

Ontogeny of osmoregulation in *Macrobrachium amazonicum*: K⁺-phosphatase activity of gill tissue of juveniles

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(Na,K)-ATPase from the gill tissue of the freshwater shrimp *Macrobrachium amazonicum*, is directly involved in osmoregulation process, and this enzyme plays an important role in ammonium excretion, a vital process for these animals. Juveniles were obtained from *Macrobrachium amazonicum* juvenile supplied by FCAV/UNESP prawn hatchery (Jaboticabal, SP), and gill microsomes were prepared according to Garçon et al. (Comp. Biochem. Physiol. 147A: 145-155, 2007). K⁺-phosphatase activity was assayed using p-nitrophenylphosphate (PNPP) as the substrate. PNPP stimulated the K⁺-phosphatase activity (PNPPase activity) according to Michaelis-Menten kinetics with $V= 54.88$ U/mg and with $K_M= 1.25$ mM through. Magnesium ($V= 43.14$ U/mg; $K_{0.5}= 0.73$ mM; $n_H= 1.3$) and potassium ions ($V= 27.82$ U/mg; $K_{0.5}= 1.62$ mM; $n_H= 1.8$) modulated the enzyme activity through site-site interactions. Ammonium ions alone also stimulated the K⁺-phosphatase activity through a single saturation curve according to cooperative kinetics ($n_H= 2.6$). Maximal activity for NH₄⁺ stimulation was $V= 27.6$ U/mg and $K_{0.5}$ of 7.05 mM. In the presence of different K⁺ concentrations (1 to 5 mM), an increase of about 3-fold ($K_{0.5}$ decreased from 4.59 mM to 1.5 mM, respectively) was observed for enzyme stimulation by NH₄⁺. Contrary, the stimulation of the enzyme by K⁺ in the presence of different concentrations of NH₄⁺ (0 to 50 mM) resulted in a slight decrease of enzyme for K⁺ ($K_{0.5}$ decreased from 1.7 mM to 2.2 mM). in the presence of 5 and 10 mM NH₄⁺, respectively). Ouabain inhibited up to 80% the total PNPPase activity, and the calculated K_i estimated for K⁺-phosphatase inhibition was 59.4 μM. These results are inédited and might contribute to the understanding of the role of (Na,K)-ATPase during the ontogeny of osmoregulation of these animals.

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