Down-regulation of carbonic anhydrase activity in the gills of the euryhaline green crab, *Carcinus maenas*, during acclimation from low to high salinity

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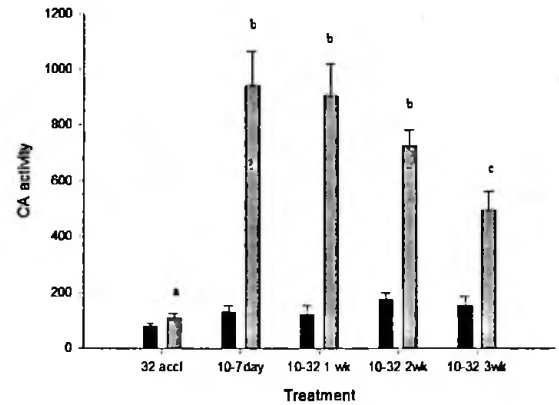
The enzyme carbonic anhydrase (CA) is known to be a central molecular component in the physiological mechanism of low salinity adaptation of euryhaline marine crustaceans[1]. Concentrated primarily in the posterior, ion transporting gills, CA has been shown to be highly sensitive to changes in environmental salinity. CA activity, which is present at low, baseline levels in crabs acclimated to 32 ppt salinity, is induced up to 10-fold in *Carcinus maenas* when crabs are transferred to 10 ppt salinity[2]. The induction process is relatively rapid; CA mRNA increases by 24 hr post-transfer, and CA activity increases between 48 and 72 hr[3]. Once induced, CA activity remains high as long as the crab remains in low salinity water. Presumably, when the crabs return to 32 ppt, CA activity also returns to baseline levels, but this phenomenon has never been investigated.

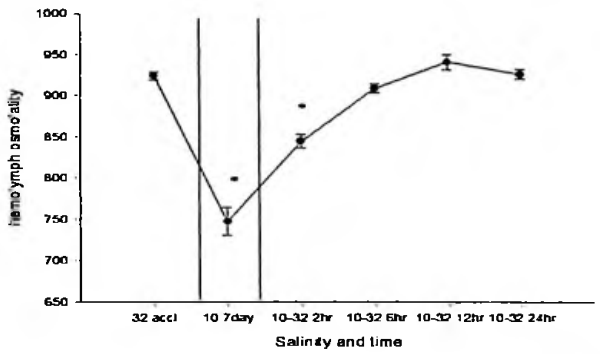
Green crabs were collected locally from the intertidal zone along the shoreline of MDIBL. Crabs were held at 32 ppt in running seawater and fed on shrimp and squid three times per week. Anterior (G4) and posterior (G8) gills were dissected out and assayed electrometrically for CA activity. Crabs were transferred to a 100 gal recirculating tank of 10 ppt equipped with a biological filter and held for 1 week. At that time, CA activity was measured in G4 and G8, and the remaining crabs were transferred back to 32 ppt. CA activity was then measured in anterior and posterior gills of crabs at 1 week intervals for 3 weeks. Hemolymph samples were also taken from each crab, frozen at -20°C, and later thawed for determination of total osmotic concentration.

Carbonic anhydrase activity (μmol CO_2 mg protein^{-1} min^{-1}) in anterior (G4, black bars) and posterior (G8, gray bars) gills of green crabs acclimated to 32 ppt, transferred to 10 ppt for 7 days, and then transferred back to 32 ppt for up to 3 wks. Mean ± SEM (N=6-8). Different letters indicate significant differences at the 0.05 level for G8 among different treatments.



As shown previously, CA activity in G4 and G8 in green crabs acclimated to 32 ppt is uniformly low (Fig. 1). After 7 days at 10 ppt, there is an approximate 8-fold induction of CA activity. These high levels of CA activity persist virtually unchanged for at least 2 weeks after transfer back to high salinity. Only by 3 weeks does CA activity begin to return towards baseline values. There were no significant changes in anterior gills.

Figure 2. Hemolymph osmolality (mOsm kg H_2O^{-1}) in green crabs acclimated to 32 ppt seawater (905 mOsm), transferred to 10 ppt (335 mOsm) for 7 days, and then transferred back to 32 ppt. Mean ± SEM (N=6-8). Asterisks denote significant differences at the 0.05 level from the 32 ppt acclimated values.



It appears that once CA induction is complete, the CA protein has a long biological half-life in the gill. Hemolymph osmolality returns to high salinity values within 12 hr after transfer from 10 to 32 ppt (Fig. 2), but branchial CA activity remains significantly elevated for 3 weeks, even though active ion transport is no longer taking place across the gills. This may be an adaptive mechanism that the crab possesses in response to the estuarine environment in which it can be exposed to both low and fluctuating salinity. Once induced, high CA activity is retained for an extended period of time as a buffer against transient changes in external salinity. Protein synthesis is metabolically expensive process; it is therefore energetically conservative to have a degradation process that is slow to respond to environmental changes and thus allows the crab to retain high levels of CA once they are achieved. Changes in CA mRNA expression are currently under investigation.

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3. **Henry, R.P., Gehnrich, S., Weihrauch, D., and Towle, D.W.** Salinity-mediated carbonic anhydrase induction in the gills of the euryhaline green crab, *Carcinus maenas*. Comp. Biochem. Physiol. 136A:243-258. 2003.