ARGINASE KINETIC CHARACTERIZATION OF THE GASTROPOD *Nacella concinna* AND ITS PHYSIOLOGICAL RELATION WITH ENERGY REQUIREMENT DEMAND AND THE PRESENCE OF HEAVY METALS

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Abstract: *Arginases are metalloenzymes broadly distributed in nature. These enzymes catalyze the L-arginine hydrolyses to L-ornithine and urea. The aim of the present work is to determine the tissue levels of arginase, its kinetic properties and subcellular localization. In December 2009, specimens were collected in Admiralty Bay, King George Island near the Brazilian Research Station. The argininolytic specific activity of foot muscle, gills and pool of other tissues was 87.0 ± 15.1; 9.8 ± 1.8 and 3.8 ± 1.0 mU/mg protein, respectively. Mainly localized in the cytosol, gills and muscular arginase Km values for L-arginine were 57.0 ± 10.5 and 66.2 ± 14.6 mM, respectively. High arginase levels in gills could be related to the systemic control of L-arginine concentrations, which is vital for energetic metabolism of phospho-L-arginine and of polyamines in the control of cell proliferation though the probable physiologic metal cation is Mn^{2+}, some arginases are activated by Co^{2+} and Ni^{2+}. The muscle* **Nacella concinna** *arginases were activated by Mn^{2+} and Co^{2+} and inhibited by Cd^{2+} whereas; gills arginase was activated only by Mn^{2+} and inhibited by Cd^{2+} and Zn^{2+}.*

Keywords: Antarctica, arginase, *Nacella concinna*, heavy metal

Introduction

The organisms that inhabit the intertidal zone on the coasts of the Antarctic Peninsula and adjacent Islands are periodically exposed to the thermal regime of the terrestrial environment as well as summer melt waters. The melt water creates micro environments with low salinity and elevated levels of heavy metals derived from lithogenic sources (Ahn *et al.* 1999, 2002). In addition, microphytobenthos are considered the principal food source and are also an important natural source of heavy metals, particularly

Cd^{2+} (Ahn *et al.*, 2004; Keil *et al.*, 2008). Intertidal zones are also most vulnerable to anthropogenic pollutants. The gastropod *Nacella concinna*, is the most conspicuous macro invertebrate in the Antarctic intertidal zone, which has been used in the biomonitoring, for example in the diesel fuel spill from the vessel, Bahia Paradise, in Arthur Harbour (Kennicutt II & Sweet, 1992).

Arginases are metalloenzymes that need a divalent cation to attain maximum activity. The probable physiological

cation is Mn²⁺, though Co²⁺ and Ni²⁺ have the capacity to activate some arginases (Carvajal *et al.*, 1995). In non ureotelic organisms, the central physiological role of arginases is the control of the levels of the amino acid L-arginine (Jenkinson *et al.*, 1996).

The metabolism of the essential amino acid L-arginine, has been studied in different classes of organisms. In general, the L-arginine participates as a substrate in various metabolic processes such as synthesis of nitric oxide, protein and phospho-L-arginine, as well as, of polyamines indirectly through the non-protein amino acid L-ornithine (Figure 1) (Wu & Morris Junior, 1998; Pellegrino *et al.*, 2004).

In *N. concinna* a probable importance of argininolytic metabolism is the control of phospho-L-arginine levels. When this invertebrate is subjected to thermal stress, the phospho-arginine is used to produce ATP from ADP, followed by a reduction in the levels of L-arginine (Figure 1) (Pörtner *et al.*, 1999; Ahn *et al.*, 2004). In this case, the reduction in the L-arginine concentration could be related to tissue argininolytic activity, so studies about arginase are important for understanding some of the physiological activities of this Antarctic invertebrate. Arginase had been used as biomarker of many mammal pathological processes (Mielczarek-Puta *et al.* 2008). The present study aims to characterize tissue distribution, subcellular localization and kinetic properties of *N. concinna* arginase as a potential biomarker of the intertidal zone pollution.

