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COELENTERATE BIOLOGY

Digestive flexibility in response to environmental salinity and temperature in the non-symbiotic sea anemone *Bunodosoma zamponii*

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Abstract In spite of their ecological importance in tidal pools, studies on digestive flexibility in non-symbiotic anemones (i.e., without symbiosis throughout their life) upon changes in key environmental factors are lacking. This study constitutes the first work to investigate the response of digestive enzymes in relation to high environmental salinity and temperature in a non-symbiotic sea anemone. We determined the occurrence and biochemical characteristics of maltase activity and compared maltase and proteolytic activities in mesenterial filaments in *Bunodosoma zamponii* from the intertidal area of Punta Cantera, Mar del Plata, Argentina (38°05′S, 57°32′W) acclimated to 35 and 40 psu and after an acute enhancement (15 min) of temperature from 20° to 30°C. Maltase activity was higher in 40 psu and upon the increase in temperature (about 80 and 99%, respectively). Proteolytic activity was not affected in any case. The results suggest the occurrence of specific digestive adjustments in relation to high salinity and temperature. The fact that differential modulation of maltase activity could be associated with a higher digestive capacity for glycogenic substrates is discussed.

Keywords Intertidal · Phenotypic flexibility · Non-symbiotic anemones · *Bunodosoma zamponii* · Mesenterial filaments · Digestive enzymes

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Introduction

In marine aquatic environments, the intertidal zone represents the interface between land and sea being covered with water at high tide and exposed to air at low tide. Intertidal zone constitutes an extremely challenging environment in which abiotic factors such as salinity and temperature vary abruptly both spatially and temporally. These variations are particularly evident in tide pools which are more vulnerable to salinity and temperature changes as tides rise and fall. Phenotypic flexibility, one category of phenotypic plasticity, implies reversible within-individual variations in

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phenotypic traits which can increase the chances of survival for animals facing changes in environmental conditions (Piersman & Drent, 2003; Kelly et al., 2012). As sessile organisms, sea anemones in tidal pools have to cope with a variety of challenges such as abrupt, wide, and extreme temporal changes in both environmental salinity and temperature. Thus, to study the possible flexible responses in different traits (i.e., digestive traits) results of significant importance to know and/or to predict their performance upon abrupt changes in these key environmental factors. However, strikingly studies in non-symbiotic anemones (i.e., without symbiosis throughout their life) are lacking.

The maintenance of glucose and amino acids homeostasis is crucial in the responses of sea anemones to variations in environmental conditions (Benson-Rodenbough & Ellington, 1982; Kasschau et al., 1984; Zamer & Hoffamn, 1989; Cowlin, 2012). Salinity adaptation appeared to be a complex process. In this context, biochemical changes involved in salinity acclimation (i.e., changes in synthesis and/or accumulation of osmolytes) require a metabolic reorganization (Cowlin, 2012). In symbiotic sea anemones, one of the main sources of glucose and amino acids comes from the metabolism of the endosymbiotic photosynthetic dinoflagellates (zooxanthellae) (Piniak et al., 2003; Bachar et al., 2007; Palka, 2010; Burriesci et al., 2012). On the contrary, non-symbiotic anemones must rely mostly on their own biochemical–physiological processes (i.e., digestion of dietary glycogenic and protein items) as sources of glucose and amino acids. Therefore, the ability for complete digestion of dietary glycogenic and protein substrates and its modulation upon variable conditions would be crucial for glucose and amino acid homeostasis in non-symbiotic anemones. The modulation of digestive enzymes activity as being a link between digestion and absorption could lead to a major availability of metabolites (i.e., glucose, amino acids) for acclimation to differential environmental conditions (i.e., distinct salinity conditions). The occurrence and modulation in coelenteron mesenterial filaments (the main site for nutrient digestion) of maltase activity, which intervenes both in the initial and final steps of degradation of various glycogenic polysaccharides (Quezada-Calvillo et al., 2008; Lin et al., 2012; Dhital et al., 2013), would determine the extent of ability for complete digestion of glycogenic substrates leading to available glucose. However, to our knowledge, studies on the occurrence and modulation of maltase activity in mesenterial filaments of non-symbiotic sea anemones are lacking. The level of proteolytic activity in the mesenterial filaments, in turn, will determine the capacity and/or extent of digestion of dietary protein substrates. A few studies, however, have demonstrated the existence of proteolytic activity in mesenterial filaments of non-symbiotic anemones (Gibson & Dixon, 1969; Van Praët, 1982a, b; Shick, 1991). Moreover, studies on the possible effect of environmental salinity on digestive enzymes activities are lacking. Thermal variations can be extreme in rocky intertidal habitats, ranging from a few minutes to several hours depending on the magnitude of the tidal exchange (Helmuth, 2009, Somero, 2010; Bingham et al., 2011; Bozinovic et al., 2011). Since non-symbiotic anemones, as we pointed out above, must rely on internal biochemical–physiological process, as sources of different metabolites, the resistance and/or modulation of digestive enzymes activities upon an abrupt increase to ambient temperature could constitute an important adjustment allowing the maintenance of glucose and amino acids homeostasis. However, the effect of an acute exposure to high temperature on digestive enzymes in intertidal non-symbiotic anemones is unknown.

