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Alkaline phosphatase activities in muscle of the euryhaline crab *Chasmagnathus granulatus*: Response to environmental salinity

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Abstract

The occurrence, characteristics and response to environmental salinity of alkaline phosphatase (AP) activity were studied in chela muscle of the euryhaline crab *Chasmagnathus granulatus* from Mar Chiquita coastal lagoon (Buenos Aires Province, Argentina). Chela muscle exhibited a levamisole-insensitive and a levamisole-sensitive AP activities with distinct characteristics. Levamisole-insensitive activity appeared to be maximal at pH 7.7, whereas levamisole-sensitive AP activity was similar with the range of pH 7.4 to 8.0. Both activities at pH 7.7 exhibited a Michaelis–Menten kinetics (K_m = 0.789 and 1.416 mM, respectively). I_{50} for levamisole-sensitive AP activity was about 12 mM. Levamisole-insensitive and levamisole-sensitive AP activities were differentially affected by temperature. Levamisole-sensitive AP activity was quite sensitive to temperature, exhibiting a peak at 37 °C but being low at 5 to 30 °C and 45 to 60 °C. Both activities were inhibited by Cu^{2+}. At 1.0 mM Cu^{2+}, levamisole-insensitive AP activity was inhibited about 82% whereas levamisole-sensitive AP activity was almost completely inhibited. Levamisole-insensitive AP activity appeared to be sensitive to environmental salinity. In crabs acclimated to low salinity (10‰) this activity was lower than in 35‰ salinity. The response to environmental salinity suggests that levamisole-insensitive AP activity could be a component of muscle regulatory mechanisms at the biochemical level secondary to hyperregulation of *C. granulatus*. The possible physiological roles and functional relationship of AP activity with Na^+/K^+ ATPase in muscle are discussed.

Keywords: Alkaline phosphatase; *Chasmagnathus granulatus*; Crabs; Levamisole; Muscle enzymes; Osmo-ionoregulation

1. Introduction

Euryhaline crabs inhabiting coastal waters, estuaries and tidal areas are exposed to frequent and abrupt changes in the environmental salinity. Biochemical, physiological, morphological and/or behavioural strategies occur in order to control movements of water and

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ions between the animals and their medium (Kirschner, 1991). In low salinities, hyperregulating crabs are able to maintain hemolymph osmotic and ionic concentrations above those in the ambient by absorbing both sodium and chloride (the major contributors to hemolymph osmolarity) from the external medium via the gills. The capability of hyperregulating crabs to adapt to low environmental salinities appears to involve the activation of their osmo-ionoregulatory machinery at different levels (from molecular to organism). In general, the posterior gills are considered to be the main site of the biochemical adaptations involved in ion transport processes (Lucu and Towle, 2003; Kirschner, 2004). In these gills of most species so far studied, adaptive increases of Na^+/K^+ ATPase activity has been clearly shown to occur, this enzyme being one of the central molecular component of the ionoregulatory process at the biochemical level (Towle, 1993, 1997; Lucu and Towle, 2003; Kirschner, 2004). In the euryhaline crab *Cyrtograpsus angulatus*, also a role of anterior gills in ionoregulation has been suggested (López Mañanes et al., 2002).

The biochemical adaptations in other organs or tissues of euryhaline crabs to varying environmental conditions are relative little known. Several responses have been shown to occur in muscle of some crabs during osmotic adaptation. In *Eriocheir sinensis*, an alkalosis of leg muscle occurred under hypoosmotic stress which suggests the exchange of acid–base equivalents between the extra- and intra-cellular compartments associated with acid–base regulation (Whiteley et al., 2001). A hypoosmotic stress led to an increase of arginine kinase flux in *Callinectes sapidus* (Holt and Kinsey, 2002). Recently, we have shown the occurrence of a salinity-dependent Na^+/K^+ ATPase activity in chela muscle of the euryhaline crab *C. angulatus* from Mar Chiquita coastal lagoon, suggesting a role for this tissue in regulatory mechanisms evoked upon osmo-ionic stress in this crab (Pinoni and López Mañanes, 2003).

