Short-term salinity-induced changes in branchial carbonic anhydrase activity and mRNA expression in the blue crab *Callinectes sapidus*

Laetitia Serrano[1] & Raymond P. Henry[1,2]

[1]Department of Biological Sciences
Auburn University, Auburn, AL 36849
[2]Mount Desert Island Biological Laboratory
Salisbury Cove, ME 04672

Euryhaline crustaceans can survive wide variations in environmental salinity by maintaining a relatively constant hemolymph osmolality and ion content through active ion uptake from the external medium, across the gills, to the hemolymph. This process occurs almost exclusively in the posterior gills. The enzyme carbonic anhydrase (CA) is believed to support the active transport of salts by providing counterions for general cation and anion exchange (e.g., Na^+/H^+ and Cl^-/HCO_3^-) through the catalyzed hydration of CO_2 in the branchial cytoplasm [1].

Cytoplasmic CA activity is highly salinity-sensitive: it undergoes a 6 to 10 fold induction in posterior gills of green crabs *Carcinus maenas* acclimated to 10 ppt [2] and blue crabs *Callinectes sapidus* acclimated to 15 ppt [4]. This induction is initiated as early as 24 and 48 hr in the blue crab [4] and green crab [3], respectively, and it takes between 4 and 7 days, respectively, to reach new acclimated levels. Recent studies have demonstrated that CA induction in the green crab is under a transcriptional regulation, as the relative amount of CA mRNA increases at 24 h after transfer to low salinity, prior to the initial increase in CA activity [3, 5].

The majority of studies on CA induction have been conducted on acclimation period from 24 h to 2 weeks, but little data are available on short-time effects of low salinity acclimation. To assess this issue, we examined the variation in CA activity and mRNA expression in gills of blue crabs acclimated to 35 ppt and transferred to low salinity over a 24 h period, the time during which the initial increase in CA activity occurs [4].

Blue crabs, fully acclimated to 35 ppt, were directly transferred to 15 ppt (T=0). Before transfer and at 2, 6, 12 and 24 hours after transfer, hemolymph samples were withdrawn from the infrabranchial sinus at the base of the fifth pereiopod and frozen at -20°C until osmolality determination. Anterior (G3) and posterior (G7) gills from both sides of the crabs were dissected out and used for the measurement of CA activity (right gills) and for total RNA extraction (left gills) (RNAgents Total RNA Isolation System, Promega). Poly-A mRNA in 2 µg of total RNA was reverse transcribed in single stranded complementary DNA (Superscript II First Strand Synthesis, Invitrogen). Specific primers were designed according to the partial sequence of CA cloned from blue crab gills (data obtained previously at Auburn University) and showing 70% identity with the cytoplasmic CA isoform reported in the green crab gills [3]. Primers were used to amplify CA cDNA templates for quantitative PCR on a Stratagene MX 4000 real-time PCR instrument. A sample of high CA expression (G7 at 6 h) was used to construct the internal standard curve and the non ion-transporting gills (G3) were used as tissue control.

After transfer to low salinity, the hemolymph osmolality sharply decreased during the initial 12 h post-transfer (from 1110 to 820 $mosm.kg^{-1}$) and stabilized around 780 $mosm.kg^{-1}$, corresponding to the new steady-state as previously described [4] (Fig. 1).

