Kinetic Characteristics of a Gill (Na⁺, K⁺)-ATPase from the Crab

*Callinectes danae* Acclimated to High Salinity

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The (Na⁺, K⁺)-ATPase plays a key role in maintaining the electrochemical gradients of all animal cells. Most investigations on osmoregulation in brachyuran crabs deal with the effects of reduced salinity; there have been few studies of high salinity exposure. Here, we examine (Na⁺,K⁺)-ATPase activity in the gills of *Callinectes danae* collected from Ubatuba Bay, Brazil, and acclimated to 28 or 40 ‰ salinity for 10 days, media in which the crab slightly hyper- and hyporegulates its hemolymph, respectively. Control crabs were held at 33 ‰ salinity, a medium isosmotic with the hemolymph. The posterior gills were dissected, homogenized and centrifuged at 20,000 g. The supernatant was centrifuged at 100,000 g and the pellet was re-suspended in homogenization buffer (10 mL/g wet tissue). ATPase activity was assayed at 25 °C using a PK/LDH or PGK/GAPDH linked system in which ATP hydrolysis was coupled to NADH oxidation or NAD⁺ reduction at 340 nm. Western blot analysis revealed a single immunoreactive band in crabs acclimated to 28 and 40 ‰ salinity, media in which α-subunit expression decreases. Kinetic data disclose that high salinity acclimation increases enzyme affinity for ATP, Mg²⁺, K⁺ and NH₄⁺, but decreases affinity for Na⁺ and ouabain.

Key words: (Na⁺,K⁺)-ATPase, *Callinectes danae*, high salinity acclimation

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