Bunodosoma zamponii (previously identified as *Phymactis clematis* or *P. papillosa*), which has been recently found to be genetically divergent from *P. papillosa* from Chile and closely related to West Atlantic Atlantic *Bunodosoma* species (Gomes et al., 2012), is an intertidal non-symbiotic sea anemone of the temperate southwestern coastal of the Atlantic Ocean (Acuña & Zamponi, 1995, 1996; Acuña, personal communication). Because of living in the intertidal area, it is exposed to seasonally and abrupt daily changes in salinity and temperature (Patronelli et al., 2005). Several studies have been conducted on this species (Excoffon & Zamponi, 1991; Acuña, 1993; Acuña & Zamponi, 1995, 1996; Olivera et al., 2002, Zamponi, 2007; Zamponi & Deserti, 2009; Zamponi, 2011; Gomes et al., 2012; Olivera et al., 2012), but none yet on digestive physiology at the biochemical level. In the natural ambient, *B. zamponii* behaves as a polyphagous opportunistic consuming varied dietary items in relation to food availability. The green algae *Ulva lactuca* (which is rich in starch-type polysaccharides) is one of the main supplementary dietary items of *B. zamponii* along with water-dissolved detritus, mollusks, and decapods (Acuña &

Zamponi, 1996; Acuña, personal communication). To increase the knowledge of different aspects of the biology of *B. zamponii* and as part of our integrative studies on the identification of enzyme activities involved in biochemical adaptations to environmental conditions in intertidal organisms, the aims of this work were to determine the occurrence and biochemical characteristics of maltase activity in coelenteron mesenterial filaments and the responses of maltase and total proteolytic activity in mesenterial filaments to high salinity (40 psu) and to an abrupt change to high temperature (20–30°C). We hypothesized that *B. zamponii* exhibits digestive adjustments at the biochemical level which represent digestive strategies to face changes in key environmental conditions with the following prediction: *B. zamponii* should exhibit an enhanced activity of maltase and total proteolytic activity in the mesenterial filaments upon acclimation to high salinity and after a short-term change to high temperature. The modulation (i.e., increase) of maltase and proteolytic activities in the mesenterial filaments upon changes in environmental conditions would imply a highest ability for digestion of glycogenic and protein substrates thus leading to an enhancement of glucose and amino acids availability to support biochemical changes underlying salinity and temperature acclimation. Due to the particular lifestyle of non-symbiotic sea anemones, they constitute an interesting biological predictive model to study the effect of changes in environmental temperature on biochemical–physiological processes (i.e., digestive physiology). Understanding the adaptive mechanisms employed by temperate, ectothermic, sessile, and non-symbiotic organisms such as *B zamponii* will help to ascertain whether these organisms could be tolerant to the predicted conditions underlying global changes (i.e., abrupt, extreme, and of wide amplitude changes in environmental factors such as salinity and temperature).

