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Glucose homeostasis in the euryhaline crab *Cytograpsus angulatus*: Effects of the salinity in the amylase, maltase and sucrase activities in the hepatopancreas and in the carbohydrate reserves in different tissues

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ABSTRACT

We studied the existence, biochemical characteristics and response to different environmental salinities of amylase, maltase and sucrase activity in the intertidal euryhaline crab *Cyrtograpsus angulatus* (Dana, 1852) along with the response to distinct salinities of glycogen and free glucose content in storage organs. Amylase, maltase and sucrase activities were kept over a broad range of pH and temperature and exhibited Michaelis–Menten kinetics. Zymography showed the existence of two amylase forms in crabs exposed to 35 (osmoconformation) and low (6–10 psu; hyper-regulation) or high (40 psu) (hypo-regulation) salinities. Carbohydrases activity in the hepatopancreas and glycemia were not affected in crab exposed to different environmental salinities. In 6 and 40 psu, the glycogen content in anterior gills was lower than in 35 psu. In 6, 10 and 40 psu, glycogen concentration in hepatopancreas, muscle and posterior gills were similar to that in 35 psu. Free glucose concentration in chela muscle was higher in 6 and 40 psu than in 35 psu. The existence and biochemical characteristics of carbohydrases activity and the adjustments in concentration of glycogen in anterior gills and free glucose in chela muscle suggests the ability to perform complete hydrolysis of glycogenic substrates and to keep glucose homeostasis in relation to acclimation to different salinity conditions.

1. Introduction

In decapod crustaceans, glucose homeostasis is fundamental for maintaining organ functions and for compensation to numerous environmental stress factors (Verri et al., 2001; Lorenzon, 2005; Dutra et al., 2008). The carbohydrates digestion such as starch, glycogen, disaccharides as well as the absorption of glucose via the hepatopancreas are principal origin of hemolymphatic glucose (Verri et al., 2001; Obi et al., 2011). The hepatopancreas is multifunctional organ. It is the principal site of digestive enzymes synthesis and where intracellular digestion begins and is accomplished (Zeng et al., 2010; Ribeiro et al., 2014; Wang et al., 2014). The occurrence and level of key carbohydrases in the hepatopancreas has a fundamental role in the metabolism of glycogenic carbohydrates.

α-amylases (α-1,4 glucan-4-gluconohydrolase) are hydrolytic enzymes responsible for the hydrolysis of internal α-D-(1, 4) glycoside bonds of α-glucans (Janeček et al., 2014; Xie et al., 2014; Peng et al., 2015; Tiwari et al., 2015). In all animals, α-amylases have a central physiological importance due to their function in the initial steps of hydrolysis of dietary starch and of dietary and/or storage glycogen (Date et al., 2015; Saborowski, 2015). Amylase activity was detected in the hepatopancreas of various decapod crustaceans and has been found that several forms occur although with a high grade of interspecific variability (Le Moullac et al., 1997; Van Wormhoudt and Sellos, 2003; Johnston and Freeman, 2005; Perera et al., 2008a, 2008b; Coccia et al., 2011; Aragón-Axomulco et al., 2012; Castro et al., 2012; Rodríguez-Viera et al., 2016). However, very little information is available about the occurrence and biochemical characteristics of amylase in the hepatopancreas of intertidal euryhaline crabs (Blandamer and Beechey, 1966; Van Wormhoudt et al., 1995; Asaro et al., 2011, 2017). Maltase, which hydrolyzes α-1,4 glycosidic linkages from non-reducing ends, has a main role in glycogenic carbohydrates digestion by participating in the initial (assisting to α- amylase) and in the final steps (Lin et al., 2012, 2014, 2015; Dhital et al., 2013).The existence of specific

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disaccharidases in the hepatopancreas would also facilitate the potential use of glycogenic disaccharides (i.e. maltase, sucrose) as glucose sources. Reports about existence and biochemical characteristics of maltase and sucrase in the hepatopancreas of intertidal euryhaline crabs are still limited (McClintock et al., 1991; Asaro et al., 2011).

