Comparative Biochemistry and Physiology, Part B 227 (2019) 64–74

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part B

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

Kinetic characterization of the gill (Na^+, K^+)-ATPase in a hololimnetic population of the diadromous Amazon River shrimp *Macrobrachium amazonicum* (Decapoda, Palaemonidae)

Leonardo M. Fabri[a], Malson N. Lucena[d], Daniela P. Garçon[e], Cintya M. Moraes[a], John C. McNamara[b,c], Francisco A. Leone[a,*]

[a] Departamento de Química, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto 14040-901, Brazil
[b] Departamento de Biologia, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto 14040-901, Brazil
[c] Centro de Biologia Marinha, Universidade de São Paulo, São Sebastião 11000-600, Brazil
[d] Universidade Federal do Mato Grosso do Sul, Campo Grande, 79070-900, Brazil
[e] Universidade Federal do Triângulo Mineiro, Iturama 38280-000, Brazil

ARTICLE INFO

Keywords:
(Na^+ K^+)-ATPase kinetics
Gill microsomal fraction
Macrobrachium amazonicum
Freshwater shrimp
Comparative biochemistry

ABSTRACT

We provide a kinetic characterization of (Na^+, K^+)-ATPase activity in a posterior gill microsomal fraction from a hololimnetic population of the diadromous Amazon River shrimp *Macrobrachium amazonicum*. Sucrose density gradient centrifugation reveals two distinct membrane fractions showing considerable (Na^+, K^+)ATP-ase activity, but also containing other microsomal ATPases. Only a single immune-reactive (Na^+, K^+)-ATPase with M_r of ≈110 kDa is present that hydrolyzes ATP with V_M = 130.3 ± 4.8 nmol Pi min^{-1} mg $protein^{-1}$ and $K_{0.5}$ = 0.065 ± 0.00162 mmol L^{-1}, exhibiting site-site interactions. Stimulation by Na^+ (V_M = 127.5 ± 5.3 nmol Pi min^{-1} mg $protein^{-1}$, $K_{0.5}$ = 5.3 ± 0.42 mmol L^{-1}), Mg^{2+} (V_M = 130.6 ± 6.8 nmol Pi min^{-1} mg $protein^{-1}$, $K_{0.5}$ = 0.33 ± 0.042 mmol L^{-1}), K^+ (V_M = 126.7 ± 7.7 nmol Pi min^{-1} mg $protein^{-1}$, $K_{0.5}$ = 0.65 ± 0.0079 mmol L^{-1}) and NH_4^+ (V_M = 134.5 ± 8.6 nmol Pi min^{-1} mg $protein^{-1}$, $K_{0.5}$ = 1.28 ± 0.44 mmol L^{-1}) also obeys cooperative kinetics. Ouabain (K_I = 0.18 ± 0.058 mmol L^{-1}) inhibits total ATPase activity by ≈70%. This study reveals considerable differences in the kinetic characteristics of the gill (Na^+, K^+)-ATPase in a hololimnetic population that appear to result from the adaptation of diadromous *Macrobrachium amazonicum* populations to different limnic habitats.

1. Introduction

Decapod crustaceans first appeared in the sea during the late Devonian, about 370 million years ago. Although most remain predominantly marine in habits, the evolution of their osmoregulatory physiology has underpinned their invasion of estuarine, brackish and fresh waters. Although many crustaceans are now independent of seawater, completing their entire life cycles in fresh water, others are likely in the process of invading fresh water. This is corroborated by their lengthy, larval developmental sequences dependent on brackish water, and characteristic metabolic, osmotic and ionic regulatory patterns (Read, 1984; Moreira et al., 1986; McNamara et al., 1986; Freire et al., 2003).

Changes in environmental salinity can impose severe challenges to population genetic structure, physiology and the overall biology of individual species (McNamara et al., 2015). Thus, the occupation of a particular osmotic niche depends on the adaptations of each of a species' ontogenetic stages to its given biotope. While marine and freshwater species spend part of their life cycles in waters where salinity varies little, others migrate between brackish and freshwater biotopes, thus exposing their semaphoronts to different salinity regimes (Charmantier, 1998; Freire et al., 2003). Brackish and fresh waters are challenging environments for crustaceans since their hemolymph osmotic and ionic concentrations are held fairly constant at levels often much higher than the surrounding medium, leading to diffusive ion loss and water gain (Charmantier, 1998; Anger, 2003). As a consequence, crustaceans inhabiting these media have evolved mechanisms that regulate not only their hemolymph Na^+ and Cl^- concentrations by compensatory ion uptake but also diminish ion loss across the gills and other body surfaces (Onken et al., 1995; Péqueux, 1995; Lucu and

* Corresponding author at: Departamento de Química, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, Avenida Bandeirantes 3900, Ribeirão Preto 14040-901, Brazil.
E-mail address: fdaleone@ffclrp.usp.br (F.A. Leone).

https://doi.org/10.1016/j.cbpb.2018.09.004
Received 13 September 2018; Accepted 18 September 2018
Available online 27 September 2018

Towle, 2003; Kirschner, 2004; Freire et al., 2008; McNamara and Faria, 2012; Henry et al., 2012; McNamara et al., 2015).