Many physiological processes in organisms are under regulation via the formation (phosphorylation) or cleavage (dephosphorylation) of phosphate esters. The dephosphorylation process is catalysed by different phosphatases. Alkaline phosphatases (AP) (EC 3.1.3.1) are ubiquitous metalloenzymes which have been involved in several essential functions in mammals (Register and Wuthier, 1984; Milan et al., 2001; Hessle et al., 2002). AP activity determination is often used in clinical and ecotoxicological studies. Comparatively, AP in invertebrates has been less studied. In the green crab *Scylla serrata* AP is important in the absorption of phosphate and calcium from seawater and for the shell formation of the crab (Park et al., 2001). In *Drosophila virilis*, AP activity has been shown to decrease upon heat stress (Sukhanova et al., 1996). AP from different invertebrate tissues has been partially characterized (Chen et al., 1996, 2000; Park et al., 2001; Mazorra et al., 2002; Xiao et al., 2002). In the clam *Scrobicularia plana*, AP activities have been suggested as potential biochemical indicators of stress due to heavy metals (Mazorra et al., 2002).

The response of AP activity to varying environmental salinity in euryhaline crabs has scarcely been studied. The occurrence of AP activity sensitive to environmental salinity has been shown in the posterior gills of *C. sapidus*. This activity was lower in crabs acclimated to 35‰ salinity (Lovett et al., 1994), conversely to the response of branchial Na^+/K^+ ATPase (Lovett et al., 1992). The variations of AP activity upon acclimation to reduced salinity suggested the role of this enzyme in modulating the osmoregulatory response of *C. sapidus*. AP may be an effector for the adaptive increases of branchial Na^+/K^+ ATPase activity in low salinity by regulating the synthesis or delivery of polyamines which, in turn, modulate Na^+/K^+ ATPase activity (Lovett et al., 1994). In skeletal muscle of ground squirrel, Na^+/K^+ ATPase has been shown to be regulated by a dephosphorylation process mediated by AP (MacDonald and Storey, 1999).

Studies on the occurrence and characteristics of AP in crustacean muscle, as well as its possible participation as a component of the biochemical adaptations to changing environmental salinity, are lacking. We have recently described the existence of an AP activity sensitive to salinity and dopamine in chela muscle of *C. angulatus* from Mar Chiquita coastal lagoon, suggesting the participation of this enzyme activity in responses at the biochemical level of this crab to reduced environmental salinity (Pinoni and López Mañanes, 2004). The role of AP activity in muscle could be related to phosphorylation/dephosphorylation mechanisms regulating key components (i.e. Na^+/K^+ ATPase) probably involved in physiological processes (i.e. cellular volume regulation, acid-balance, mobilization of energetic sources) secondary to osmo-ionoregulation (Pinoni and López Mañanes, 2004).

Chasmagnathus granulatus is a semiterrestrial euryhaline crab which is found from southern Brazil to Patagonia (Argentina) (Boschi, 1964). In Mar Chiquita coastal lagoon, it is one of the dominant crabs in the outer parts where it is exposed to highly and abruptly variable environmental salinity ranging from 4 to 36‰ (Anger et al., 1994; Spivak et al., 1994).

Previous work in our laboratory demonstrated that *C. granulatus* exhibits a strong hyperregulatory capacity in reduced salinity (López Mañanes et al., 2000; Schleich et al., 2001). The differential response of Na^+/K^+ ATPase and carbonic anhydrase activities in individual anterior and posterior gills of *C. granulatus* both upon acclimation and after an abrupt change to reduced salinity suggested the occurrence of functional differences as well as different regulation mechanisms operating in individual gills of this crab (López Mañanes et al., 2000; Schleich et al., 2001). The regulatory mechanisms, under osmotic shock at the biochemical level in other tissues of *C. granulatus*, have been poorly investigated. Recently, an increase in the mobilization of lipids from muscle was found upon acclimation to low salinity (Luvizotto-Santos et al., 2003). Furthermore, an increase in gene expression of phosphoenolpyruvate carboxykinase (PEPCK) and gluconeogenic and PEPCK activities in jaw muscle of *C. granulatus* has been shown to occur upon hyperosmotic stress (Schein et al., 2004). As part of our integrative studies on regulatory mechanisms at the biochemical level of *C. granulatus* from Mar Chiquita coastal lagoon, we have previously shown the occurrence of a Na^+/K^+ ATPase activity which increases in crabs acclimated to low salinity in chela muscle of *C. granulatus*, suggesting both a role for muscle in regulatory mechanisms evoked upon osmo-ionic stress and the participation of this enzyme in physiological functions underlying osmotic adaptations (i.e. cellular volume regulation, acid-balance, mobilization of energetic source) of this crab (Pinoni and López Mañanes, 2003). Since AP has been associated with osmoregulation in euryhaline crabs (Lovett et al., 1994) and with mechanisms of adjustment secondary to ionoregulation in *C. angulatus* (Pinoni and López Mañanes, 2004) we hypothesized that muscle AP is another component of the biochemical adaptation of *C. granulatus* to varying environmental salinities.

