# A carbonic anhydrase repressor is localized in the sinus gland of the eyestalk in the euryhaline green crab, *Carcinus maenas*

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Euryhaline crustaceans, such as the green crab, can survive large reductions in environmental salinity, primarily because they can regulate the osmotic concentrations in their hemolymph above those in low salinity waters. This is accomplished through the active uptake of salts (e.g., $Na^+$ and $Cl^-$), specifically by the posterior three pairs of ion-transporting gills. The enzyme carbonic anhydrase (CA) has been shown to be a central component of the molecular mechanism of low salinity adaptation in these species. High levels of CA activity are found in the posterior gills, and that activity is induced up to 10 fold during acclimation to low salinity[1].

CA induction is believed to be under transcriptional regulation, with CA mRNA increasing initially after low salinity exposure, and CA activity following thereafter, presumably as a result of the synthesis of new CA protein[2]. Furthermore, it is believed that CA expression is under inhibitory regulation by a repressor that is present in the eyestalk of the crab at high salinity[3]. Injections of eyestalk extract inhibit normal salinity-stimulated CA induction by up to 70%. The eyestalk contains the major endocrine complex of the crab, the X-organ/sinus gland complex. The sinus gland is also known to contain a family of inhibitory peptides known as the crustacean hyperglycemic hormone (CHH) family. The CA repressor appears to have some functional similarity to these inhibitory peptides, and so it is tempting to suggest that it is a related member of this group that is also stored in the sinus gland. This report represents the initial direct testing of that hypothesis.

Adult, intermolt green crabs were either collected locally from the shoreline around MDIBL or were purchased from the Marine Biological Laboratory, Woods Hole, MA. Crabs were maintained in running seawater at 31 ppt salinity and 12°C. They were transferred directly to 10 ppt for a period of 4 days. Crabs were either untreated or given daily injections of either sinus gland extract or medullary tissue extract from the eyestalks. For the eystalk dissections, crabs were chilled on ice and the eyestalks were removed at their base with dissecting scissors. The eyestalks were kept on ice, and the interior tissue was removed intact with a dissecting needle. The sinus gland was then separated out from the remaining medullary tissue under a dissecting microscope. For each injection, individual crabs were given the equivalent of two sinus glands, or the medullary tissue from two eyestalks, homogenized in 500 µL of filtered seawater. After 4 days, anterior (G4) and posterior (G8) gills were dissected out of the crabs and assayed electrometrically via the delta pH method for CA activity.

For crabs acclimated to 32 ppt, CA activity was uniformly low in anterior and posterior gills. Transfer to 10 ppt for 4 days resulted in an approximate four-fold induction of CA activity in posterior gills only. Daily injections of sinus gland extract inhibited this CA induction by nearly 50% (Fig. 1). Injections of extract of the remaining medullary tissue of the eyestalk had no effect on CA induction.

These results demonstrate that the previously reported effectiveness of whole eyestalk extracts on inhibiting low salinity mediated CA induction can be traced to a CA repressor localized in the major neurohemal organ of the eyestalk, the sinus gland.

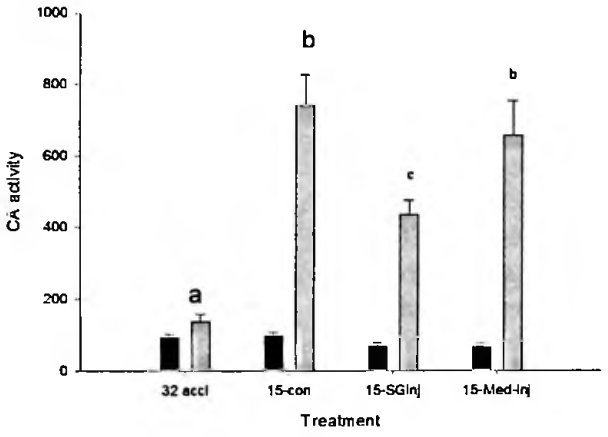


Figure 1. Carbonic anhydrase activity (μmol $CO_2$ mg protein$^{-1}$ min$^{-1}$) in anterior (G4, black bars) and posterior (G8, gray bars) gills of green crabs acclimated to 32 ppt and transferred to 15 ppt for 4 days. Con = controls; SG-inj = crabs injected with sinus gland extract; Med-inj = crabs injected with extract of medullary tissue. Mean ± SEM (N=6-8). Letters over the bars indicate significant differences in G8 across the treatments.

Now that the putative CA repressor has been localized to the sinus gland, HPLC fractionation of the gland's contents will be undertaken, and inhibition of CA induction will be used as the bioassay for identification of the peak with CA repressor activity.

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1. **Henry, R.P., Garrelts, E.E., McCarty, M.M., and Towle, D.W.** Differential induction of branchial carbonic anhydrase and $Na^+/K^+$ ATPase in the euryhaline crab, *Carcinus maenas*. J. Exp. Zool. 292:595-603. 2002.
2. **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.
3. **Henry, R.P.** Suppression of branchial carbonic anhydrase induction by a compound in the eyestalk of the green crab, *Carcinus maenas*. Bull. Mt. Desert Island Biol. Lab. 40:35-36. 2001.