

### **Gill microsome (Na,K)-ATPase of *Callinectes ornatus* crab**

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(Na,K)-ATPase-rich posterior gills of crustaceans participate actively in the osmoregulation and ammonia excretion processes. *Callinectes ornatus* (Na,K)-ATPase-rich gill microsomes were obtained according to Furriel et al. (J. Exp. Zool. 301A:63, 2004). The enzyme activity was measured in 50 mM Hepes buffer, pH 7.5, containing 2 mM ATP, 5 mM Mg<sup>2+</sup>, 50 mM Na<sup>+</sup> and 10 mM K<sup>+</sup>, both in the presence and absence of 3 mM ouabain. ATP hydrolysis by (Na,K)-ATPase followed Michaelis-Menten kinetics with  $K_m = 0.16 \pm 0.01$  mM and  $V = 116.3 \pm 5.6$  U/mg, while enzyme modulation by magnesium ( $V = 111.0 \pm 5.4$  U/mg and  $K_{0.5} = 0.54 \pm 0.03$  mM), sodium ( $V = 110.6 \pm 5.3$  U/mg and  $K_{0.5} = 6.3 \pm 0.3$  mM), potassium ( $V = 116.0 \pm 5.5$  U/mg and  $K_{0.5} = 1.5 \pm 0.1$  mM) and ammonium ions ( $V = 173.3 \pm 8.5$  U/mg e  $K_{0.5} = 5.4 \pm 0.3$  mM) occurred through site-site interactions. Interestingly, the stimulation of enzyme activity by potassium ions in the presence of increasing concentrations of ammonium ions resulted in a 50% higher specific activity. Ouabain inhibited up to 86% of total ATPase activity ( $K_i = 74.5$   $\mu$ M), suggesting the presence of about 14% of phosphatases and/or other ATPases as contaminants. The inhibition of ATPase activity by oligomycin and ethacrynic acid associated with ouabain suggests the presence of FoF1- and K-ATPase, respectively, in the microsomes.

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