The aim of this work was to determine the occurrence, characteristics and response to environmental salinity of AP activity in muscle of *C. granulatus* from Mar Chiquita coastal lagoon.

2. Materials and methods

2.1. Chemicals

pNPP (*p*-nitrophenylphosphate), Tris-(hydroxymethylamino-methane) (Tris), ethyleneglicol *N,N′, N′*-tetraacetic acid (EGTA), ethylenediamine tetraacetic acid (EDTA), bovine serum albumin and levamisole (L [−]-2, 3, 5, 6-Tetrahydro-6-phenylimidazol [2, 1-*b*] thiazole) were from Sigma (St. Louis, MO, USA); sucrose and cupric sulphate were obtained from Merck (Darmstadt, Germany); magnesium sulphate and Coomassie Blue G250 were from Fluka (Germany). All solutions were prepared in glass-distilled water.

2.2. Animal collection and maintenance

Crabs were caught from a single area from Mar Chiquita lagoon. Only adult male crabs with a carapace width greater than 2.5 cm were collected. Animals were transported to the laboratory in lagoon water on the day of collection. Crabs were maintained in natural seawater (35‰ salinity) or in dilute water (10‰ salinity) for at least 10 days prior to use. The aquaria contained 36 l of water, continuously aerated and filtered. A regime of 12-h light/12-h dark was applied and the temperature was kept at 22 ± 2 °C. Aquaria were shielded by a black plastic to reduce disturbance. Crabs were fed three times a week with commercial food (Cichlind T.E.N., Wardley, USA) (about 0.07 g per individual) but they were starved 48 h prior to experiments. Dilute seawater was obtained by dilution of natural seawater with distilled water.

2.3. Preparation of enzyme muscle extract

The crabs were cryoanesthesized by putting them on ice for about 15 min. After removing the chelae, the muscle was immediately excised, mixed with homogenizing medium (0.25 M sucrose/0.5 mM EGTA–Tris, pH 7.4) (8 ml g^{-1} of muscle tissue) and homogenized (CAT homogenizer ×120, tool T10) on ice. The muscles from both chelae of one individual were

pooled and used for each preparation of enzyme extract. The homogenate was fractionated into 400 μl aliquots and stored at −20 °C until use. Glycerol (1.3% v/v) was added to samples before freezing.

2.4. Assay of alkaline phosphatase activity

In the standard assay, AP activity was determined by measuring pNPP hydrolysis in a reaction medium containing 4 mM $MgSO_4$ in 100 mM Tris–HCl buffer (pH 7.7) in the absence (total AP activity) and in the presence of 16 mM levamisole (levamisole-insensitive AP activity). Levamisole-sensitive AP activity was estimated as the difference between both assays.

An aliquot of the corresponding sample (250–400 μg of protein) (linearity zone on activity vs. protein concentration plot) was added to the reaction mixture (final volume = 1 ml) and pre-incubated for 5 min at 37 °C. The reaction was initiated by the addition of 100 μl of 95 mM pNPP (final concentration = 9.5 mM). Incubation was carried out at 37 °C for 30 min. The reaction was stopped by addition of 2 ml of 0.1 M KOH. The amount of released pNPP was determined by reading the absorbance at 410 nm.

To study the effect of pH on AP activity, the procedure was the same as described above except that the activity was determined in the presence of varying pH of the reaction mixture. To study the effect of pNPP concentration on AP activity, the procedure was the same as described above except that the activity was determined in the presence of varying pNPP concentrations in the reaction mixture. The effect of temperature on AP activity was determined as described above, but varying the incubation temperature.

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

2.5. Protein analysis

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

2.6. Statistical analysis

Analysis of variance (one-way ANOVA or repeated measures ANOVA) was used to estimate the statistical significance of the differences and $p < 0.05$ was considered significant.