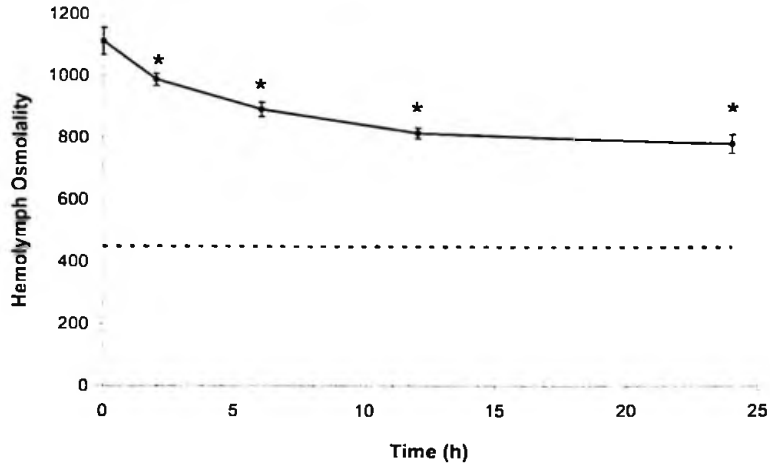

Fig. 1. Hemolymph (solid line) and diluted seawater (dashed line) osmolality (mosm.kg^{-1}) for *Callinectes sapidus* acclimated to 35 ppt (T=0) and transferred to 15 ppt for 24 hours. Mean ± S.D., N=4-10. Statistical comparisons with T=0: * $p<0.001$.

The CA activity was low and not significantly different in G3 and G7 of crabs acclimated to 35 ppt (Fig. 2). No changes in CA activity were observed in G3 over the time course of 15 ppt acclimation. However, the low salinity exposure induced a significant 77% increase in G7 occurring at 24 h after transfer ($p<0.005$).

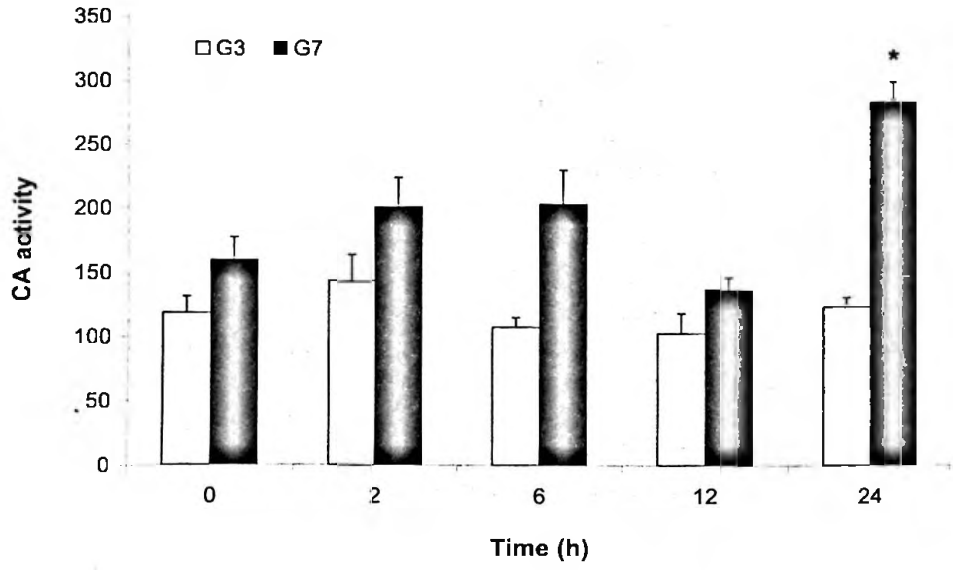

Fig. 2. CA activity (µmol CO_2.mg protein^{-1}.min^{-1}) in anterior (G3, white bars) and posterior (G7, black bars) gills of *Callinectes sapidus* acclimated to 35 ppt (T=0) and transferred to 15 ppt for various time. Mean ± S.E.M., N=4-6. Statistical comparisons with T=0: * $p<0.005$.

Expression of CA mRNA was not significantly different in G3 and G7 of crabs acclimated to 35 ppt ($p=0.5$; Fig. 3). There was no significant change in G3 mRNA expression at any of the times after low salinity transfer. CA mRNA expression in G7 exhibited an approximate 4-fold and 6-fold significant increase at 2 and 6 hr post-transfer, respectively ($p<0.05$), and then decreased at 12 h. By the end of the 24 h acclimation, the level of CA mRNA was still 2-fold higher compared to the level in crabs acclimated to high salinity, but this was not significantly different due to the high degree of variability.

