A BRANCHIAL CARBONIC ANHYDRASE REPRESSOR IS DOWN-REGULATED IN THE EYESTALKS OF THE EURYHALINE CRAB, *CARCINUS MAENAS*, AFTER ACCLIMATION TO LOW SALINITY

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Carbonic anhydrase (CA) activity is induced tenfold (from approximately 120 to 1,200 umol CO_2 mg protein^{-1} min^{-1}) in the posterior, ion transporting gills (G7-9) of the euryhaline green crab, *Carcinus maenas*, after transfer from 33 to 10 ppt salinity (Henry et al., Bull. Mt. Desert Island Biol. Lab. 38:55, 1999). This induction is a result of salinity-mediated gene activation and increased expression of CA mRNA (Gehnrich et al., Bull. Mt. Desert Island Biol. Lab. 40:114-115, 2001). The increase in mRNA expression occurs 24 hours after low salinity transfer and immediately precedes the initial increase in CA activity. Induction of CA activity is enhanced by removal of the major endocrine complex of the crab, the eyestalk. Eyestalk ablation (ESA) resulted in a doubling of CA activity in posterior gills in crabs acclimated to 32 ppt and not given a low salinity stimulus, and it potentiates the salinity-stimulated response by about 20% (Henry et al., Bull. Mt. Desert Island Biol. Lab. 39:21-22, 2000). Anterior gills served as control tissues since CA activity in this tissue does not respond to either low salinity or ESA.

These data served as the basis for postulating the existence of a repressor substance in the eyestalks of *C. maenas* acclimated to high salinity, which inhibits CA expression and keeps CA activity at baseline levels. The effect of this putative repressor is either reduced or removed upon exposure to low salinity, allowing CA induction to occur. Furthermore, injection of extracts taken from the eyestalks of crabs acclimated to 33 ppt, inhibits CA induction by 50% in intact (untreated) crabs transferred from 33 to 10 ppt (Henry, Bull. Mt. Desert Island Biol. Lab. 40:35-36, 2001). The putative CA repressor was found to be absent from the eyestalks of a stenohaline species, *Cancer irroratus*, as injections of eyestalk extract from that species into *C. maenas* had no inhibitory effect on low salinity-stimulated CA induction (Henry, Bull. Mt. Desert Island Biol. Lab. 41:52-53, 2002). Branchial CA induction is therefore either a result of the down-regulation of the repressor in low salinity or a cessation of its secretion from the endocrine complex of the eystalk. If the former is true, the repressor should be absent from the eystalk; if the latter, the repressor should be present in the eyestalk but not released. This idea was directly tested in the following experiments.

C. maenas were collected from Frenchman's Bay, maintained at 33 ppt in running seawater, and fed mussels. *C. maenas* were transferred directly to a 75 gallon recirculating aquarium of 10 ppt salinity untreated (control). After 4 and 7 days post-transfer, CA activity was measured in anterior (G3) and posterior (G7) gills. At 4 days after transfer to low salinity, there was a significant increase in CA activity in G7 (from about 200 to almost 1,000 umol CO_2 mg protein^{-1} min^{-1}) and no change in G3 (Table 1). CA activity continues to increase in G7 over 7 days post-transfer, reaching 1,869 umol CO_2 mg protein^{-1} min^{-1} (a ninefold increase in this case).

In a second set of experiments, *C. maenas* were acclimated to 10 ppt for a period of 7 days. At that point, crabs were either left untreated (controls) or subjected to ESA. Both groups

were left at 10 ppt for another 7 days and then assayed for CA activity in G3 and G7. There was no change in CA activity in G3, and there was no significant difference in activity in G7 between the control and the ESA-treated crabs (Table 1). ESA, which potentiates CA induction in the acute phase of low salinity acclimation, has no effect on CA activity after acclimation is complete. This suggests that whatever repressor is present in the eyestalk at high salinity is gone once the crab has acclimated to low salinity, as removal of the eyestalk has no further effect on CA activity.

In a third set of experiments, intact *C. maenas* acclimated to 33 ppt were then transferred to 10 ppt and given a daily injection of eyestalk extract taken from *C. maenas* acclimated to either 33 ppt or 10 ppt. Crabs were injected through the arthroidial membrane covering the hemolymph sinus at the base of the walking leg. Two eyestalks were homogenized in 0.5 ml of filtered seawater and centrifuged at 10,000 g for 10 min at 4°C. Each crab was given a 400 ul injection of the supernatant immediately before transfer to 10 ppt and then injected daily for a period of 4-7 days thereafter, at which point CA activity in G3 and G7 was measured. Eyestalk extract from *C. maenas* acclimated to 33 ppt reduced CA induction by 50% at either 4 or 7 days after transfer to low salinity, compared to untreated crabs (Table 1). However, eyestalk extract from *C. maenas* acclimated to 10 ppt had a significantly reduced effect (15%) on salinity-stimulated CA induction in the gills of *C. maenas* ($p < 0.05$, t-test).

Table 1. Effects of salinity transfer, ESA, and injection of eyestalk extracts on CA activity in anterior (G3) and posterior (G7) gills of the green crab, *Carcinus maenas*. Injections were performed daily for a period of 4 to 7 days. CA activity reported as umol CO_2 mg protein^{-1} min^{-1}. Mean ± SEM (N). T = 12°C.

	CA Activity	
Treatment	Anterior (G3)	Posterior (G7)
33 ppt acclimated	117 ± 15 (6)	215 ± 20 (6)
33-10 ppt transfer, untreated, 7 days	155 ± 21 (6)	1,869 ± 218 (6)
10 ppt acclimated, untreated, 7 days	122 ± 20 (7)	1,650 ± 205 (12)
10 ppt acclimated, ESA, 7 days	136 ± 11 (5)	1,693 ± 168 (9)
33-10 ppt transfer, untreated, 4 days	205 ± 15 (8)	991 ± 72 (8)
33-10 ppt transfer, 33 ppt eyestalk injections, 4 days	111 ± 7 (5)	524 ± 20 (5)
33-10 ppt transfer, 10 ppt eyestalk injections, 4 days	127 ± 11 (5)	850 ± 75 (5)
33-10 ppt transfer, 33 ppt eyestalk injected, 7 days	61 ± 5 (10)	593 ± 53 (10)
33-10 ppt transfer, 10 ppt eyestalk injected, 7 days	122 ± 8 (5)	1,228 ± 332 (5)

These results confirm and extend the original hypothesis of the presence and action of a carbonic anhydrase repressor in the eyestalk of *C. maenas*. The effects of this substance are removed after exposure to low salinity, as shown by the results of the two experiments in Table 1. In green crabs acclimated to low salinity, the substance and/or its effects are absent, as evidenced by ESA having no effect on CA induction in crabs acclimated to 10 ppt. The absence of effect appears to be a result of the repressor being down-regulated in the eyestalks of crabs acclimated to 10 ppt, as injections of eyestalk extract from crabs acclimated to 10 ppt have much less inhibitory potency compared to those from crabs acclimated to 33 ppt. The inhibitory effect is not entirely lost, however, and that may be related to the crab's lower lethal limit of salinity

acclimation. Green crabs can survive down to 5 ppt, so if the CA repressor is down-regulated in a manner that is salinity-dependent, there could still be some expression of the compound present until the crab reaches its absolute limit with respect to survival in low salinity.

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