Materials and methods

Animal collection and maintenance

The sea anemones were collected from the intertidal zone of Punta Cantera (38°05′S, 57°32′W) (Mar del Plata, Buenos Aires, Argentina) during spring (September 2012). The tidal pools in this site exhibited a complex and variable pattern of salinity and temperature variations. The values of salinity of the water in tidal pools in the intertidal zone of Mar del Plata coast, including the sites of collection, can range between 33 and 35 psu, but frequently higher values can be reached and maintained (up to 40 psu) during warm seasons. The water temperature can abruptly vary (i.e., increase) depending on air temperature and wind particularly in warm seasons (Martos et al., 2004; Sánchez & Momo, 2013; personal observations). At time of collection, the values of salinity and temperature in tidal pools water were 35 psu and 20°C, respectively. Animals were transported to the laboratory in sea water (35 psu) on the day of collection. The sea anemones were maintained in natural seawater (35 psu) or concentrated seawater (40 psu) for at least 20 days prior to use. Concentrated seawater was obtained by addition of commercial marine salt (Red Sea Salt, Israel) to natural seawater (López Mañanes et al., 2000; Pinoni & López Mañanes, 2004, 2008; Pinoni, 2009; Michiels et al., 2013). The aquaria contained 36 l of water, continuously aerated and filtered. The water was continuously filtered by means of an Atman filter (HF-0400). A regime of 12 h light/12 h dark was applied, and the temperature was kept at 20 ± 2°C. Aquaria were shielded by black plastic to reduce disturbance. Sea anemones were individually fed with 0.22 ± 0.1 g of fresh food three times a week, but they were starved 24 h prior to the experiments. To determine the occurrence and biochemical characteristics of maltase activity in mesenterial filaments, individuals acclimated to 35 psu and at 20°C were used. To study the effect of short-term exposure to high temperature, animals acclimated at 35 psu at 20 ± 2°C under continuous aeration and individually housed in small aquaria were subjected to an abrupt increase of temperature up to 30°C for 15 min *or* kept under the same conditions (control group). Both the salinity and temperature conditions were used simulating field conditions usually faced by *B. zamponii* in Punta Cantera (Patronelli et al., 2005; personal observations).

Preparation of enzyme mesenterial filament extract

The sea anemones were cryoanesthetized by putting them on ice for about 10 min. The mesenterial

filaments were immediately excised, mixed with homogenizing medium (0.5 M Tris/HCl pH 7.4; 4 ml g^{-1} of mesenterial filaments), and homogenized (CAT homogenizer × 120, tool T10) on ice. The homogenate was centrifuged at 10,000×g for 15 min (Sorval, rotor SS34, refrigerated). The mesenterial filaments from one individual were used for each preparation of enzyme extract. The supernatant was fractionated into 500 ul aliquots and stored at −20°C until use.

Biochemical assays

Maltase activity was assayed by measuring the glucose released from the hydrolysis of maltose as we have previously described (del Valle et al., 2004; Asaro et al., 2011a, b; del Valle & López Mañanes, 2011; Pinoni et al., 2013). To study the effect of pH, maltase activity was determined at different pH levels (5.9–8.4) of the reaction mixture (0.1 M maleate/NaOH buffer) containing 28.0 mM of maltose at 37°C. To study the effect of maltose concentration on the activity, maltase activity was determined in the presence of varying substrate concentrations (0.56–42 mM) in the reaction mixture (0.1 M maleate/NaOH buffer, pH 6.4) at 37°C. To study the effect of temperature on the activity, maltase activity was assayed at different temperature (4–45°C) of the reaction mixture containing 28.0 mM in 0.1 M maleate/NaOH buffer (pH 6.4). In all cases, the reaction was initiated by adding an aliquot of the corresponding sample (≤20 µl) (linearity zone on activity vs. protein concentration plot) to the reaction mixture (final volume = 100 µl). After incubation for 10 min, the reaction was stopped by addition of 1.5 ml of the combined enzyme color glucose reagent solution (oxidate glucose 10 kU l^{-1}; peroxidase 1 kU; l,4-aminophenazone 0.5 mmol l^{-1}; phosphates pH 7.0100 mmol l^{-1}, hydroxybenzoate 12 mmol l^{-1}) (Wiener Lab Kit cod. 1400101) (del Valle et al., 2004; Asaro et al., 2011a, b; Pinoni et al., 2013). After 5 min, the amount of glucose released was determined by reading the absorbance at 505 nm of the colored quinone complex (Beckman UV–visible spectrophotometer). For further experiments, the activity was determined as described above in a reaction mixture containing 28.0 mM of maltose in 0.1 M maleate/NaOH buffer (pH 6.4) at 37°C.

