassium ferricyanide. The cells were dehydrated in acetone and embedded in Epon. Ultra-thin sections were harvested on 300 mesh copper grids, stained in 5% uranyl acetate and 1% lead citrate and then observed in a JEOL 1210 transmission electron microscope. Alternatively, cells were collected by centrifugation, washed with PBS, pH 7.2 and fixed for 2 h at room temperature in 2.5% glutaraldehyde in 0.05 M Na-cacodylate buffer, pH 7.4, to which 5 mM $CaCl_2$ was added (Oschman and Wall, 1972). After fixation, the cells were washed in 0.1 M cacodylate buffer plus 5 mM $CaCl_2$ and post-fixed for 1 h in 1% OsO_4 (w/v) in 0.1 M cacodylate buffer plus 5 mM $CaCl_2$ and 0.8% (w/v) of potassium ferricyanide. Afterwards, the cells were washed, stained *en bloc* for 2 h with 2% (w/v) aqueous uranyl acetate, dehydrated in acetone and embedded in Epon. Thin sections were then further stained with uranyl acetate and lead citrate prior to observation in JEOL 1210 transmission electron microscopy.

2.2.1.1. Physical fixation. Living cells were initially incubated with L-hexadecene to dissipate the high-pressure equally according to Moor (1987). Next, the pellet was mounted in a sandwich aluminum holder and frozen in a Balzers HPM 010 machine under a pressure of 2100 bar for 20 ms (Studer et al., 1989). After freezing, the sample was transferred to liquid nitrogen and submitted to freeze-substitution (Kellenberger, 1991) on a Balzers (FSU010) machine containing pure acetone with 2% osmium tetroxide (Van Harreveld and Crowell, 1964). The temperature rise was then programmed to be gradual as follows: −90 °C (8 h), −60 °C (8 h), −20 °C (8 h), followed by 1 h at room temperature. Afterwards, the specimens were embedded in epoxy resin (Poly/Bed 812) and routine processed for transmission electron microscopy.

2.2.2. X-ray microanalysis

Hundred nanometers of thick unstained sections were collected on nylon grids and observed in a JEOL 1200 EX electron microscope operating at 120 kV, using a beryllium support. X-rays were collected within a range of 200–300 s on a 0–10 keV energy range using a Noran/Voyager III system. Analyses of selected areas were carried out using a small spot diameter (electron beam spot size of about 50 nm).

2.2.3. 3D reconstruction

Ribbons of 20 golden serial thin sections were collected on formvar-coated 0.5 mm × 2.0 mm slot grids and stained in aqueous uranyl acetate for 20 min and lead citrate for 5 min. Serial micrographs were taken and printed at a final magnification of 40,000×. The hydrogenosomes, lysosomes, endoplasmic reticulum, flagella and other cell components were outlined with distinct colors and each plane was separately traced onto a digitizing table (Numonics, 2205) and then into a 3D reconstruction program—BIGED for IBM PC (Young et al., 1987). The resulting data files consisted of contour outlines representing cross sections of the objects of interest within the volume. These files were mounted on a fixed axis creating a reconstructed image that could be rotated along the *X*-, *Y*-, and *Z*-axis. The files were then transferred to a Silicon Graphics workstation and the surfaces between planes were generated using the software package SYNU (Synthetic Universe) (Hessler et al., 1992). Selected images were photographed on a Polaroid digital palette film recorder system.