A carbonic anhydrase repressor is found in the hemolymph of the euryhaline green crab, *Carcinus maenas*

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The green crab, *Carcinus maenas*, can survive in salinities between 40 and 5 ppt. An osmotic and ionic conformer at high salinity, the green makes the transition to osmotic and ionic regulation at a critical salinity of 26 ppt. At 10 ppt the crab can maintain its hemolymph osmolality nearly 300 mOsm higher than that in the ambient water, primarily by the active uptake of Na^+ and Cl^- across the gills. Adaptation to low salinity involves the up-regulation of a suite of ion-transport proteins in the posterior, ion transporting gills, including the enzyme carbonic anhydrase (CA). CA activity in the posterior gills is induced approximately 8 fold after transfer from 32 to 10 ppt[1].

The induction of CA activity appears to be under transcriptional regulation, as CA mRNA increases at 24 hr after transfer from 32 to 10 ppt, and protein-specific CA activity increases immediately thereafter (48 hr post-transfer)[2]. CA activity is therefore a measure of CA gene expression. Furthermore, this process also appears to be under inhibitory control by a CA repressor, present at high salinity in the major endocrine organ of the crab, the eyestalk, which maintains CA expression at baseline levels[3]. The question remains, however, whether this putative CA repressor is transported via the hemolymph to the gills, the presumptive site of its inhibitory action.

A series of experiments were designed to determine whether there is a CA repressor present in the hemolymph of the green crab at high salinity and whether it is reduced or absent at low salinity, thus allowing CA induction to occur. Green crabs were acclimated to 33 ppt and then transferred directly to 12 ppt, either intact (controls) or with one of a variety of treatments. Eyestalk ablation (ESA) was used to remove the presence of the repressor, and injections of hemolymph from crabs, acclimated to either high or low salinity, were used to directly test for the repressor's presence. Crabs were treated for a 4 day time course, at which time they were killed, and the anterior (G3) and posterior (G7) were dissected out and assayed for CA activity.

At 33 ppt, CA activity was uniformly low in G3 and G7 (Fig. 1). Transfer to 12 ppt for 4 days resulted in a 4-fold induction of CA activity in G7 only. The anterior gills serve as internal controls. Injection of filtered seawater (sham operated crabs) had no effect on CA induction. Injection of hemolymph from green crabs acclimated to 32 ppt had a dose-dependent inhibitory effect on CA induction. Injections of each specific volume of hemolymph were given twice daily over the 4 day experimental time course. Injections of 0.5 and 1.0 ml resulted in approximately a 30% reduction in normal CA induction (Fig. 1, 0.5 and 1 ml), and 2 ml injections resulted in a 50% inhibition. The latter value is similar to that seen with injections of eyestalk extract[1].

Injections of hemolymph from crabs that had been acclimated to 12 ppt for 7 days, a condition in which the CA repressor is known to be down-regulated[4], had no effect on normal CA induction (Fig. 1, 12 ppt). Furthermore, hemolymph taken from crabs that had been treated with eyestalk ablation for 1 week, thus removing the source of the CA repressor, also had no effect on CA induction in intact crabs (Fig. 1, ESA). And finally, injections of hemolymph taken from a stenohaline species, *Cancer irroratus*, had no effect on CA induction in *C. maenas* (Fig 1. C.i.).

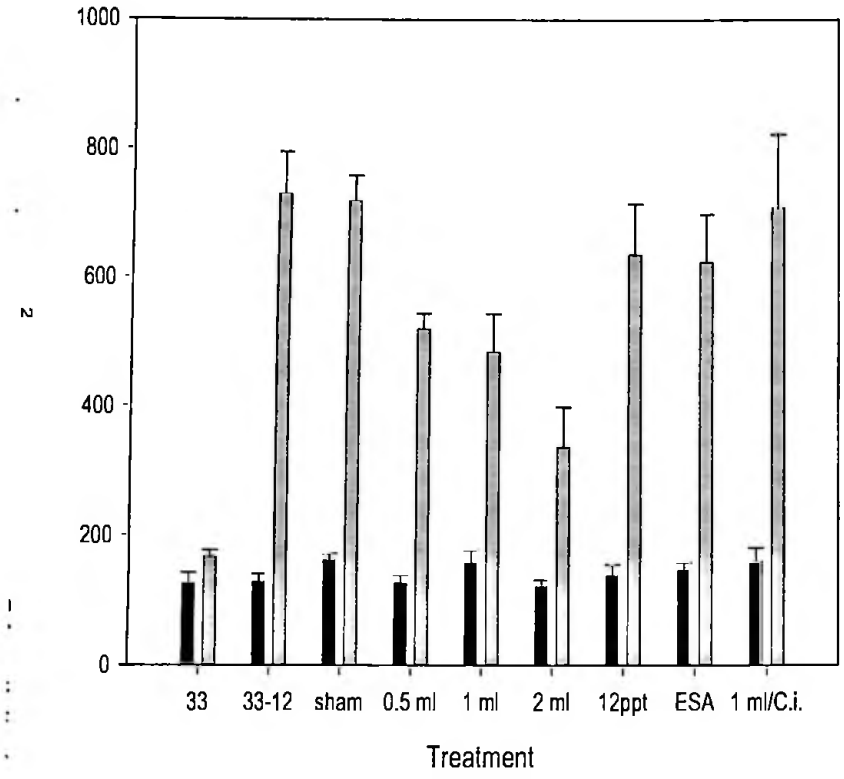

Figure 1. Carbonic anhydrase activity in G3 (dark bars) and G7 (light bars) from green crabs acclimated to 33 ppt and transferred to 12 ppt after various treatments. See text for details. Mean ± SEM (N=6-12).

These results show a similar pattern to those observed for direct injections of the eyestalk extract itself, indicating that the CA repressor is present in the hemolymph of green crabs when they are acclimated to high salinity. Injections of 2 ml of hemolymph from 32 ppt-acclimated animals, twice daily, are equivalent to daily injections of the contents of two eyestalks. And as is the case with eyestalk injections, the CA repressor appears to be reduced or absent in hemolymph of crabs acclimated to low salinity. The compound also appears to be absent from the hemolymph of stenohaline species in which there is no CA induction. It is plausible to suggest that the CA repressor in the hemolymph is the same compound that is synthesized in the eyestalks, and that it is released into the hemolymph and travels to the gills via this route.

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