Comparative Biochemistry and Physiology, Part A 208 (2017) 61–69

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part A

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

Aquaporin in different moult stages of a freshwater decapod crustacean: Expression and participation in muscle hydration control

Kamila Foguesatto [a], Robert T. Boyle [a,b], Monique T. Rovani [a], Carolina A. Freire [c], Marta M. Souza [a,b,*]

[a] Programa de Pós-Graduação em Ciências Fisiológicas, Instituto de Ciências Biológicas, Universidade Federal do Rio Grande – FURG, Brazil
[b] Instituto de Ciências Biológicas, Universidade Federal do Rio Grande – FURG, Brazil
[c] Departamento de Fisiologia, Universidade Federal do Paraná - UFPR, Curitiba, PR, Brazil

ARTICLE INFO

Article history:
Received 19 September 2016
Received in revised form 21 February 2017
Accepted 7 March 2017
Available online 9 March 2017

Keywords:
AQP expression
Hyposmotic
Moulting
Palaemonetes argentinus
Shrimp
Water

ABSTRACT

Crustaceans, during their moult cycle, at the stages of both pre-moult and post-moult, need water uptake. This movement of water creates a challenge for the regulation of cell volume. The cells of freshwater decapods require a high regulatory capacity to deal with hyposmotic stresses, given the need to face dilution of the haemolymph during their moult cycles. This study investigated the variation in the expression of water channels (aquaporins) along the moult cycle of a freshwater palaemonid shrimp, focusing on their role in cell volume regulation. Moults in *Palaemonetes argentinus* have been investigated along three stages of its moult cycle: intermoult, late pre-moult and recent post-moult. For the evaluation of tissue volume regulation, the weight of isolatedmuscle, subjected to isosmotic and hyposmotic salines, was followed for 60 min. The expression of AQP during the different moult stages was evaluated by immunocytochemistry. Muscle from the three moult stages in isosmotic conditions showed the same pattern of tissue volume regulation. When muscle from animals in pre-moult and intermoult were submitted to hyposmotic stress they swell, followed by volume regulation, while in post-moult the regulation is compromised. The difference in volume regulatory control between pre-moult and post-moult may be related to a possible regulation of water channels, as AQP expression was equal at these stages. This study presents novel findings for crustaceans in general, in the demonstration that AQP expression changes during the moult cycle of a decapod crustacean, together with the regulation of cell volume with the participation of AQPs.

1. Introduction

The exoskeleton is a common characteristic in all arthropods, serving as protection from predators and environmental variations. However, its presence generates restrictions upon growth, thus during their development, arthropods must shed this extracellular cuticle from its living tissues, and rapidly expand its new exoskeleton (Chang and Mykles, 2011). This process occurs in a cycle known as ecdysis or moulting (Withers, 1992). The moulting cycle of crustaceans is classically divided into five stages: recent post-moult (A), late post-moult (B), intermoult (C), pre-moult (D) and ecdysis (moulting itself) (Drach, 1939).

In the recent post-moult stage (A) the animal has recently shed the old shell, the new exoskeleton is still soft and flexible, but at this stage the animal needs to expand the new exoskeleton to make room for the growth of new tissues (Chang, 1995; Rao et al., 2008). Therefore, it takes up water, promoting the expansion of the internal volume (Chang, 1995). The absorbed water appears in the haemolymph, which becomes more diluted (Chen et al., 2012; Wilder et al., 2009) and then in tissues, especially in the hepatopancreas, but also in epithelial and muscular tissue (Passano, 1960; Bhat et al., 2012).

The intermoult (C) is the stage of highest osmotic stability (Wang et al., 2003). The pre-moult stage (D) has several substages (D_0, D_1', D_1'', $D1'''$, D_{2-4}) (Drach and Tchernigovtzeff, 1969). D_2 is the last substage of pre-moult in the freshwater shrimp, *Macrobrachium rosenbergii*. In this substage, there is increased osmolality of the haemolymph (500 mOsm/kg H_2O) with respect to stages A, B and C (~430 mOsm/kg H_2O), favoring water influx from the environment into the haemolymph, and generating its dilution in the subsequent stages, D_3 and ecdysis (Cheng et al., 2001; Wilder et al., 2009). Thus the animal body expands, disrupting the exoskeleton during ecdysis, enabling the animal to escape from the old exoskeleton (Rao et al., 2008).