Materials and Methods

Specimens of *N. concinna* (n = 5) were collected on December 2009, in the Keller Peninsula, Admiralty Bay, King George Island, Antarctica. The tissues were homogenized in 20 mM buffer Tri-HCL, pH 7.4, containing 1 mM trimethylamin-N-oxide, 5 mM of potassium phosphate, 0.5 mM EDTA, 250 mM sucrose, sonicated for 30 seconds and centrifuged at 14000 g for 10 minutes. All kinetic studies were conducted at 0 °C using supernatant. The L-ornithine formed in the reaction was measured using a spectrophotometer after reacting with ninhydrin and the activity expressed in mmol of L-ornithine formed per minute. The reaction systems used for kinetic studies are
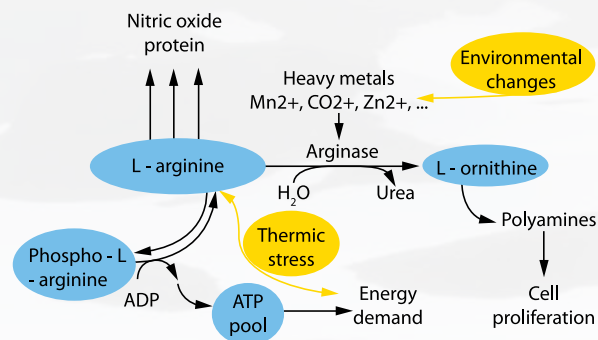

Figure 1. Conceptual diagram illustrating the pathways of L-arginine metabolism and production of phospho-L-arginine and its utilization in thermal stress involving L-arginine pools.

described in the figures. The statistical differences between the treatments and controls were obtained using one-way ANOVA followed by Tukey's post-tests.

Results and Discussion

The argininolytic activity of the gills was 9 to 23 times higher than that of the foot muscle and the pool of the other tissues respectively (Figure 2a). At subcellular level, the cytosol concentrates most of the arginase activity, both in gills (99%) and foot muscle (86.4%). Carvajal *et al.* (2004) also verified that gill cells of *Semele solida* had 91% of the arginase activity in cytosolic fraction. Most of the studies dealt with tissue levels rather than subcellular levels. The high levels of gills arginase activity could be related to the excretion of urea arising from the hydrolysis of L-arginine for the systemic control of this amino acid. The levels of arginase in the foot muscle could be related to the control of energy metabolism of phospho-L-arginine. As the presence of arginase in muscle tissue is uncommon, arginase in muscle tissue of *Chiton latus* has been associated with the removal of arginine to accelerate the utilization of phospho-L-arginine (Carvajal *et al.*, 1988).

The km values (Michaelis constant) for arginase in the foot muscle and gills were 57.0 ± 10.5 and 66.2 ± 14.6 mM, respectively. The L-arginine activity was inhibited by L-arginine concentrations above 80 and 100 mM in the foot muscle and gills, respectively (Figure 2b). The km values
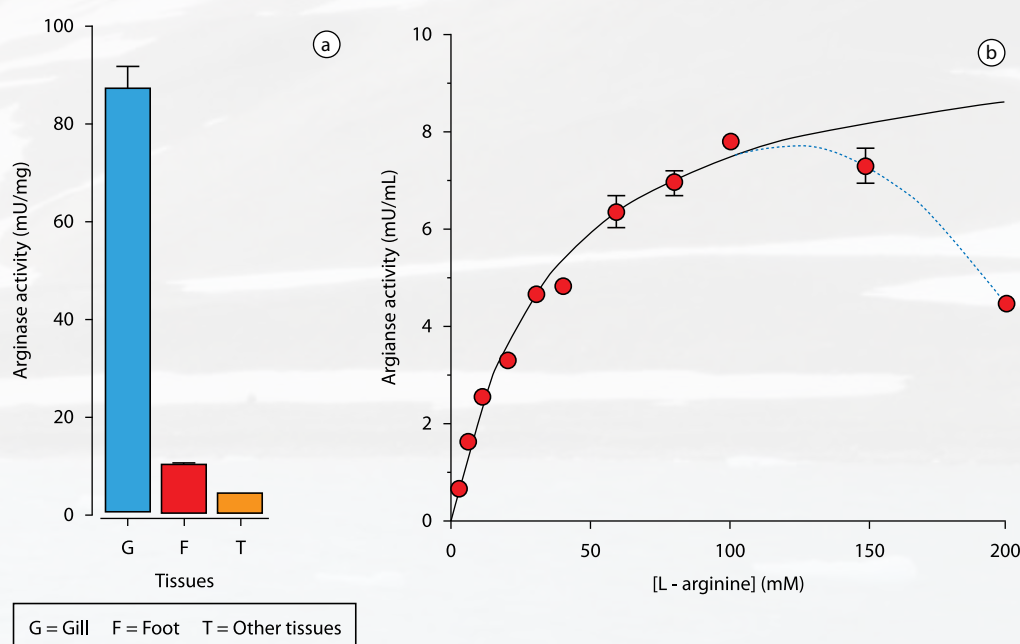
Figure 2. Tissue levels of arginase (a) and the effect of L-arginine concentration on the gills argininolytic activity of *N. concinna* (b). Results expressed as mean ± standard error of the mean.