Results of effect of varying concentrations of pNPP on AP activity were analyzed by means of nonlinear regression analysis (GraphPad Prism 2.01 software). The corresponding curves shown are those which best fit the experimental data. K_m values (Michaelis–Menten constant) were estimated from these curves (GraphPad Prism 2.01 software). I_{50} (levamisole concentration at which levamisole-sensitive AP activity was 50% inhibited) was calculated from inhibition curve (GraphPad Prism software).

3. Results

3.1. AP activities of chela muscle of C. granulatus: effect of pH and levamisole

In preliminary experiments, AP activity of chela muscle was determined within the range of pH 7.4–9.0. AP activity was similar within the pH range of 7.4–8.0, whereas at pH 9.0 AP activity was 36.5 ± 8.4% of the activity at pH 8.0 (data not shown).

The effect of levamisole concentrations on total AP activity in chela muscle at pH 7.4, 7.7 and 8.0 is shown in Fig. 1. AP activities were dose-dependently inhibited by levamisole. The inhibition of total AP activity by levamisole revealed the presence in chela muscle of *C. granulatus* of two AP activities (a levamisole-insensitive and a levamisole-sensitive AP activity). Since, in the presence of 16 mM levamisole, inhibition of total AP activity was maximal in all cases, this concentration of inhibitor was used for experiments described below.

Levamisole-insensitive AP activity was maximal at pH 7.7. At pH 9.0 levamisole-insensitive AP activity was only about 34% of the activity at pH 7.7 (Fig. 2A). Levamisole-sensitive AP activity was similar within the range of pH 7.4–8.0, decreasing about 60% at pH 9.0 (Fig. 2B). Based on these results, further characterization of levamisole-insensitive and levamisole-sensitive AP activities in chela muscle of *C. granulatus* was carried out at pH 7.7.

I_{50} (the concentration that produced 50% levamisole-sensitive AP activity inhibition) was about 12 mM (Fig. 3).

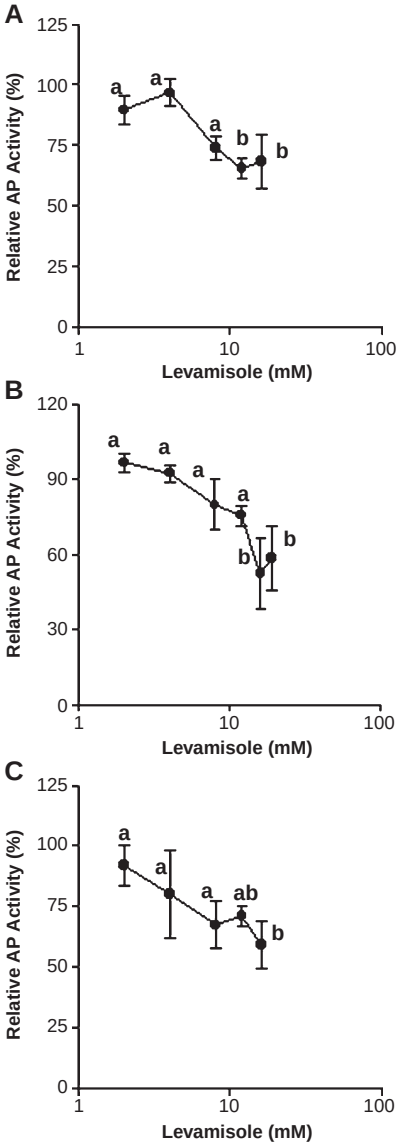

Fig. 1. AP activity at pH 7.4 (A), 7.7 (B), and 8.0 (C) in chela muscle of *C. granulatus* acclimated to 35‰ salinity in the absence and in the presence of levamisole. The values of AP activity are expressed as relation to the activity in the absence of inhibitor (100%). Data are the mean ± S.E. for three individuals. Different letters indicate significant differences ($p < 0.05$).

3.2. Effect of pNPP on AP activities in chela muscle of C. granulatus

The effect of pNPP concentrations on AP activities at pH 7.7 of chela muscle of *C. granulatus* is shown in Fig. 4. Both levamisole-insensitive and levamisole-sensitive AP activities exhibited Michaelis–Menten kinetics ($K_m = 0.789$ and 1.416 mM, respectively).