Due to the necessary water movements to/from the animal, or across its cell membranes during the various stages of the moult cycle, variation in water permeability and participation of osmoionic regulatory mechanisms are expected to occur (Chang, 1995). Osmoconformer marine species have been proposed to display a greater capacity to regulate cell volume (water tissue content) when facing osmotic challenges,

---

* Corresponding author at: Instituto de Ciências Biológicas, Universidade Federal do Rio Grande - FURG, Rio Grande, RS, Brazil.
E-mail address: martasouza@furg.br (M.M. Souza).

http://dx.doi.org/10.1016/j.cbpa.2017.03.003

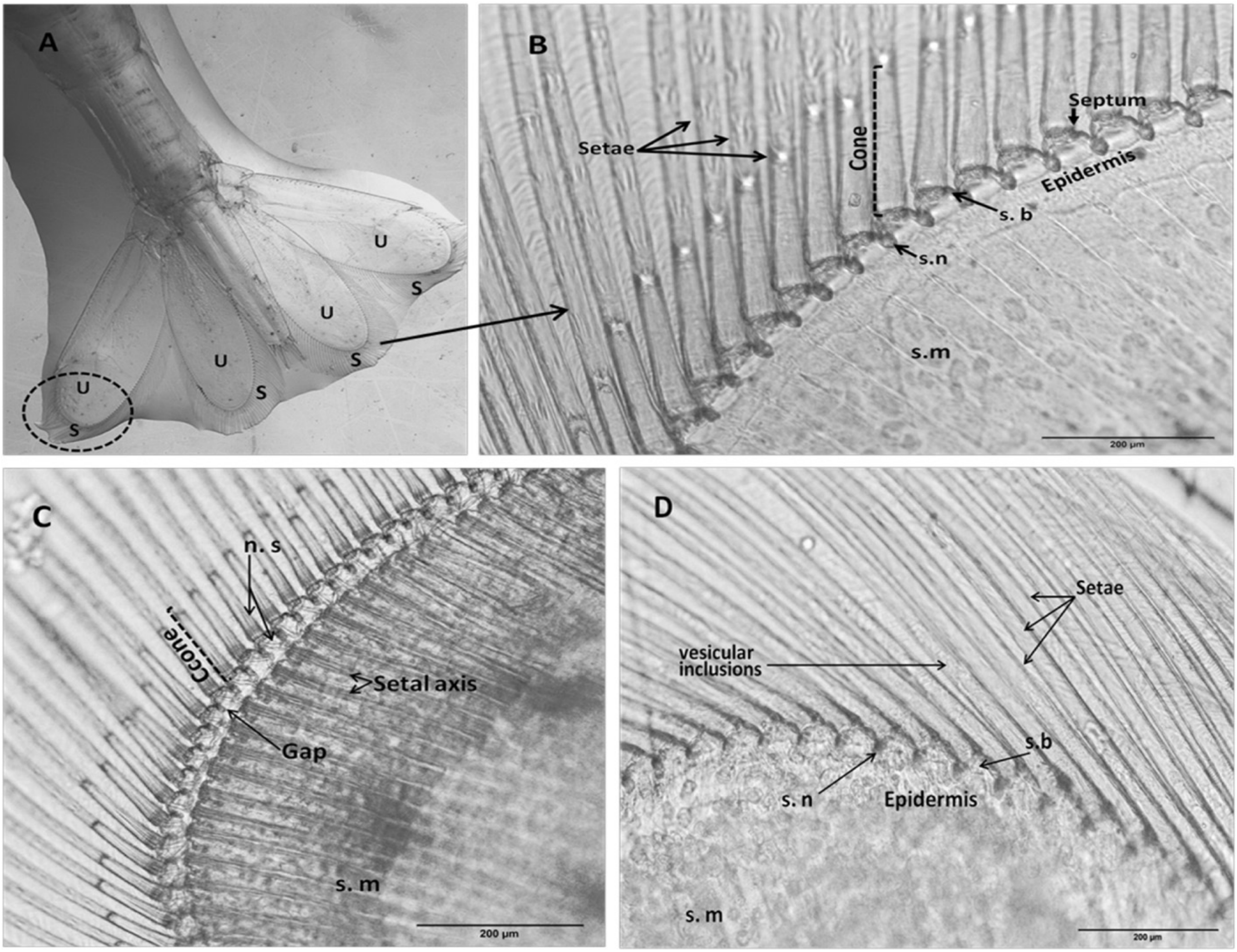

Fig. 1. a- Image of the posterior region of *P. argentinus*, circle indicates the region observed in one of the uropods (U) and setae (S) for determining the moult stages. Photo-micrographs (bars 200 µm) b- intermoult, c- Pre-moult D_2, d- recent post-moult. s.m- setal matrix; s.n- setal node; s.b- setal base; n.s- new setae.

when compared to osmorregulating freshwater species, especially those with long evolution in fresh waters (Freire et al., 2008, 2013; Foster et al., 2010). Studies have shown that the cells of freshwater crustaceans have diminished ability to cope with hyperosmotic shock but have maintained the ability to handle the unavoidable hyposmotic shocks during the moult cycle (Freire et al., 2013). However, intracellular isosmotic volume control during the moult cycle has not been thoroughly investigated; it is addressed in the present study.

The water transporter family of proteins (aquaporin - AQP) has been strongly implicated as critical in regulating cell volume in a range of organisms, since they are responsible for significant water flux across cell membranes (Borgnia et al., 1999; Conner et al., 2012; Masaro et al., 2012). Within the aquaporin family, AQP0, AQP1, AQP2, AQP4, AQP5, AQP6, and