and the inhibition by substrate L-arginine are similar to the arginase of polyplacophoran *Chiton latus*, which also has relatively high levels of arginase activity in the foot muscle and gills (Carvajal *et al.* 1988). Tormanen (1997) also observed arginase inhibition with high concentrations of substrate L- arginine in *Zebra mussel.*

The effects of heavy metals on the arginase activity in the foot muscle and gills are summarized in Figure 3. Similar to the arginases in other organisms, the gills and the foot muscle arginases of *N. concinna*, were activated in the presence of Mn^{2+}, confirming that Mn^{2+} is the probable physiological cation necessary for the activation of this enzyme. On the other hand, the foot muscle arginase was also activated by Co^{2+}, the same activation was not observed in gills tissue (Figure 3a, b). Cations like Co^{2+} have also been reported to activate some arginases, Carvajal *et al.* (1984, 1988) observed activation of arginase by Co^{2+} in gills and foot muscle arginase of *Chiton latus*, gills arginase of *Concholepas concholepas*, whereas Tormanen (1997) observed the same activation in arginase of *Zebra mussel.*

Zn^{2+} is one of the metals released by the combustion of coal, oil and gasoline. This metal can also be released from lead battery. It is also present in soils near stations which have used brass, steel coated nails and paints (Claridge *et al.*, 1995; Webster *et al.*, 2003), leaching of volcanic rocks also results in high levels of Fe^{3+} and Zn^{2+} (Ahn *et al.*, 1999; Weihe *et al.*, 2010). Arginase activity in the presence of Zn^{2+} and Fe^{3+} alone or combined with Mn^{2+} in foot muscle did not show any significant alteration, whereas, gills arginase was inhibited by Zn^{2+} and Fe^{3+} alone or combined with Mn^{2+} (Figure 4a, b). Carvajal *et al.* (1984, 1988, 1994) observed inhibition of arginases by Zn^{2+} in gills and foot muscle of *Chiton latus*, gills of *Concholepas concholepas* and gills of *Semele solida* whereas Tormanen (1997) observed same inhibition by Zn^{2+} in *Zebra mussel.*