3.3. Effect of temperature on AP activities in chela muscle of C. granulatus

Levamisole-insensitive and levamisole-sensitive AP activities at pH 7.7 were differentially affected by temperature (Fig. 5). Levamisole-insensitive activity increased upon an enhancement of temperature from 5 to 30–37 °C reaching maximal activity. At higher temperatures levamisole-insensitive AP

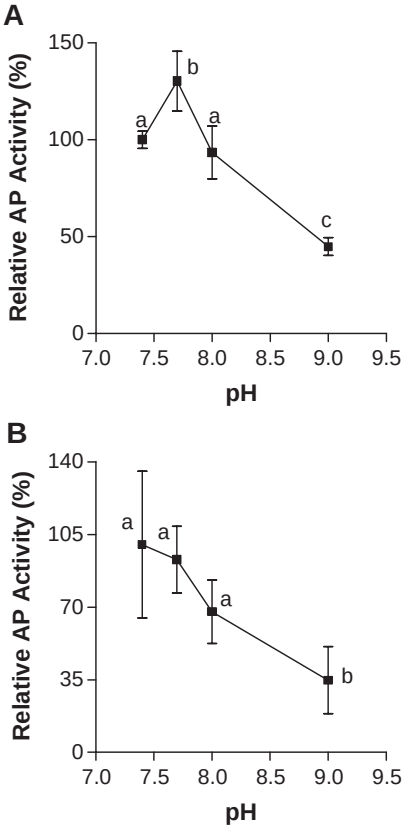

Fig. 2. Effect of pH (7.4–9.0) on AP activities in chela muscle of *C. granulatus* acclimated to 35‰ salinity. The values of AP activity are expressed as relation to the activity at pH 7.4 (100%). Data are the mean ± S.E. for three individuals. (A) Levamisole-insensitive AP activity. (B) Levamisole-sensitive AP activity. Different letters indicate significant differences ($p < 0.05$).

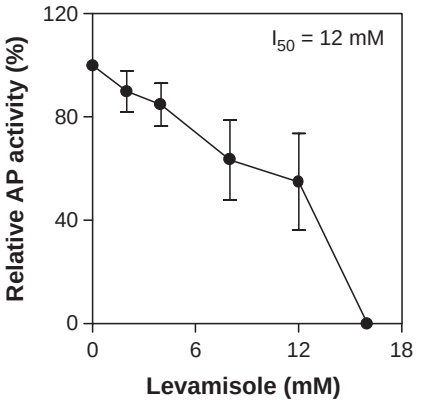

Fig. 3. Effect of levamisole (2–16 mM) on levamisole-sensitive AP activity in chela muscle of *C. granulatus* acclimated to 35‰ salinity. Maximal levamisole-sensitive AP activity was taken as 100%. I_{50}: levamisole concentration that produced 50% of inhibition, were calculated by GraphPad Prism 2.01. Data are the mean ± S.E. for three individuals.

activity decreased being at 45 and 60 °C about 29% of the activity at 37 °C (Fig. 4A). Levamisole-sensitive AP activity appeared to be quite sensitive to temperature exhibiting a peak at 37 °C. At lower (4–30 °C) or higher (45–60 °C) temperatures levamisole-sensitive AP activity was strongly diminished, being about 16% of the activity at 37 °C (Fig. 4B).

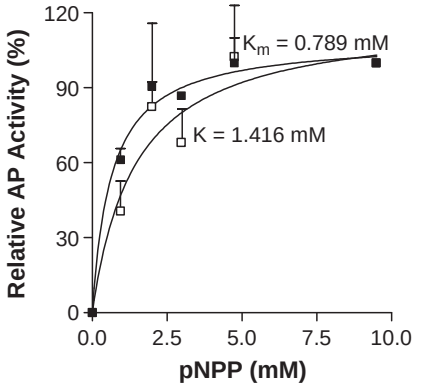

Fig. 4. Effect of pNPP on AP activities at pH 7.7 in chela muscle of *C. granulatus* acclimated to 35‰ salinity. The curves are the ones which best fit the experimental data. The values of AP activity are expressed as relation to the activity at 9.5 mM pNPP (100%). Data are the mean ± S.E. for three to four individuals. (■) Levamisole-insensitive AP activity. (□) Levamisole-sensitive AP activity.