Total proteolytic activity was assayed by adding an aliquot of the corresponding sample (linearity zone on activity vs. protein concentration plot) to a reaction mixture containing 1% w/v azocasein in 0.1 M Tris–HCl buffer (pH 7.5) as we previously described (del Valle et al., 2004; Pinoni et al., 2011, 2013). After incubation at 45°C for 30 min, the reaction was arrested by adding 0.75 ml of cold trichloroacetic acid (TCA) (10% v/v) (del Valle et al., 2004; Pinoni et al., 2011, 2013). Overnight absorbance was measured at 440 nm (A440) in the supernatant resulting after centrifuging at 1800×g for 20 min (IEC-Centra 7R, refrigerated). One unit activity (U) was defined as the amount of enzyme extract that produced an increase of 1 in A440. The proteolytic activity was expressed as U h^{-1} mg $protein^{-1}$.

The determination of enzyme activities was always performed with samples that had been stored at −20°C, without previous thawing.

Protein was assayed according to Bradford (1976). Bovine serum albumin was used as standard.

Coelenteron fluid osmolality

Coelenteron fluid was sampled (100–200 µl) by means of a micropipette (P 200). Osmolality was measured with a micro-osmometer (Osmomat 030 D, GONOTEC).

Statistical analysis

The results of the effect of different substrate concentrations on the enzymatic activities were analyzed by a nonlinear regression analysis (GraphPad Prism 4.0 software). The curve that appears is the one that best adjusts to the experimental data. The values of K_m (constant of Michaelis–Menten) were estimated by the analysis of the data using the graph of Lineweaver–Burk (GraphPad Prism 4.0 software). The statistical analysis of the data was done using the Sigma 3.0 program for Windows, which automatically performs a previous test of equality of variances and normality. To study the effect of salinity acclimation, a *t* test parametric or non-parametric (Mann–Whitney test) was performed. To evaluate the effect of abrupt change of temperature on enzyme activities, a *t* test parametric or non-parametric (Mann–Whitney test) was used (Zar, 1999).

Results

Maltase activity in mesenterial filaments of *Bunodosoma zamponii*

Maltase activity in the mesenterial filaments was determined within the range of pH 5.9–8.4. The activity increased (about 50%) from 5.9 to 6.4. Highest values were detected within the range 6.4–6.8. At pH 8.4, the decrease in maltase activity was subtle, as it was 18% lower than the activity at pH 6.4 (Fig. 1a). The effect of maltose concentrations on maltase activity is shown in Fig. 1b. Maltase activity in mesenterial filaments exhibited Michaelis–Menten kinetics (apparent K_m 0.31 ± 0.4 mM). Maltase activity gradually increased upon an enhancement of temperature of the reaction mixture from 4° to 37°C (Fig. 1c).

Effect of environmental salinity on digestive enzymes in mesenterial filaments of *Bunodosoma zamponii*

To study the effect of environmental salinity on maltase and total proteolytic activity in mesenterial filaments, individuals of *B. zamponii* were acclimated to 35 and 40 psu at 20°C. The osmolality of coelenteron fluid of individuals acclimated to 35 (885 ± 19 mOsm × kg^{-1}) and 40 psu (1067 ± 39 mOsm × kg^{-1}) was similar to that of the corresponding external medium (35 psu = 841 ± 19 mOsm × kg^{-1}, 40 psu = 1039 ± 30 mOsm × kg^{-1}) ($P > 0.05$). The osmolality of coelenteron fluid in 40 psu was higher to that of individuals acclimated to 35 psu [$t_{(12)} = -41$, $P = 0.001$]. Individuals acclimated at high salinity (40 psu) exhibited a higher specific maltase activity in mesenterial filaments (about 80%) than individuals

