# A hemolymph-borne carbonic anhydrase repressor is effective at the level of CA mRNA expression in the euryhaline green crab, *Carcinus maenas*

Katherine Smith[1,3] & Raymond Henry[2,3]

[1]Department of Biology
University of New Hampshire, Durham, N.H.
[2]Department of Biological Sciences
Auburn University, Auburn, AL 36849
[3]Mount Desert Island Biological Laboratory
Salisbury Cove, ME 0467

The enzyme carbonic anhydrase (CA) is a central molecular component of the mechanism of active ion uptake in the gills of euryhaline crustaceans. In the posterior, ion transporting gills of the euryhaline green crab, *Carcinus maenas*, CA activity is induced by about 6 fold in response to transfer from 32 ppt to 10 ppt salinity.[1] This induction is under transcriptional control: low salinity results in an increase in CA expression, as measured by an increase in CA mRNA, and that, in turn, leads to the synthesis of new enzyme.[2] CA gene expression, in turn, appears to be under the control of a repressor compound found in the major endocrine complex of the crab, the eyestalk. Present in the eyestalk of crabs acclimated to high salinity, this compound keeps CA expression (and therefore, CA activity) at low, baseline levels. When crabs are exposed to low salinity, the repressor is down-regulated, thus allowing for increased CA expression, which results in the induction of CA activity.[3,4] Recently, it was shown that the putative CA repressor is also found in the hemolymph of green crabs and that it inhibits normal salinity-mediated induction of CA activity.[5] As with the results from studies on the repressor from the eyestalks, the CA inhibitor is present and effective in the hemolymph of crabs that are acclimated to 32 ppt but absent from crabs treated with eyestalk ablation or from crabs acclimated to low salinity. It is believed that the hemolymph is the route of transport from the eyestalk to the target tissue, the gill. This report tests the hypothesis that the hemolymph-borne CA repressor acts at the level of gene expression in the posterior gills, preventing the salinity-stimulated increase in CA mRNA and thus preventing CA induction.

Green crabs were collected locally and held at 32 ppt salinity. One subset of crabs was transferred directly to 10 ppt for 4 days with no other treatment. A second subset of crabs was transferred to 10 ppt and given twice daily injections of 2 mL of hemolymph taken from crabs acclimated to 32 ppt, a salinity at which the CA repressor is known to be present.[5] The gills were then dissected out and used in the following manner. Anterior (G4) and posterior (G8) gills from the left side of the crab were assayed for CA activity, and G4 and G8 from the right side of the crab were used for total RNA extraction. The RNA was then reverse transcribed, and gene specific primers were used to monitor CA mRNA levels through quantitative PCR (Stratagene MX 4000). The sample with the highest level of CA activity was used to construct an internal standard curve against which the other samples were compared.

For the 32 ppt acclimated crabs, CA activity was uniformly low in anterior and posterior gills (Fig. 1). There was a 3-fold induction of CA activity in the posterior gills in crabs transferred to 10 ppt for 4 days ($174 \pm 22$ to $575 \pm 51$ μmol $CO_2$ mg protein$^{-1}$ min$^{-1}$). This level of induction was reduced by 84% (239 μmol $CO_2$ mg protein$^{-1}$ min$^{-1}$; Fig 1) by the hemolymph injections. CA activity in the anterior gills, the control, non-ion transporting tissue, was unaffected by either low salinity or hemolymph injection.