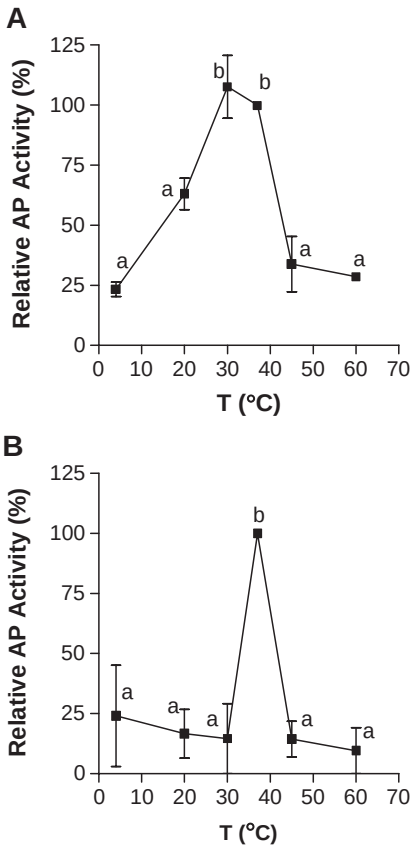

Fig. 5. Effect of temperature (4–60 °C) on AP activities at pH 7.7 in chela muscle of *C. granulatus* acclimated to 35‰ salinity. The values of AP activities are expressed as relation to the corresponding activity at 37 °C (100%). In some cases, deviation bars were smaller than symbols used. Data are the mean ± S.E. for three individuals. (A) Levamisole-insensitive AP activity. (B) Levamisole-sensitive AP activity. Different letters indicate significant differences ($p < 0.05$).

3.4. Effect of Cu^{2+} on AP activities of chela muscle of *C. granulatus*

The effect of the heavy metal Cu^{2+} on levamisole-insensitive and levamisole-sensitive AP activities at pH 7.7 is shown in Table 1. Cu^{2+} appeared to strongly inhibit both activities (63.1–81.8% for levamisole-insensitive AP activity and 77.8–98.8% for levamisole-sensitive AP activity). Within the range of Cu^{2+} concentrations used (0.05–1.00 mM) no significant changes in extent of inhibition was observed.

Table 1
Effect of Cu^{2+} on levamisole-insensitive and levamisole-sensitive AP activities at pH 7.7 in chela muscle of *C. granulatus* acclimated to 35‰ salinity

$CuSO_4$ (mM)	% Inhibition	
	Levamisole-insensitive AP activity	Levamisole-sensitive AP activity
0.05	63.1 ± 2.4*	77.8 ± 5.1*
0.20	74.3 ± 3.1*	77.4 ± 11.3*
1.00	81.8 ± 2.7*	98.8 ± 1.3*

The values of AP activity are expressed as relation to the corresponding activity in the absence of $CuSO_4$ (100%). Data are the mean ± S.E. for three individuals.
* Significantly different from the corresponding activity in the absence of $CuSO_4$ ($p < 0.05$).

3.5. Effect of environmental salinity on AP activities in chela muscle of C. granulatus

In individuals acclimated to 35‰ salinity, chela muscle of *C. granulatus* exhibited a high levamisole-insensitive AP activity (2126 ± 6 nmol pNPP min^{-1} mg $protein^{-1}$) (Fig. 6A). In crabs acclimated to reduced salinity (10‰) levamisole-insensitive AP activity was about 30% lower (1483 ± 108 nmol pNPP min^{-1} mg $protein^{-1}$) than the activity in crabs acclimated to 35‰ salinity (Fig. 6A).

Levamisole-sensitive AP activity of chela muscle of *C. granulatus* was 550 ± 113 nmol pNPP min^{-1} mg $protein^{-1}$ in individuals acclimated to 35‰ salinity. This activity was not affected by acclimation of crabs to reduced salinity (10‰) (Fig. 5B).

4. Discussion

Our results show the occurrence of two AP activities (a levamisole-insensitive and a levamisole-sensitive AP activity) in chela muscle of the euryhaline crab *C. granulatus* from Mar Chiquita coastal lagoon. In mammals, several isoenzymatic forms have been reported which are different from each other in immunogenicity, thermostability, electrophoretic mobility and sensitivity to inhibitors (McComb et al., 1979). Levamisole, a well-known AP inhibitor, is commonly used to discriminate between different mammals AP isoforms (Cyboron et al., 1982; Chan and Stinson, 1986; Calhau et al., 2000). In euryhaline crabs, the presence of a levamisole-insensitive and a levamisole-sensitive AP activities have been also described in gill homogenates of the euryhaline crab *C. sapidus* (Lovett et al., 1994).