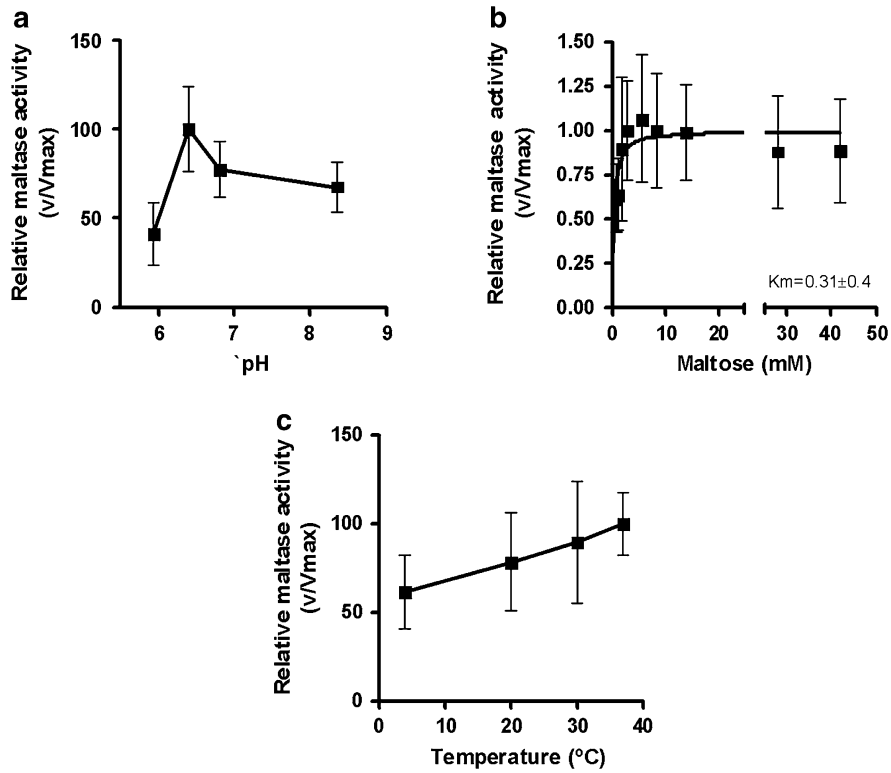

Fig. 1 **a** Effect of pH (5.9–8.4) on maltase activity in mesenterial filaments of *B. zamponii*. The maltase activity values are expressed as a relation to the specific activity at pH 6.4 (100%, 21.58 ± 5.18 ug glucose mg $prot^{-1}$ min^{-1}). Data are the mean ± SE for five individuals. **b** Effect of maltose concentration on maltase activity in mesenterial filaments of *B. zamponii*. The activity values are expressed as a relation to the corresponding activity in the presence of 28 mM maltose (100%, 20.30 ± 7.43 ug glucose mg $prot^{-1}$ min^{-1}). The curves are the ones which best fit the experimental data (GraphPad Prism 2.01). Data are the mean ± SE for five individuals. **c** Effect of temperature (4–45°C) on the maltase activity in mesenterial filaments of *B. zamponii*. The activity is expressed in relation to the activity at 37°C (100%, 36.89 ± 6.57 ug glucose mg $prot^{-1}$ min^{-1}) Data are mean ± SE for five individuals

acclimated to 35 psu [$T_{(7.7)} = 34, P = 0.017$] (Fig. 2a). No differences in total proteolytic activity in mesenterial filaments were found between individuals acclimated to 35 or 40 psu (Fig. 2b) ($P > 0.05$).

Effect of an abrupt change to high temperature on digestive enzyme activities of mesenterial filaments of *Bunodosoma zamponii*

After 15 min under exposure to high temperature (30°C), maltase activity in mesenterial filaments was highly increased (about 99%) [$T_{(7.6)} = 25, P < 0.003$] (Fig. 3a). Proteolytic activity in mesenterial filaments was not affected ($P > 0.05$) (Fig. 3b).

Discussion

The results of this work show the occurrence of maltase activity in mesenterial filaments and its response upon acclimation to high environmental salinity and a short-term abrupt change to high temperature (whereas proteolytic activity was not affected) in the non-symbiotic sea anemone *B. zamponii* suggesting the occurrence of specific and differential digestive adjustments at the biochemical level and mechanisms of regulation in relation to environmental changes usually faced by this anemone in its natural habitat. The higher maltase activity in mesenterial filaments of *B. zamponii* in high salinity and after an abrupt change to high temperature could imply a higher ability for digestion of glycogenic substrates therefore leading to a higher glucose availability under these conditions.

