Journal of Structural Biology 171 (2010) 11–17

Contents lists available at ScienceDirect

Journal of Structural Biology

journal homepage: www.elsevier.com/locate/yjsbi

Dry and survive: Morphological changes during anhydrobiosis in a bdelloid rotifer

Roberto Marotta [a,*], Francesca Leasi [a], Andrea Uggetti [a], Claudia Ricci [b], Giulio Melone [a]

[a] Dipartimento di Biologia, Università degli Studi di Milano, Via Celoria 26, 20133 Milano, Italy
[b] DIPSA, Università degli Studi di Milano, Via Celoria 2, 20133 Milano, Italy

ARTICLE INFO

Article history:
Received 2 December 2009
Received in revised form 21 March 2010
Accepted 6 April 2010
Available online 9 April 2010

Keywords:
Extreme adaptation
Bdelloids
Anhydrobiosis
Cell junctions
Microscopy

ABSTRACT

Bdelloid rotifers are aquatic microinvertebrates able to cope with the loss of environmental water by entering dormancy, and are thus capable of living in temporary habitats. When water is evaporating, bdelloids contract into "tuns", silence metabolism and lose water from the body, a condition known as anhydrobiosis. Under controlled conditions, a bdelloid species (*Macrotrachela quadricornifera*) was made anhydrobiotic, and its morphology was studied by light, confocal and electron microscopy. A compact anatomy characterizes the anhydrobiotic rotifer, resulting in a considerable reduction of its body volume: the internal organs, precisely packed together, occupy the body cavity almost completely and the lumen of hollow organs disappears. Remarkable ultrastructural changes characterize the anhydrobiotic condition. The mitochondria are wholly surrounded by a ring of electron-dense particles, and the epidermal pores, open in the hydrated specimens, become gradually closed by structures similar to epithelial junctions. The cilia are densely packed: microtubules are still identifiable, but the axonemal organization appears disrupted. This is the first extensive comparative study on the morphological changes associated with the anhydrobiosis process in a rotifer, providing the basis for an improved understanding of the processes involved in this extreme adaptation.

1. Introduction

Anhydrobiosis, first described by Van Leeuwenhoek (1702) in a bdelloid rotifer, is the state of suspended animation which certain organisms enter in response to desiccation. Anhydrobiotic organisms, found in many taxa ranging from unicellular organisms to higher invertebrates and plants, are able to survive almost complete dehydration (Clegg, 2001; Watanabe, 2006).

Within metazoans, only species of Nematoda, Tardigrada and Rotifera can survive desiccation at any age (Crowe and Madin, 1975; Wright et al., 1992; Ricci, 1998; Wright, 2001). Among rotifers, anhydrobiosis has been proposed to be an apomorphic feature for bdelloids, subsequently lost by some species (Ricci, 1998). During anhydrobiosis, bdelloids contract in a tun-shaped morphology, reducing the surface and controlling the evaporation rate. When dry, bdelloids show remarkable reduction of volume and size (Ricci et al., 2008).

The biochemical and genetic mechanisms involved in bdelloid anhydrobiosis are still unclear (Tunnacliffe and Wise, 2007). Indeed bdelloid rotifers, in contrast to most anhydrobiotic organisms which synthesize trehalose and other non-reducing disaccharides as osmoprotectant molecules, lack trehalose and trehalose synthase genes (Lapinski and Tunnacliffe, 2003; Caprioli et al., 2004; Tunnacliffe et al., 2005; McGee, 2006). Instead bdelloids produce hydrophilic LEA (Late Embryogenesis Abundant) proteins, implicated in desiccation tolerance in plants, nematodes and other micro-organisms (Tunnacliffe and Wise, 2007; Tunnacliffe et al., 2005; Wise and Tunnacliffe, 2004; Pouchkina-Stantcheva et al., 2007).