AP activity in mammals are characterized by exhibiting a high pH optimum (Ohkubo et al., 1974; Cyboron and Wuthier, 1981; Chan and Stinson, 1986). In invertebrates, AP activities exhibited a range of optimum pH values between 7.1 and 10.5 (Lovett et al., 1994; Funk, 2001; Mazorra et al., 2002; Xiao et al., 2002). Levamisole-insensitive and levamisole-sensitive AP activities in chela muscle of *C. granulatus* exhibited a different pH profile (Fig. 2). Highest levamisole-insensitive AP activity in chela muscle of *C. granulatus* at pH 7.7 is in agreement with optimum pH for this activity in gills of *C. sapidus* (Lovett et al., 1994). Conversely, the response to pH of levamisole-sensitive AP activity in chela muscle of *C. granulatus* was quite different to

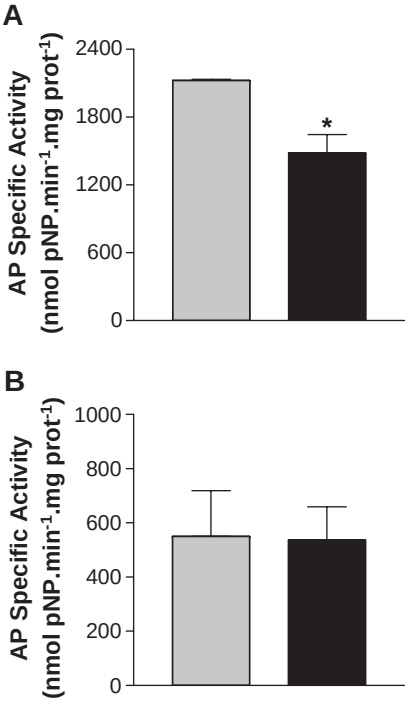

Fig. 6. Effect of acclimation to reduced salinity on AP activities at pH 7.7 in chela muscle of *C. granulatus*. Data are the mean ± S.E. for four individuals. (A) Levamisole-insensitive AP activity. (B) Levamisole-sensitive AP activity. Grey bars: individuals acclimated to 35‰ salinity; Black bars: individuals acclimated to 10‰ salinity. *Significantly different from the activity of individuals acclimated to 35‰ salinity ($p < 0.05$).

the response of this activity in gills of *C. sapidus* which exhibited an optimal pH at 9.1 (Lovett et al., 1994).

The Michaelis–Menten kinetics of AP activities of chela muscle of *C. granulatus*, in response to varying pNPP concentrations (Fig. 4), are in agreement with those previously described for levamisole-insensitive and levamisole-sensitive AP activities in gill homogenates of the euryhaline crab *C. sapidus* (Lovett et al., 1994) and for the enzyme purified from the digestive tract of the green crab *S. serrata* (Chen et al., 2000).

AP from different animal tissues show a variable sensitivity to temperature (Olsen et al., 1991; Ásgeirsson et al., 1995; Funk, 2001; Bortolato et al., 2002). In invertebrates optimum temperature appeared to be species-dependent. Levamisole-insensitive and levamisole-sensitive AP activities of chela muscle of *C. granulatus* were strongly affected by temperature. However, the sensitivity of both activities appeared to be quite different (Fig. 5). The inhibition of the AP activities of chela muscle of *C. granulatus* at high temperatures is similar to that described for AP purified from viscera of *Pinctada fucata* (Xiao et al., 2002) and from the digestive tract of *S. serrata* (Chen et al., 1997). Since *C. granulatus* in Mar Chiquita coastal lagoon is exposed to frequent changes in the environmental temperature, the differential sensitivity to temperature of these AP activities could be associated with a distinct role in thermal acclimation. However, further studies (i.e. "in vivo" experiments) are required to test this hypothesis.