In the natural ambient, *B. zamponii* behaves as a polyphagous opportunistic with a varied diet in relation to food availability. In this context, dietary items rich in glycogenic polysaccharides such as the green algae *Ulva lactuca* (Castro-González et al., 1996) and water-dissolved detritus are common dietary supplements besides animal items such as mollusks and decapods (Acuña & Zamponi, 1996; Acuña, personal communication). Digestive enzymes are a complementary tool useful for determining which dietary components are effectively metabolized (Brethes et al., 1994; Johnston & Freeman, 2005; Pinoni et al., 2011). The occurrence of maltase and proteolytic activity in mesenterial filaments of *B. zamponii* suggests its potential ability to metabolize different substrates contained in its varied diet. Maltase activity (which hydrolyzes α1,4 glycosidic linkages from non-reducing ends) has a main role in carbohydrates digestion participating both in the initial steps by assisting to α- amylase and in the final steps of hydrolysis to yield glucose (Lin et al., 2012; Dhital et al., 2013). The occurrence of maltase activity in mesenterial filaments of *B. zamponii* points out its ability to potentially utilize different kinds of glycogenic carbohydrates (i.e., starch, glycogen, dextrin, and maltose) as glucose sources which would be of significant physiological importance as being a non-symbiotic anemone. Information about biochemical characteristics of maltase activity in mesenterial filaments of seawater anemones is lacking. The pH value for maximal maltase activity found in mesenterial filaments of *B. zamponii* (Fig. 1a) is within the range of optimal acid pH values reported for maltase activity in hepatopancreas of decapod crustaceans (Figueiredo & Anderson, 2009; Asaro et al., 2011a).

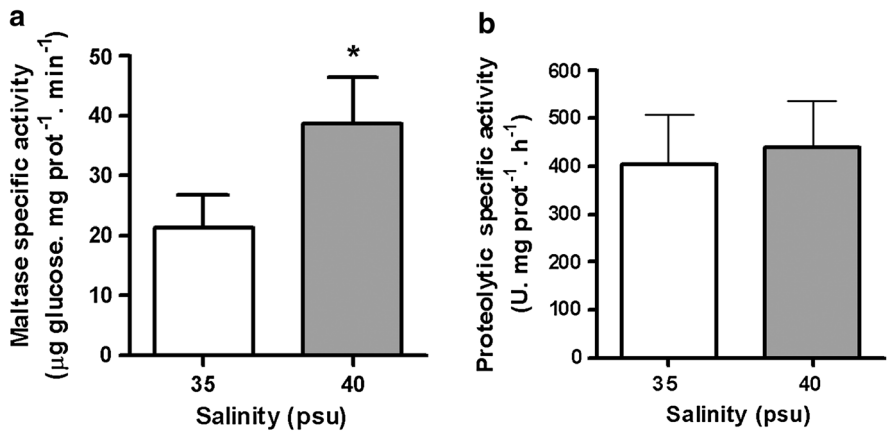

Fig. 2 Maltase (**a**) and proteolityc specific (**b**) activities in mesenterial filaments of individuals of *B. zamponii* acclimated to different salinities. Data are mean ± SE for six to seven individuals. *Open bars* individuals acclimated to 35 psu salinity, *gray bars* individuals acclimated to 40 psu. *Significantly different from the activity of individuals acclimated to 35 psu salinity ($P < 0.05$)

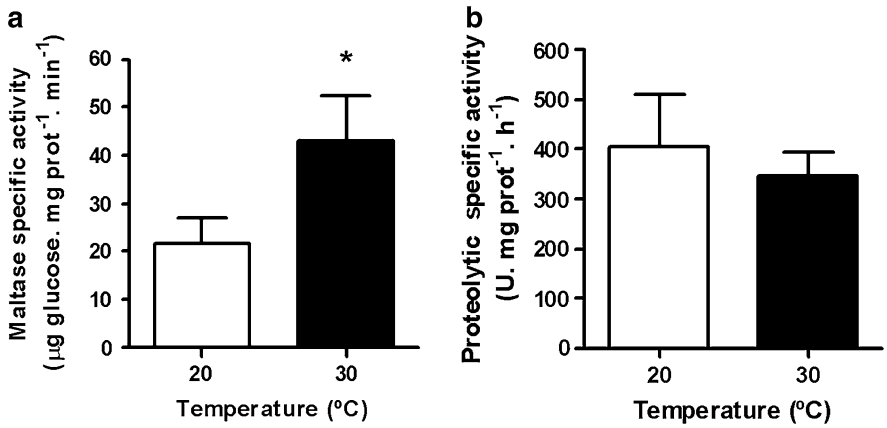

Fig. 3 Effect of abrupt change of temperature from 20°C (*open bars*) to 30°C (*black bars*) on maltase (**a**) and proteolityc specific (**b**) activities in mesenterial filaments of individuals of *B. zamponii*. Data are mean ± SE for six to seven individuals *Significantly different from the activity at 20°C, $P < 0.05$