AQP8 are selective for water transport. AQP1 is expressed in mammalian red blood cells, brain, lungs and kidneys (mammals) with proven importance in water reabsorption and secretion of fluids (Gomes et al., 2009). In fish, AQP1 expression has been correlated to changes in ambient water salinity (Tipsmark et al., 2010; An et al., 2010). However, studies investigating the presence and participation of AQPs in aquatic invertebrates are few and recent (Chung et al., 2012; Boyle et al., 2013).

Given that dilution of the haemolymph occurs during the moult cycle, with resulting swelling of cells (Wilder et al., 2009; Kirschner, 2004), this study aimed at investigating the putative role of the expression of water transport proteins (AQP) on tissue/volume regulation of a freshwater crustacean during its moult cycle, comparing several stages of the moult cycle: intermoult [C], late pre-moult [D_2] and recent post-moult [A].

2. Materials and methods

2.1. Animals

The freshwater shrimp *Palaemonetes argentinus* Nobili 1901 (Palaemonidae, Palaemoninae) was used for this study. As other freshwater members of the family, it is a strong hyper-osmoregulator species, maintaining the osmolality of its haemolymph at ~400 mOsm/kg H_2O (Charmantier and Anger, 1999; Ashelby et al., 2012). Shrimps were collected on the banks of the São Gonçalo canal in the town of Vila Santa Isabel, Rio Grande/RS-Brazil (32°07′13.8″S, 52°35′38.9″N), and were held at the aquatic vivarium of the Federal University of Rio Grande in ~40-liter tanks of constantly aerated freshwater and gravel-filled substrate. Animals were acclimated at room temperature (~23 °C), with a 12/12 h light/dark photoperiod, being fed once a day with commercial fish food (Alcon Basic®).

2.2. Setogenesis

Setogenesis was used to determine the moult stage of the shrimps. This method consists of the classification of the moult stage according