In estuaries and coastal lagoons, intertidal euryhaline crabs must deal with sharp and broad changes in environmental salinity. Biochemical adaptation to environmental salinity implies adjustments in different tissues (Michiels et al., 2013, 2015a; Pinoni et al., 2013, 2017; Larsen et al., 2014). In intertidal euryhaline crabs, simultaneous determinations of diverse parameters in different organs are needed for an integral analysis of the adaptation at biochemical level to different salinities (Romano and Zeng, 2012; Pinoni et al., 2013, 2015).

Cyrtograpsus angulatus (Dana, 1852) is a euryhaline crab common in intertidal areas of southwestern Atlantic coast from Rio de Janeiro (Brazil) to Patagonia (Argentina) and of the Pacific coast in Chile and Perù. *C. angulatus* is one of the dominant crabs in these regions (Boschi, 1964; Spivak, 1997). This crab is a predator-omnivorous-carrion (Spivak, 1997; Botto et al., 2005). The digestive battery at the biochemical level subjacent to its dietary behavior is not totally understood. We have shown the occurrence of different digestive enzymes activities (i.e. endo and ectoproteases and lipase) (Michiels et al., 2013; Michiels, 2015) in the hepatopancreas of *C. angulatus*, but studies on carbohydrase activities (i.e. amylase, maltase and sucrase) are still lacking. In Mar Chiquita coastal lagoon (Argentina) *C. angulatus* is exposed to sharp, recurrent and deeply changes in salinity (from 4 to 40 psu and from time to time reaching lower and higher values) (López Mañanes et al., 2002; Pinoni and López Mañanes, 2004, 2008; Michiels et al., 2013). We showed that biochemical acclimation to salinity implicates multiple and integral responses (López Mañanes et al., 2002; Pinoni and López Mañanes, 2004, 2008; Pinoni, 2009). The distinct regulation of proteolytic and lipase activities in the hepatopancreas suggest that different adjustments in proteins and lipids metabolism are triggered by exposure to high salinity (Michiels et al., 2013). Nothing is known yet about the occurrence of adjustments in components involved in glucose homeostasis at the biochemical level. The aims of this work were to determine i) the occurrence and biochemical characteristics of amylase, maltase and sucrase activities in the hepatopancreas of *C. angulatus* and ii) the effect of low and high salinity on these activities in the hepatopancreas and on the content of carbohydrate reserves in different tissues of this crab.

2. Methods

2.1. Collection and maintenance of animals

Crabs in intermolt (carapace width > 2.5 cm) (Drach and Tchernigovtzeff, 1967) were captured from the mudflat area of Mar Chiquita coastal lagoon (37°32′–37°45′S; 57°19′–57°26′W, Argentina) in autumn. Crabs were transported to the laboratory on the same day of capture in lagoon water. The salinity for all experiments was determined in practical salinity units (psu). The individuals of *C. angulatus* were distributed in 3 groups (4–8 individuals per condition): I) dilute seawater (6–10 psu), II) seawater (35 psu), and III) concentrated seawater (40 psu) for 10 days (Michiels et al., 2013). Dilute and concentrated seawater preparation and the maintenance of crabs in aquaria were done as previously described (Michiels et al., 2015a). The aquaria contained 36 L of water which was continuously aerated and filtered (Atman filter HF-0400). A regime of 12 h light/12 h dark was applied and the temperature was kept at 22 ± 2 °C. The animals were fed with commercial food (43% carbohydrates, 44% proteins, 13% lipids; 0.07 g per individual), three times a week ad libitum and were starved for one or two days before sampling (Michiels et al., 2013). No changes in the feeding behavior and no mortality occurred under the experimental conditions utilized. The regulations and statements of Ethics Committee CICUAL (OCA 1499/12) FCEyN Universidad Nacional de Mar del Plata were followed.