The gills constitute a multi-functional organ effector system and together with other excretory organs are responsible for gas exchange, acid-base and osmotic homeostasis and the excretion of nitrogen compounds (Taylor and Taylor, 1992; Péqueux, 1995; Lucu and Towle, 2003; Freire et al., 2008; Henry et al., 2012). In the Crustacea, they provide a selective interface across which salt is actively transported between the external environment and the internal *milieu* (Freire et al., 2008; Weihrauch et al., 2004) to different degrees depending on the gradient maintained. Ion transporters such as the (Na^+, K^+)- and V(H^+)-ATPases, Cl^-/HCO_3^- and Na^+/H^+ exchangers, and carbonic anhydrase drive ion transport by the crustacean gill (Tsai and Lin, 2007; Freire et al., 2008; McNamara and Faria, 2012). Of these transporters, the evolution of function in the V(H^+)- and (Na^+, K^+)-ATPases has been considered critical for the colonization of fresh water (Morris, 2001; Tsai and Lin, 2007). (Na^+, K^+)-ATPase, expressed in all crustacean Na^+-transporting epithelia, is located in the basal membrane invaginations of the gill ionocytes, together with K^+ and Cl^- channels (Henry et al., 2012). In strong hyper-osmoregulators, key ion transporters like the V(H^+)-ATPase, Na^+ channels and Cl^-/HCO_3^- exchangers are located in the apical membrane (Onken and Riestenpatt, 1998).

Models of salt uptake across the lamellar epithelia of freshwater palaemonid shrimps propose that active gill Na^+ absorption ensues through Na^+ channels in the apical flange membranes of the pillar cells in concert with (Na^+, K^+)-ATPase activity located in membrane invaginations of the ion-transporting septal cells to which the pillar cells are anatomically coupled (McNamara and Lima, 1997; McNamara and Torres, 1999; Belli et al., 2009; McNamara and Faria, 2012). Na^+ influx is driven by H^+ extrusion via the apical pillar cell V(H^+)-ATPase that leads to cellular hyperpolarization, facilitating basal Cl^- extrusion (Torres et al., 2003; Faleiros et al., 2010). Apical Cl^-/HCO_3^- exchangers, using HCO_3^- derived from carbonic anhydrase-catalyzed CO_2 hydration are thought to transport Cl^- into the pillar cell flanges while Cl^- efflux proceeds through basal Cl^- channels either directly to the hemolymph or to the septal cells. Together with active Na^+ transport into the hemolymph by the (Na^+, K^+)-ATPase, K^+ recycling through septal cell K^+ channels generates a negative electrical potential that drives Cl^- efflux to the hemolymph (for review see Freire et al., 2008; McNamara and Faria, 2012).

The (Na^+, K^+)-ATPase, an oligomeric membrane-bound protein member of the P_{IIc} ATPase family (Geering, 2008; Poulsen et al., 2010; Chourasia and Sastry, 2012) pumps three Na^+ out of and two K^+ into cells per molecule of ATP hydrolyzed. In addition to establishing the ionic gradients that drive various membrane transport processes, this ATPase generates the membrane resting potential in excitable cells (Jorgensen et al., 2003; Martin, 2005). The (Na^+, K^+)-ATPase structure consists of a catalytic α-subunit and a chaperone β-subunit together with an FXYD peptide (Morth et al., 2007; Geering, 2008; Poulsen et al., 2010). The α-subunit consists of ten transmembrane segments (M_1-M_{10}) and contains the cation (Na^+, K^+, Mg^{2+} and NH_4^+) binding sites, the nucleotide (ATP) binding site, the specific ouabain-binding site and the protein kinase phosphorylation domains (Kaplan, 2002). The β-subunit is a highly glycosylated, single span, type II membrane protein (McDonough et al., 1990), associated with transmembrane helices αM_7 to αM_{10}. It is required for routing the α-subunit to the plasma membrane and for occlusion of the K^+ binding sites (Poulsen et al., 2010). The third subunit is single-span transmembrane protein belonging to the FXYD2 peptide family, which interacts with transmembrane helix αM_9, fine-tuning the kinetic behavior of the (Na^+, K^+)-ATPase to the specific demands of a given cell type, tissue or physiological state (Garty and Karlish, 2006; Geering, 2008; Shindo et al., 2011). This FXYD peptide constitutes part of the gill (Na^+, K^+)-ATPase from the blue crab *Callinectes danae* (Silva et al., 2012).

