Effects of the long-term exposure of the *Callinectes ornatus* to 33‰ salinity on gill (Na^+,K^+)-ATPase activity

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Crustacean gills are multifunctional organs, performing respiratory gas exchange, hemolymph acid-base and osmo-ionic regulation. In addition to the excretion of nitrogenous metabolic end products, the basolateral (Na^+,K^+)-ATPase is a key enzyme in the ion-capture process. We report a comparative kinetic characterization of gill (Na^+,K^+)-ATPase in *C. ornatus,* a marine crab, acclimated to 33‰ salinity for 10 days. (Na^+,K^+)-ATPase-rich microsomes were prepared according to Garçon et al. (Comp. Biochem. Physiol. 147A: 145-155, 2006) and the enzyme activity was measured in 50 mM Hepes buffer, pH 7.5, containing 1 mM ATP, 2 mM Mg^{2+}, 50 mM Na^+ and 10 mM K^+, both in the presence and absence of 3 mM ouabain. ATP hydrolysis by (Na^+, K^+)-ATPase followed Michaelis-Menten kinetics with K_M= 0.046±0.002 mM and V= 76.2±3.5 U/mg, while enzyme modulation by magnesium (V= 74.5 U/mg, $K_{0.5}$= 0.33 mM), potassium (V= 76.4 U/mg, $K_{0.5}$= 1.03±0.05 mM), sodium (V= 76.4 U/mg, $K_{0.5}$= 5.3 mM) and ammonium ions (V= 98.9 U/mg, $K_{0.5}$= 4.1 mM) occurred according cooperative kinetics. Stimulation of enzyme activity by potassium ions in the presence of increasing ammonium ion concentrations (from 0 to 20 mM) resulted in a 30% increase on enzyme specific activity (99 U/mg), and a decrease in $K_{0.5}$ from 1.03 mM to 0.005 mM. Ouabain inhibited up to 87% of total ATPase activity (K_I= 114.9 μM), suggesting the presence of about 13% of ATP-hydrolyzing activities other than (Na^+,K^+)-ATPase. The inhibition of ATPase activity by oligomycin, bafilomycin and ethacrynic acid associated with ouabain suggests the presence of F_0F_1-ATPase, V-ATPase and K^+-ATPase, respectively, in the microsomes fractions.

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