Modulation of V-ATPase activity in the gills of the juvenile and adult Amazon River shrimp *Macrobrachium amazonicum*

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**Introduction:** The gills are the main sites of ion uptake. Salt uptake apparently involves the extrusion of H⁺ to the subcuticular space by an apical V-ATPase in the thin ionocytes of the distal epithelium. While gill (Na⁺,K⁺)-TPase kinetics have been analyzed in many marine and estuarine crabs, few studies have focused on the biochemical mechanisms that underlie the notable hyperosmoregulatory ability of freshwater shrimp. We examine the kinetic properties of a gill V-ATPase from the juvenile and adult Amazon River shrimp *Macrobrachium amazonicum*. 

**Material and Methods:** Gill ATPase activity was assayed spectrophotometrically at 340 nm and 25 °C using PK/LDH linked system in which ATP hydrolysis was coupled to the oxidation of NADH. Standard assay conditions were 50 mmol L⁻¹ Hepes buffer pH 7.5, containing 10 mmol L⁻¹ KCl, 50 µmol L⁻¹ orthovanadate and optimal concentrations of ATP and MgCl₂, with or without 2.0 µmol L⁻¹ bafilomycin A1. The difference in activity measured with or without bafilomycin A1 represents V-ATPase activity. 

**Results and Discussion:** The gill V-ATPase from juvenile hydrolyzed ATP at a rate of 36.59 U mg⁻¹ and K₀.₅= 0.605 mmol L⁻¹, obeying cooperative kinetics. Stimulation by magnesium (K₀.₅= 1.4 mmol L⁻¹) was also cooperative. Bafilomycin (Kᵢ= 45 nmol L⁻¹) inhibited orthovanadate-insensitive ATPase activity by around 65%. ATP hydrolysis by the gill microsomal enzyme from adult *M. amazonicum* occurred with a specific activity of 41.6 U mg⁻¹ and K₀.₅= 0.212 mmol L⁻¹, and substrate stimulation of enzyme activity occurred with positive cooperative (n=1.3). The stimulation of V-ATPase activity by Mg²⁺ (V= 39.76 U mg⁻¹; K₀.₅= 0.258 mmol L⁻¹) also involved site-site interactions. Bafilomycin inhibited about 70% orthovanadate-insensitive ATPase activity with Kᵢ= 24.23 nmol L⁻¹. 

**Conclusion:** The present investigation contributes to a better understanding of the biochemical adaptations underpinning the establishment of the Decapoda in fresh water.

**Keywords:** *Macrobrachium amazonicum*, V-ATPase, Kinetic characterization, Osmoregulation

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