

Regulation of gill (Na⁺, K⁺)-ATPase During Acclimation of *C. danae* to Different Salinities

Garçon D.P.¹, Lucena, M.N.², Farias, D.L.², McNamara J.C.³; Leone F.A.²

¹Campus Universitário de Iturama, Iturama/Universidade Federal do Triângulo Mineiro, MG, Brasil; ²Departamento de Química e ³Departamento de Biologia – Faculdade de Filosofia Ciências e Letras de Ribeirão Preto/Universidade de São Paulo, Ribeirão Preto, SP, Brasil.

INTRODUCTION: Euryhaline crabs exhibit adaptive changes in gill (Na⁺, K⁺)-ATPase activity in response to environment salinity. In general activity increases under conditions demanding greater osmoregulatory capability. **OBJECTIVE:** To perform an extensive kinetic characterization of the (Na⁺, K⁺)-ATPase expressed in the posterior gill tissue of *C. danae* acclimated to 28 and 40‰ salinity. **MATERIAL AND METHODS:** Crabs were collected from Ubatuba Bay, SP, and acclimated for 10 days at 28 or 40‰ S. Posterior gills were dissected, homogenized (15 mL/g wet tissue) and centrifuged at 20,000 ×g. The supernatant was then centrifuged at 100,000 ×g and the resulting pellet was re-suspended in the same volume of homogenization buffer. (Na⁺, K⁺)-ATPase activity was assayed at 25 °C using a PK/LDH or PGK/GAPDH linked system in which ATP hydrolysis was coupled to NADH (NAD⁺) oxidation (reduction) at 340 nm. **RESULTS AND DISCUSSION:** No changes in activity were observed for crabs acclimated to 28 or 40‰ S, while 15‰ S-acclimated crabs show an approximately 3-fold greater specific activity. ATP was hydrolyzed obeying Michaelis-Menten kinetics and ions stimulated (Na⁺, K⁺)-ATPase activity showing site-site interactions. At 40‰ salinity, *C. danae* hyporegulates hemolymph osmolality and the affinity of the (Na⁺, K⁺)-ATPase for ATP, magnesium, potassium and ammonium ions increases while that for sodium ions decreases. **CONCLUSIONS:** The (Na⁺, K⁺)-ATPase of crabs acclimated to 40‰ S exhibits a single binding site for K⁺ that can be occupied by NH₄⁺, however no additional stimulation of the (Na⁺,K⁺)-ATPase activity by ammonium ions was found.

Keywords: (Na⁺, K⁺)-ATPase; acclimation; *Callinectes danae*.

Financial support: CNPq, FAPESP, FAPEAM, INCT-ADAPTA.