Little is known about the morphological and ultrastructural changes accompanying anhydrobiosis in rotifers and other invertebrates, since the loss of internal water characterizing this process interferes with fixative activity during sample preparation (see Section 2). So far, fine morphology of anhydrobiotic rotifers has been briefly documented in two bdelloid species: *Philodina roseola* (Dickson and Mercer, 1967) and *Habrotrocha rosa* (Schramm and Becker, 1987).

This is the first extensive comparative study on the morphological changes associated with the anhydrobiosis process in another bdelloid species, *Macrotrachela quadricornifera*. In describing the anatomy and ultrastructure of anhydrobiotic *M. quadricornifera*, we intend to provide the structural basis for the comprehension of the mechanisms involved in this extreme adaptation.

2. Materials and methods

The species used for this study is *M. quadricornifera*, that we culture since years. Anhydrobiosis was induced to 8-day-old animals following an already established desiccation protocol that in 7 days

* Corresponding author.
E-mail address: roberto.marotta@unimi.it (R. Marotta).

1047-8477/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.jsb.2010.04.003

carries the rotifers from fully hydrated to fully desiccated (Ricci et al., 1987, 2003). For morphological investigation the rotifers were sampled in the course of the desiccation process (at 36, 72, and 76 h). Each experimental group was accompanied by a parallel group of 20 bdelloids, that was kept anhydrobiotic for 7 days and rehydrated to record recovery rate, that was always found higher than 95%.

2.1. Light microscopy (LM) and transmission electron microscopy (TEM)

Relaxed hydrated, contracted hydrated and anhydrobiotic rotifers at different times of the desiccation protocol were processed for both light and electron microscopy. Since anhydrobiotic specimens are difficult to prepare for ultrastructural observation due to the loss of internal water, different fixatives were tested. Best results required fine tuning of the fixative composition and osmolarity. Hydrated specimens were fixed in a 0.1 M cacodylate buffered paraformaldehyde–glutaraldehyde mixture in a saturated solution of picric acid (SPAFG, Ermak and Eakin, 1976) for 2 h. Anhydrobiotic specimens were fixed with 1.5% glutaraldehyde in 0.05 M cacodylate buffer (pH 7.2–7.4) for 2 h. Both hydrated and anhydrobiotic specimens were washed in 0.125 M cacodylate buffer, post-fixed with 1% OsO_4 in 0.075 M cacodylate buffer for 2 h, washed in distilled water, stained *in toto* overnight in 2% aqueous uranyl acetate, dehydrated in a graded ethanol series, and embedded in both SPURR and EPON resin. Thin sections were cut with a Reichert Ultracut E microtome, stained with lead citrate and uranyl acetate, and observed with a JEOL 100SX electron microscope.

2.2. Scanning electron microscopy (SEM)

Hydrated rotifers were fixed with 2% OsO_4 in 0.1 M cacodylate buffer, washed in distilled water, dehydrated in a graded ethanol series, critical point-dried with CO_2, mounted on stubs and sputter coated with gold. Anhydrobiotic bdelloids were fixed with OsO_4 vapor for 2 h, then mounted on stubs, sputter coated with gold, and observed under a LEO 1430 scanning electron microscope at 20 kV.

2.3. Confocal laser scanning microscopy (CLSM)

Fully extended animals were obtained through anesthesia with marcain (about 0.025%) at room temperature. Non-anaesthetized (controls) and anhydrobiotic rotifers were fixed at 4 °C overnight in 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS), pH 7.4. Specimens were then rinsed repeatedly in 0.1 M PBS, dehydrated in methanol at −20 °C to permeabilize cell membranes and permeabilized once more for 2 h in PBT (0.1 M PBS with 0.2% Triton X-100, 0.25% bovine serum albumin, and 0.05% NaN_3). The specimens were stained with 1 μM DAPI, rinsed in 0.1 M PBT, mounted in 3% DABCO and observed with a confocal laser scanning microscope (CLSM, Leica TCS-SP2). In parallel, a few animals were processed as described, but not stained, to check for autofluorescence. Series of optical sections were projected as a fluorescence maximum projection (MPJ) for a whole reconstruction.