The Amazon River shrimp *Macrobrachium amazonicum* is endemic to South America (Holthuis, 1952) and its presumptive natural distribution includes the Orinoco, Amazon and Paraguay/Lower Paraná river basins (Magalhães et al., 2005). This diadromous shrimp has diversified into coastal populations that inhabit river stretches close to estuaries, and into continental, hololimnetic populations occupying rivers, lakes and other inland water bodies (Charmantier and Anger, 2011; Anger, 2013). Although, these two population groups apparently differ in external morphology and meristic characters, phylogenetic reconstruction suggests that they constitute a single species (Vergamini et al., 2011). *Macrobrachium amazonicum* is primarily a diadromous, freshwater species (Magalhães, 1985; Collart and Rabelo, 1996; Zanders and Rodriguez, 1992) that can migrate to brackish water for spawning, and on which it is dependent for larval development (McNamara et al., 1983; McNamara et al., 1986). The adult shrimp is a good hyper-osmotic regulator, including excellent chloride regulatory capability (Augusto et al., 2007), and has been used as a model organism for physiological and molecular studies of salinity tolerance and osmoregulatory ability (McNamara et al., 1983; McNamara et al., 1986; Zanders and Rodriguez, 1992; Faleiros et al., 2010; Charmantier and Anger, 2011), including larval growth patterns (Moreira et al., 1986) and chemical composition (Anger et al., 2009).

The gill (Na^+, K^+)-ATPase has been characterized kinetically in several ontogenetic stages of *M. amazonicum* (Santos et al., 2007; Belli et al., 2009; Leone et al., 2012), and the effects of salinity on gill chamber ultrastructure and on the differential localization of the (Na^+, K^+)- and V(H^+)-ATPases in gills and branchiostegites have been examined (Boudour-Boucheker et al., 2014). Modulation of gill (Na^+, K^+)ATP-ase activity by K^+ plus NH_4^+ (Leone et al., 2014), and inhibition by polyamines of enzyme activity and phosphorylation/dephosphorylation rates are ontogenetic stage-specific (Lucena et al., 2017a, 2017b). The kinetic proprieties of a microsomal gill V(H^+)-ATPase from juvenile and adult *M. amazonicum* have been characterized (Lucena et al., 2015).

In this study, we provide an extensive biochemical and kinetic characterization of (Na^+, K^+)-ATPase activity in a gill microsomal fraction prepared from fresh caught, free-living *M. amazonicum* taken from a hololimnetic, continental population found in the Tietê River (São Paulo State). These data are compared with those for other populations of *M. amazonicum*.

2. Materials and methods

2.1. Material

All solutions were prepared using Millipore MilliQ ultrapure, apyrogenic water. Tris, ATP ditris salt, pyruvate kinase (PK), phosphoenolpyruvate (PEP), NAD^+, NADH, imidazole, N-(2-hydroxyethyl) piperazine-N19-ethanesulfonic acid (HEPES), lactate dehydrogenase (LDH), ouabain, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), phosphoglycerate kinase (PGK), glyceraldehyde-3-phosphate (G3P), nitroblue tetrazolium (NBT), 5-bromo-4-chloro-3-indole phosphate (BCIP), alamethicin, 3-phosphoglyceraldehyde diethyl acetal were purchased from the Sigma Chemical Company (Saint Louis, USA). Dimethyl sulfoxide (DMSO) and triethanolamine were from Merck (Darmstadt, Germany). The protease inhibitor cocktail (1 mmol L^{-1} benzamidine, 5 μmol L^{-1} antipain, 5 μmol L^{-1} leupeptin 1 μmol L^{-1}, pepstatin A and 5 μmol L^{-1} phenyl-methane-sulfonyl-fluoride) was from Calbiochem (Darmstadt, Germany). Mouse monoclonal IgG α-5 antibody raised against the α-subunit of chicken (Na^+, K^+)-ATPase was from the Development Studies Hybridoma Bank, maintained by the University of Iowa (Iowa, USA). Anti-mouse IgG, alkaline phosphatase conjugate was purchased from the Promega Corporation (Madison, USA).

Crystalline suspensions of LDH and PK in 3.2 mol L^{-1} ammonium sulfate (200 μL) were centrifuged at 20,000 × *g* for 15 min, at 4 °C, in an Eppendorf Model 5810 refrigerated centrifuge (Hamburg, Germany).