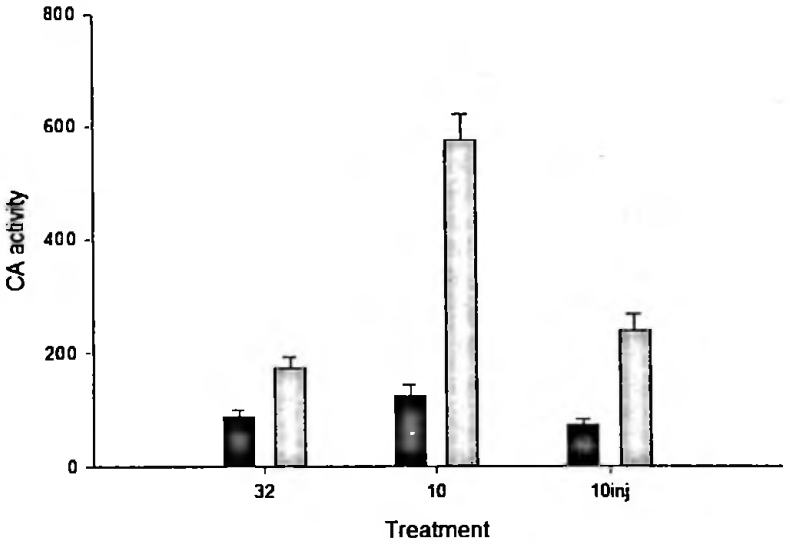


Figure 1. Carbonic anhydrase activity (μmol $CO_2$ mg protein$^{-1}$ min$^{-1}$) in anterior (G4, black bars) and posterior (G8, gray bars) gills of *C. maenas* given the following treatments: 32 = crabs acclimated to 32 ppt; 10 = crabs transferred to 10 ppt for 4 days; 10inj = crabs transferred to 10 ppt for 4 days and given injections of 2 mL of hemolymph from 32 ppt acclimated crabs twice daily. Mean ± SEM (N = 6).

The same pattern was seen for CA mRNA expression (Fig. 2). Levels were low and uniform in anterior and posterior gills in crabs acclimated to 32 ppt, but there was a 4.5-fold increase in CA mRNA expression in posterior gills after 4 days of exposure to 10 ppt. This increase in expression was inhibited by 87% by the hemolymph injections.

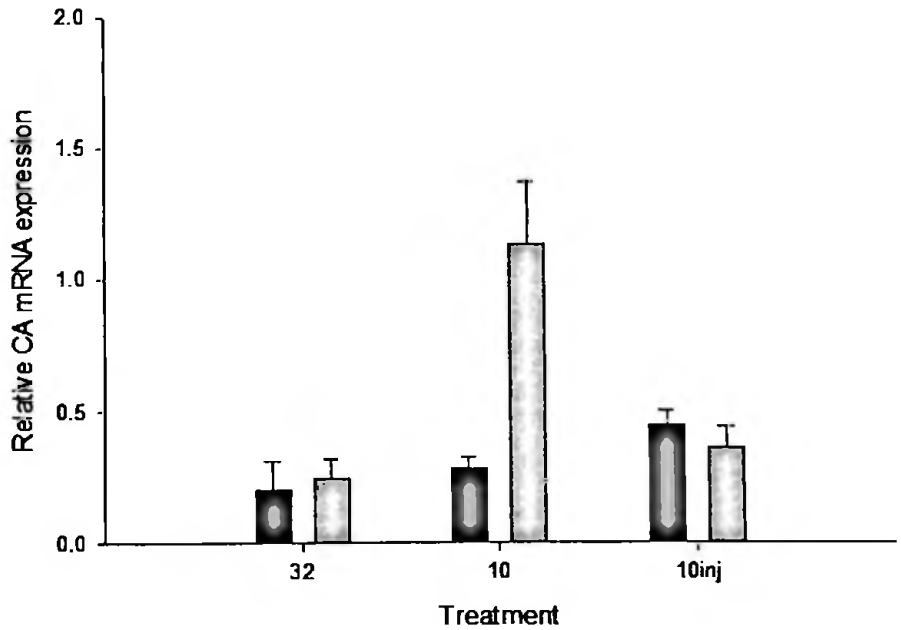


Figure 2. Carbonic anhydrase mRNA expression (in relative units) in anterior (G4, black bars) and posterior (G8, gray bars) gills of *C. maenas* given the following treatments: 32 = crabs acclimated to 32 ppt; 10 = crabs transferred to 10 ppt for 4 days; 10inj = crabs transferred to 10 ppt for 4 days and given injections of 2 mL of hemolymph from 32 ppt acclimated crabs twice daily. Mean ± SEM (N = 6).

The CA repressor appears to function at the transcriptional level, inhibiting the increase in CA mRNA that normally occurs in response to low salinity exposure.

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5. **Smith, K., and Henry, R.P.** A carbonic anhydrase repressor is found in the hemolymph of the euryhaline green crab, *Carcinus maenas*. *Bull. Mt. Desert Island Biol. Lab* 43:108-109. 2004.