Since the pH of the coelenteron in several Anthozoa ranges between 6.7 and 8.75, the maintenance of maltase activity over a wide range of pH (6.4–8.4) (Fig. 1a) suggests that a similar level of hydrolytic capacity would be maintained under varying physiological conditions. The maintenance of maltase activity within a broad range of pH is similar to that we found for this activity in hepatopancreas of intertidal euryhaline crabs (Asaro et al., 2010, 2011a). The Michaelis–Menten kinetics exhibited by maltase activity in mesenterial filaments of *B. zamponii* (Fig. 1b) is in accordance to that described for this activity in homogenates from hepatopancreas of euryhaline crabs (Asaro, 2009; Asaro et al., 2011a) and from intestine of several fishes (German & Bittong, 2009) and mammals (del Valle & López Mañanes, 2008). However, its apparent K_m appeared to be lower suggesting a higher affinity for its substrate. A higher affinity for glycogenic substrates could lead to the maintenance of glucose homeostasis even when glycogenic substrates are limited. This could be an advantage at the biochemical level in relation to the fact that *B. zamponii*, as a non-symbiotic anemone, should obtain glucose mainly from digestion of dietary glycogenic substrates. Whether *B. zamponii* is also capable to obtain glucose by direct absorption from seawater as described in the non-symbiotic species, *Actinia equina* cannot be discarded (Van Praët, 1982b, 1985; Shick, 1991). The results on the effect of varying temperature of the reaction mixture on maltase activity of mesenterial filaments of *B. zamponii* show that enzyme activity is maintained over a wide range from 4° to 37°C (Fig. 1c). This along with the fact that maltase activity was strikingly high at low temperature (4°C) (about a 60% of activity at 37°C) and tended to be higher at 37°C, suggests the maintenance of hydrolytic capacity upon a broad range and even extreme temperature conditions. Since during winter, *B. zamponii* is largely exposed to low temperatures in its natural habitat (Patronelli et al., 2005; personal observation), a cold tolerant maltase activity could be related to a role upon acclimation to low temperature (i.e., higher digestion of glycogenic substrates). A cold resistant maltase activity occurs in intestine of antarctic fishes (Maffia et al., 1993). In mammals, the activity of intestinal brush border sucrase–isomaltase complex comprises 80% of maltase activity and the rest of maltase activity being accounted for maltase–glucoamylase enzymes (sucrase-independent maltase activity) (Quezada-Calvillo et al., 2008; Nichols et al., 2009; Dhital et al., 2013). Since we did not detect sucrase activity (not shown), maltase in mesenterial filaments of *B. zamponii* could correspond to a sucrase-independent maltase activity. However, a further characterization is needed to test this.

Bunodosoma zamponii inhabiting the rocky intertidal habitat suffers daily and seasonally variations in salinity due to changes in tide amplitude and water loss by evaporation (Patronelli et al., 2005). As expected, based on the successful occupancy of the intertidal area, individuals of *B. zamponii* were able to tolerate long-term exposure (20 days) to high salinity (40 psu), no mortality occurring throughout the experimental period. Similarly to other Anthozoa (Amado et al., 2011), *B. zamponii* behaved as an osmoconformer both in 35 and 40 psu since coelenteron fluids osmolality was similar to that of the corresponding external media (this work). Despite being osmoconformers, sea anemones depend on various mechanisms such as mucus secretion, cell