During short-time course acclimation to low salinity, the pattern of increase for the CA specific activity was different than that observed for the relative amount of CA mRNA transcript: the induction of CA activity lagged behind changes in RNA expression.

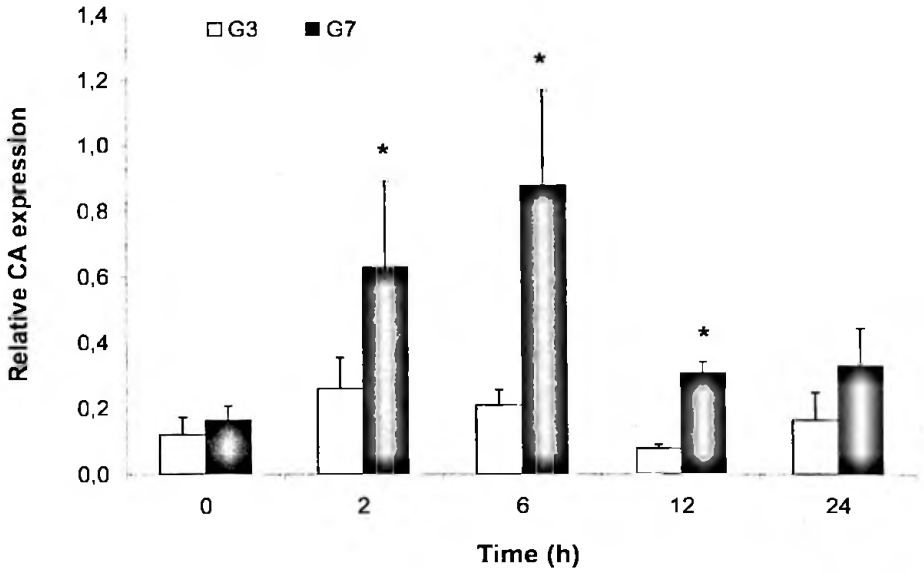

Fig. 3. CA mRNA relative expression in anterior (G3, white bars) and posterior (G7, black bars) gills of *Callinectes sapidus* acclimated to 35 ppt (T=0) and transferred to 15 ppt for various time. Mean ± S.E.M., N=4-6.
Statistical comparisons with T=0: * $p<0.05$.

These results strongly support the hypothesis that the low salinity-stimulated CA induction is under transcriptional regulation, with gene activation occurring first, followed by synthesis of new enzyme. Further experimentations will determine whether any variation in CA expression occurs during a long-time acclimation and whether the regulation process is affected by the magnitude of salinity reductions.

Supported by NSF IBN 02-30005 to RPH, and by funds from the Thomas H. Maren Foundation.

1. **Henry, R. P.** Multiple functions of gill carbonic anhydrase. *J. Exp. Zool.* 248: 19-24, 1988.
2. **Henry, R. P.; Garrelts, E. E.; MCCarty, M. M.; Towle, D. W.** Differential induction of branchial carbonic anhydrase and Na^+/K^+ ATPase activity in the euryhaline crab, *Carcinus maenas*, in response to low salinity exposure. *J. Exp. Zool.* 292: 595-603, 2002.
3. **Henry, R. P.; Gehnrich, S.; Weihrauch, D., 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.
4. **Henry, R. P.; Watts, S. A.** Early carbonic anhydrase induction in the gills of the blue crab, *Callinectes sapidus*, during low salinity acclimation is independent of ornithine decarboxylase activity. *J. Exp. Zool.* 289: 350-358, 2001.
5. **Thomason, K.; Towle, D. W.; Henry, R. P.** Quantitative expression of carbonic anhydrase mRNA and protein-specific activity in the gills of the euryhaline green crab, *Carcinus maenas*, during low salinity acclimation. *Bull. Mt. Desert Island Biol. Lab.* 43: 73-74, 2004.