3. Results

3.1. Light and scanning electron microscopy

In the relaxed hydrated specimen the mouth opens anteriorly, in a slightly ventral position, between the pedicels of the trochi (Fig. 1a and b). Anterior and dorsal to the mastax, lies the cerebral ganglion (Fig. 1b and c). The trunk is mostly occupied by the large digestive apparatus (gut), flanked by the two vitellaria, large syncytial glands producing yolk (Fig. 1b and c). Stomach and intestine lumens open inside the large mass of their syncytial cytoplasm, to end inside the cloaca (Fig. 1b and c). The foot houses the retractor muscles, and the pedal glands (Fig. 1b). A clearly visible body cavity separates the internal organs from each other and from the body wall (Fig. 1b and c).

In the hydrated contracted specimen the head and the foot are telescopically retracted into the trunk, which assumes a characteristic barrel shape (Fig. 1d). Epidermal folds are present at both extremities (Fig. 1d–f). The relative position of the internal organs is similar to that of the relaxed hydrated specimens. The body cavity is still discernible, but the organs appear more compressed, and partially penetrated into each other (Fig. 1e and f). The trochi, together with cerebral ganglion, mastax and foot are shifted inside the trunk, compressing from both ends the gut and the two vitellaria dorso-laterally (Fig. 1e and f).

Compared to the contracted hydrated specimens, in anhydrobiotic rotifers the body is flattened dorso-ventrally, and assumes a characteristic dome-shaped morphology (Fig. 1g). Several epidermal folds are present at both ends (Fig. 1h and i). The internal anatomy appears less resolved than in the hydrated specimens, probably as a consequence of changes of cell biochemical composition. The internal organs are precisely packed together, completely filling the body cavity, and the cavities of the hollow organs appear greatly reduced (Fig. 1h and i). The gut, together with the vitellaria, shifts ventrally pushing trochi, brain and mastax anteriorly, and the foot to a more posterior position (Fig. 1h and i). The mastax and the retrocerebral gland appear slightly rotated with respect to the anterior posterior axis (Fig. 1i).

3.2. Confocal microscopy

Hydrated and dried *M. quadricornifera* specimens possess about 650 nuclei well discernible after DAPI staining (Fig. 2). As many as 200 nuclei form the cerebral ganglion; and both mastax and pedal ganglion have about 100 nuclei each (Fig. 2a). Of the 15–25 nuclei belonging to the paired gonads, eight larger polyploid nuclei (9–11 μm in diameter; Fig. 2a) correspond to the vitellarium and about 7–12 smaller nuclei (2–3 μm in diameter; Fig. 2a) correspond to the germarium.

In both relaxed and contracted hydrated specimens, the internal organs are arranged symmetrically along the main longitudinal axis (Fig. 2a and b). In contrast, in anhydrobiotic rotifers the bilateral symmetry is partially lost: brain and mastax appear shifted laterally to the main longitudinal axis (cf. Fig. 2b and c). In the hydrated specimens the somatic nuclei are largely euchromatic (Fig. 2d and g). DAPI staining of the eight large nuclei of the vitellarium, characterized by the presence of a large nucleolus, shows a central region lacking DAPI fluorescence, probably corresponding to the nucleolar region (Fig. 2e and h; cf. Akkiprik et al., 2009). In the anhydrobiotic bdelloids, in contrast, the nuclei are more heterochromatic, and the nucleolus inside them is not clearly visible (Fig. 2f and i). In addition, the nuclei of the vitellarium are elongated and thin, and do not show the characteristic central region lacking DAPI fluorescence (Fig. 2f and i).

3.3. Transmission electron microscopy

3.3.1. Epidermis and epidermal pores

In the hydrated *M. quadricornifera* specimens the epidermis contains the intracytoplasmic skeletal lamina (average thickness 0.65 μm), pierced by numerous epidermal pores, and coated by a filamentous glycocalix (Fig. 3a and b). The pores, completely open in hydrated specimens, are cylindrical openings across the skeletal lamina (average diameter 0.16 μm) (Fig. 3a and c). The plasma membrane coating the pore inner surface reaches the epidermal