2.2. Sample procedures

Animals were weighed and anesthetized by cold for about 25 min. For glucose and osmolality determination a sample of hemolymph was taken to be used as described below. The hepatopancreas, chelae muscles, anterior (1–5) and posterior (6–8) gills were at once cut out and gently dried on a paper towel and weighed. Wet mass was measured to the nearest 0.01 g. Immediately after weighing, the hepatopancreas was homogenized in Tris/HCl buffer (0.1 M, pH 7.4; 4 mL g tissue^{-1}) (CAT homogenizer 9120, tool T10) and centrifuged at 10,000 × *g* for 15 min at 4 °C. A sample of the homogenate was kept for posterior determination of glycogen content. The supernatant was fractionated and stored at −20 °C for further enzymatic assays. Glycerol (1.3% v v^{-1}) was added to supernatant samples before freezing (Ljungström et al., 1984).The chela muscle and anterior and posterior gills were mixed with homogenizing medium (0.25 M sucrose/0.5 mM EGTA-Tris, pH 7.4) (8 mL or 4 mL g tissue^{-1}, respectively) and homogenized on ice with homogenizer as described for hepatopancreas (chela muscle) or in a motor-driven hand-operated Teflon-glass homogenizer (Potter–Elvehjem, 1700 rpm) (anterior and posterior gills), the homogenates were fractionated and stored at −20 °C until use.

2.3. Hemolymph osmolality

Hemolymph was sampled from the intrabranchial sinus at the base of the cheliped with a syringe flushed with an anticoagulant (sodium citrate buffer, 10% w v^{-1} pH 7.4) and put in an iced centrifuge tube to separate plasma (2,000 × *g* during 3 min at 0 °C) (IEC-Centra 7R). A cryoscopic osmometer (Osmomat 030, Gonotec) was used to determine the osmolality (mOsm kg^{-1}) of the hemolymph and external medium. The values are given as hemolymph osmolality (measurement of solutes concentration) defined as the number of osmoles (Osm) of solutes per kilogram of solvent and as osmoregulatory capacity which is calculated as the difference between the value of osmolality of the hemolymph and that of the medium (Lignot et al., 2000; Charmantier and Anger, 2011). Osmoregulatory capacity is a common parameter to analyze the osmoregulatory performance at a given salinity (Lignot et al., 2000; Charmantier and Anger, 2011).

2.4. Biochemical assays

2.4.1. Amylase activity

Amylase activity in hepatopancreas was measured according to Biesiot and Capuzzo (1990) using starch (15 mg mL^{-1}) as substrate, as we previously detailed (Asaro et al., 2011). Briefly, the sample was incubated for 15 min at 30 °C in the presence of starch in 50 mM phosphate buffer (pH 5.2), 1.5 mL of dinitrosalicylic acid reagent (Miller, 1959) was added for further incubation for 10 min at 100 °C. After cooling, the released maltose was assessed reading absorbance at 540 nm (ZL5000 PLUS, Zeltec). To determine the effect of varying pH, temperature and starch concentration, the activity was assayed at varying pH (5.2–7.0) (50 mM phosphate buffer), temperature (4–45 °C) and starch concentration (0.03–17.97 mg mL^{-1}) in the reaction mixture. Individuals acclimated to 35 psu were used in these experiments.

2.4.2. Maltase and sucrase activity

Maltase and sucrase activity in hepatopancreas were determined measuring the glucose released from the specific substrate as we detailed (Asaro et al., 2011). Briefly, the sample was incubated during 10 min at 37 °C with 42 mM of maltose or sucrose in 0.1 M maleate-NaOH buffer (pH 5.2). The reaction was arrested with 1.5 mL of a glycemia kit (glucose oxidase 10 kU L; peroxidase 1 kU; l,4-amino-phenazone 0.5 mmol L^{-1}; phosphates pH 7.0100 mmol L^{-1},

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