Goniopsis cruentata crab: characterization of K^+-phosphatase activity of gill microsome (Na^+,K^+)-ATPase

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The (Na^+,K^+)-ATPase furnishes part of the driving force for transepithelial movement of monovalent ions across the gills and other transporting epithelia of many aquatic animals, including the Crustacea. Despite its importance in such physiological processes, the structure and kinetic characteristics of the crustacean gill (Na^+,K^+)-ATPase are poorly known. We characterized the K^+-phosphatase activity of (Na^+,K^+)-ATPase in gill microsomes from fresh-caught *Goniopsis cruentata* crabs using p-nitrophenylphosphate (PNPP) as substrate. Adult, intermolt *G. cruentata* were collected in an estuary from Ubatuba bay (São Paulo State, Brazil), and (Na^+,K^+)-ATPase-rich microsomes were prepared from the posterior gills according to Garçon et al. (Comp. Biochem. Physiol. 147A: 145-155, 2007). K^+-phosphatase activity was assayed continuously at 25°C in 50 mM Hepes buffer, pH 7.5 containing 10 mM p-nitrophenylphosphate (PNPP), 7 mM $MgCl_2$ and 10 mM KCl. PNPP hydrolysis by the microsomal enzyme obeyed Michaelis-Menten kinetics with K_M= 0,97 mM and V= 61,3 U/mg. K^+-phosphatase activity modulation by Mg^{2+} (V= 58,5 U/mg, $K_{0.5}$= 0,82 mM), K^+ (V= 57,5U/mg, $K_{0.5}$= 3,4 mM) and NH_4^+ (V= 62.5 U/mg, $K_{0.5}$ = 10.3 mM) took place according to site-site interactions. Na^+ (K_I= 1,93 mM) inhibited the K^+-phosphatase activity around 90%. The single inhibition curve observed for ouabain inhibition suggests the absence of (Na^+,K^+)-ATPase isoforms in the microsomal preparation. However, up to 27% of total K^+-phosphatase activity was not inhibited by 3 mM ouabain, suggesting the presence of other ATP-hydrolyzing enzymes in the microsomal preparation.

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