Foot muscle and gill arginase is also inhibited by Cd^{2+}. The same inhibition is not present in gills arginase of *Semele solida* (Carvajal *et al.*, 1994). High concentrations of Cd^{2+} in surface waters of the Southern ocean is referred to as "Cd anomaly", during the austral summer the upwelling of waters favours uptake of Cd^{2+} by primary consumers
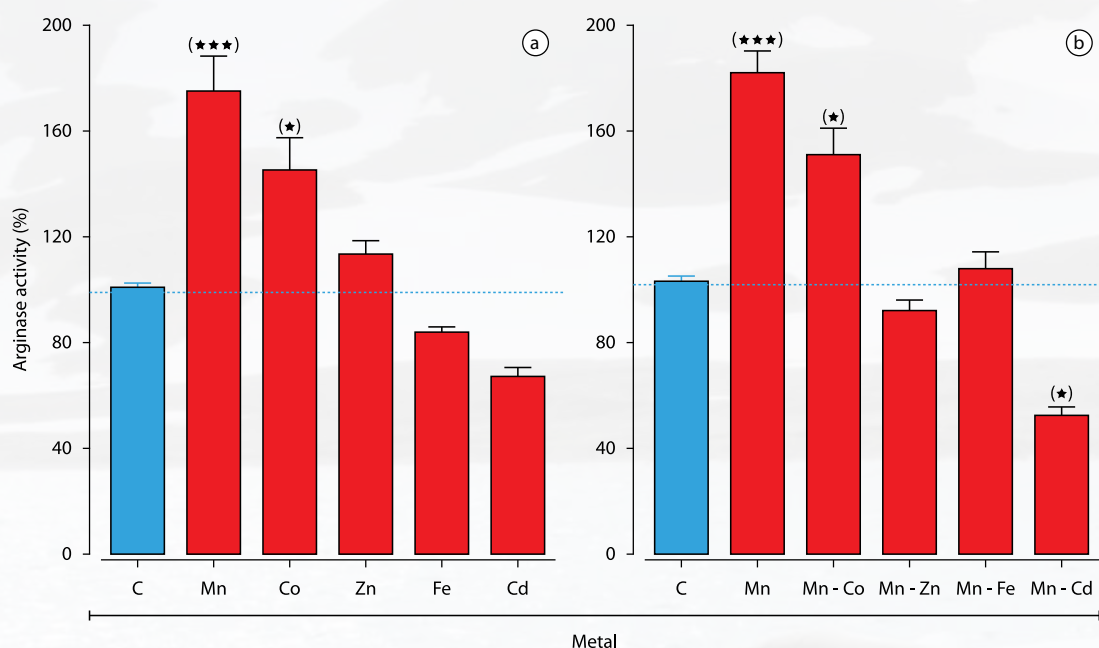
Figure 3. Effect of metallic cations on the foot muscle arginase activity of *N. concinna*. The activities were determined in 20 mM of Hepes buffer, pH 7.4, containing 30 mM of L arginine. The control activity (C) was determined in a reaction system without the addition of metallic cations. The isolated effect of 1 mM metallic cations (a) and combined effect of 1 mM Mn^{2+} with a second 1 mM metallic cation (b). Differences between control activity of arginase and the arginase activity with metals were significant for $p < 0.05$ (*) and $p < 0.001$(***).
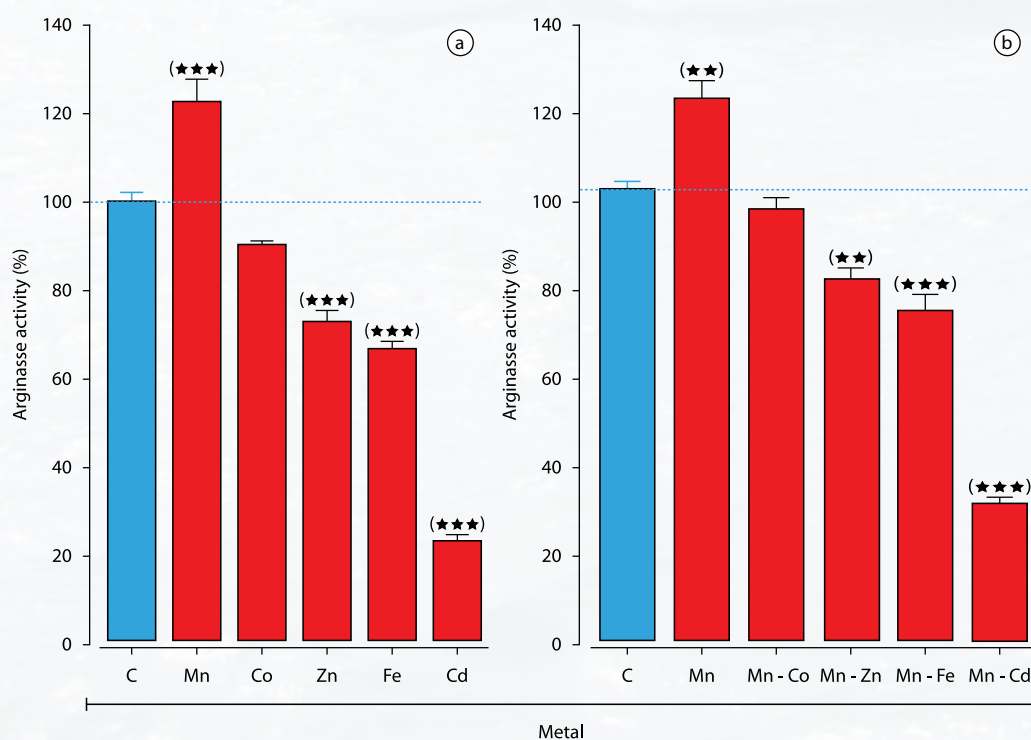

Figure 4. Effect of metallic cations on the gills arginase in *N. concinna*. The activity was determined in 20mM of Hepes buffer, pH 7.4 containing 30 mM of L-arginase. The control activity (C) was determined in a reaction system without the addition of metallic cations. The isolated effect of 1 mM metallic cations (a) and combined effect of 1 mM Mn^{2+} with a second 1 mM metallic cation (b). Differences between control activity of arginase and the arginase activity with metals were significant for $p < 0.05$ (**) and $p < 0.001$(***).

and high availability of this metal in the food chain. High concentration of this metal is found in digestive glands and kidneys of some Antarctic mollusks, this indicating binding of these metals with metallothioneins which are associated with detoxifying role (Bargagli *et al.*, 1996; Lohan *et al.*, 2001; Keil *et al.*, 2008).

Conclusion

Gills and foot muscle of *N. concinna* express arginases with distinct kinetic properties. The presence of arginase in the foot muscle supports the hypothesis that argininolytic activity can be involved in control of phopho-L-arginine metabolism. The gills and muscular argininolytic activity of *N. concinna* is basically in the cytosolic faction. The cations Mn^{2+} and Co^{2+} were capable of activating foot muscle arginase, where as Zn^{2+}, Fe^{3+} and Cd^{2+} did not inhibit significantly. The gills arginase showed a distinct behaviour, was activated by Mn^{2+} and inhibited by Zn^{2+}, Fe^{3+} and Cd^{2+}. The different behaviour of gills and foot muscle arginase of *N. concinna* can have a relation to the entry of heavy metals to these tissues.

Acknowledgements

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