Copper is one of the major marine pollutants which inhibited various enzymes (Grosell et al., 2002; Brooks and Mills, 2003). Cu^{2+} has been shown to inhibit AP activity from different tissues in several invertebrates such as *P. fucata* (Xiao et al., 2002), *S. serrata* (Chen et al., 2000) and *S. plana* (Mazorra et al., 2002). The AP activities of chela muscle of *C. granulatus* were strongly inhibited "in vitro" by Cu^{2+} (Table 1). In fishes, AP activities change in response to water with heavy metals making these activities useful as indicators of heavy metals exposure (Lan et al., 1995). The inhibition by Cu^{2+} of "in vitro" AP activity of chela muscle of *C. granulatus* could indicate their potential use in ecotoxicological studies. However, further studies about the response of this activity to Cu^{2+} and other heavy metals are necessary to establish whether they may serve as biochemical indicators of stress due to heavy metals. Furthermore, studies about toxicity of Cu^{2+} in *C. granulatus* from Mar Chiquita coastal lagoon are lacking.

The role of AP as a component of euryhaline crabs to environmental salinity is still unknown. Lovett et al. (1994) reported that levamisole-sensitive and levamisole-insensitive AP activities in the gills of *C. sapidus* decreased upon acclimation to low salinity. Because Na^+/K^+ ATPase activity in gills increased in reduced salinity, they suggested that AP may be an effector for the adaptive changes in Na^+/K^+ ATPase activity. AP was suggested to be involved in the regulation of the synthesis or delivery of polyamines which are Na^+/K^+ ATPase modulators (Lovett et al., 1994).

Recently, we have shown the occurrence in chela muscle of the euryhaline crab *C. angulatus* from Mar Chiquita coastal lagoon of an AP activity which decreased both upon acclimation and after an abrupt change to reduced salinity. This response of AP to salinity suggests that this activity could be a component of muscle regulatory mechanisms at the biochemical level secondary to hyperregulation of *C. angulatus* (Pinoni and López Mañanes, 2004). Furthermore, the antagonistic response to environmental salinity of Na^+/K^+ ATPase activity, which increased upon acclimation to reduced salinity (Pinoni and López Mañanes, 2003), and AP activities in chela muscle of *C. angulatus* suggests a physiological link between both activities (Pinoni and López Mañanes, 2004). Similar to that described in *C. angulatus*, we have found a Na^+/K^+ ATPase activity sensitive to the salinity in chela muscle of the *C. granulatus* which could suggest a role of this enzyme as well as the participation of muscle in mechanisms of adjustments secondary to hyperregulation in this crab (Pinoni and López Mañanes, 2003).

Levamisole-insensitive and levamisole-sensitive AP activities in chela muscle of *C. granulatus* appeared to exhibit a differential response to acclimation to 10‰ salinity, a salinity at which *C. granulatus* exhibits a strong hyperregulatory capacity (López Mañanes et al., 2000; Schleich et al., 2001) (Fig. 6). The lower levamisole-insensitive AP activity in chela muscle of this crab in reduced salinity suggests that this activity could also be a component at the biochemical level of adaptive responses associated with ionoregulatory process in *C. granulatus*. As described for *C. angulatus* (Pinoni and López Mañanes, 2004), the antagonistic responses of leva-

misole-insensitive AP activity and Na^+/K^+ ATPase activity of *C. granulatus* to salinity could suggest the occurrence of a physiological link between both activities as has been suggested in the gills of *C. sapidus* (Lovett et al., 1994). In muscle of the squirrel, Na^+/K^+ ATPase has been described to be regulated via dephosphorylation by AP (MacDonald and Storey, 1999). The participation of levamisole-sensitive AP activity in response at the biochemical level of *C. granulatus* associated to differential changes in environmental salinity cannot be discarded. In this context, we have described an AP activity involved in short-term responses after an abrupt change to reduced salinity in chela muscle of *C. angulatus* (Pinoni and López Mañanes, 2004).

In summary, our results show the existence of two distinct AP activities in muscle of *C. granulatus*. The response of levamisole-insensitive AP activity to low salinity suggests the participation of this enzyme in responses at the biochemical level of this crab to varying environmental salinity. Although, levamisole-insensitive and levamisole-sensitive AP activities exhibited quite distinct characteristics (i.e. response to pH, pNPP, Cu^{2+}, temperature, and salinity) a further characterization is needed to establish the occurrence of different AP isoforms in chela muscle of *C. granulatus*. Furthermore, future studies must be focused in order to establish the exact physiological roles of muscle AP activities in the integrative responses to varying environmental conditions of *C. granulatus*, thus allowing a better understanding of the multiple mechanisms underlying adaptive process of this crab.

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