SALINITY ADAPTATIONS IN THE EURYHALINE GREEN CRAB, CARCINUS MAENAS

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The green crab, *Carcinus maenas*, is a euryhaline intertidal species that can survive in salinities ranging from 40 to 8 ppt. At high salinity the crab is an osmotic and ionic conformer; but at a critical low salinity the organism makes the transition to osmotic and ionic regulation. One of the major biochemical adaptations in this transition is the induction of two major transport-related enzymes, carbonic anhydrase (CA) and the Na/K ATPase. The activities of these two enzymes increase 4 - 10 fold in specific gills during low salinity adaptation, but virtually nothing is known about the process which initiates and regulates enzyme expression.

Individuals of *C. maenas* were collected from high salinity (32 ppt) and maintained in either 32 ppt (running seawater) or 40 ppt (aquaria). Crabs were transferred directly to 10 ppt seawater in filtered recirculating tanks and sampled at various time intervals up to 14 days post-transfer. Hemolymph samples were taken and saved for osmotic, ionic, and nitrogen analysis. Gill and muscle samples were dissected out and were either assayed for CA and Na/K ATPase activity (Holleland and Towle, CBP 96B:177-181, 1990; Henry, in The Carbonic Anhydrases, Dodgson et al., eds., 1991) or frozen for later analysis of intracellular osmotic effectors. A separate group of crabs was used to measure whole-organism ammonia excretion.

Immediately upon transfer from 32 to 10 ppt the crabs undergo a typical cell volume regulatory response. Hemolymph osmotic concentrations decreased from 950±8 (N=10) to 680±20 (N=10) mOsm within 12 hrs post-transfer but remained stable thereafter. Hemodilution presumably causes cell swelling and consequent volume readjustment as evidenced by the reduction of the intracellular pool of ninhydrin positive substances (TNPS, mostly free amino acids - FAA). At the same time, hemolymph TNPS concentrations increase, indicating FAA transfer from cytoplasm to hemolymph. Hemolymph ammonia remains fairly constant, but ammonia excretion increases, suggesting that the FAAs are deaminated at a central site, possibly the gill. The cell volume regulatory response is essentially complete within 24 - 48 hr after transfer.

For crabs transferred from 32 to 10 ppt, the initial increase in CA activity was seen at 4 days post-transfer (146±16 to 456±36 μmol CO_2 mg protein^{-1} min^{-1},N=6), and values stabilized near 800 by 7 days post-transfer. Interestingly, the Na/K ATPase values were already high in gills of crabs acclimated to 32 ppt (161±42 nmol ATP mg protein^{-1} min^{-1},N=6) and did not increase further upon transfer to 10 ppt. Crabs acclimated to 40 ppt and transferred to 10 ppt showed an approximate tripling of Na/K ATPase activity, with the initial increase occurring at 7 days post-transfer. These results suggest that transport-related enzyme induction in *C. maenas* is controlled differently than in other euryhaline crustaceans, and that the induction of CA and the Na/K ATPase are controlled separately. Regardless, the cell volume regulatory response occurs prior to enzyme induction and could represent the initial signal in the regulatory pathway. Future experiments will focus on cell swelling as a trigger for CA induction.

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