volume regulation, modulation of the concentration of compatible solutes osmolytes, and changes in concentration of body free amino acids to balance osmotic challenges upon differential environmental salinities (Kasschau et al., 1984; Oren, 1999, 2002; Mayfield & Gates, 2007; Foster et al., 2010; Amado et al., 2011; Cowlin, 2012). In euryhaline crustaceans, the modulation of specific digestive enzyme activities has been proposed to be associated with a major availability of metabolites (i.e., glucose and amino acids) for the underlying metabolic reorganization upon acclimation to extreme salinities (Li et al., 2008; Asaro et al., 2011a, b; Romano & Zeng, 2012; Michiels et al., 2013; Pinoni et al., 2013). The higher maltase activity in mesenterial filaments of *B. zamponii* acclimated to 40 psu (Fig. 2a) suggests that modulation of this activity is one component of the biochemical adaptation to high salinity in this anemone. The enhanced maltase activity could be associated with an increased capacity for digestion of glycogenic substrates, which, in turn, would lead to an enhancement in glucose availability for supporting biochemical changes underlying salinity acclimation. Since total proteolytic activity in mesenterial filaments of *B. zamponii* was not affected by acclimation to high salinity, the level of this activity could be enough to support dietary provision for amino acids homeostasis. The fact that maltase activity in mesenterial filaments of *B. zamponii* was affected at high salinity while proteolytic activity was not (Figs. 2a, b) suggests the occurrence of specific regulation of the activity of digestive enzymes and of differential digestive adjustments. The mechanisms of regulation (i.e., chemical messengers) or factors involved in the modulation of the activity of digestive enzymes in seawater anemones are far from having been elucidated. A genomic study in the sea anemone *Nematostella vectensis* revealed that diverse neuropeptide and hormone families are represented with genes encoding prepropeptides and receptors related to insulin-like peptides and glycoprotein hormones (Anctil, 2009). Further studies are needed to establish whether these peptides and hormones are involved in the modulation of digestive enzymes activities such as maltase in *B. zamponii*.

The ability to respond to changes in temperature is crucial for adapting and surviving in highly fluctuating environments (Choresh et al., 2001; Bozinovic et al., 2011). Changes in ambient temperature can affect behavior, cellular structures, enzyme function, neuronal function, and metabolic pathways (Somero, 2002; Hofmann & Todgham, 2010; Bingham et al., 2011; Chung et al., 2012; Tattersal et al., 2012). The enhanced maltase activity in mesenterial filaments of individuals of *B. zamponii* exposed to 30°C (Fig. 3a) compared to individuals maintained at 20°C points out that modulation of this activity is a component of biochemical adjustments upon an acute increase in environmental temperature in this anemone. In their natural habitat, *B. zamponii* is exposed to daily abrupt changes in temperature, mainly in summer, when maximal air temperature sometimes reaches up to 38°C and even higher values in tidal pools upon evaporation (Martos et al. 2004; Sánchez and Momo, 2013; personal observations). The increased maltase activity suggests that capacity for digestion of the target glycogenic substrates would not be impaired by acute exposure to high temperature but, moreover, could be potentially enhanced. Whether the higher maltase activity is related to an up-regulation of pre-existing enzyme, synthesis de novo or changes in membrane lipid composition require further experimental approach. Similarly to that found upon acclimation of individuals of *B. zamponii* to high salinity (Fig. 2b), total proteolytic activity in mesenterial filaments appears not to be affected by acute exposure to high temperature (Fig. 3b). Since proteins are involved in several physiological processes such us membrane transport, movements, enzyme-catalyzed reactions, and intracellular signaling (Diniz et al., 2012), the maintenance of adequate intake of dietary protein is crucial to support and survive under different environmental stresses. The fact that no changes in proteolytic activity occur upon acute exposure to high temperature (Fig. 3b) suggests that hydrolysis of dietary proteins and therefore the provision of nitrogenous nutrients would not be impaired. The higher maltase activity in mesenterial filaments upon acute exposure to high temperature, while total proteolytic activity was not affected, further supports the idea of the occurrence of a specific modulation of maltase activity and consequently of adjustments in carbohydrate metabolism. However, we cannot discard that a modulation in the final step of protein degradation (i.e., aminopeptidases) and/or in amino acids absorption (i.e., modulation of transport systems) could be occurring upon high salinity acclimation or acute exposure to high temperature.

In conclusion, the results of this study show the occurrence of maltase activity in the mesenterial filaments of the non-symbiotic sea anemone *B. zamponii* which is increased upon acclimation to high salinity and acute exposure to high temperature suggesting its role in biochemical adaptation upon changes in environmental conditions. Whether modulation of maltase activity implies digestive adjustments leading to differential capacity for digestion of glycogenic dietary items and consequently to a major availability of carbohydrate, metabolites remain to be investigated. Furthermore, the fact that maltase activity in the mesenterial filaments was affected while no changes occurred in total proteolytic activity suggests the existence of specific regulatory pathways. Future studies should focus on establishing the regulatory pathways involved to provide a better understanding of the integrative responses and mechanisms underlying biochemical adaptation of *B. zamponii* in particular and non-symbiotic sea water anemones in general, to challenging intertidal habitat conditions.

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