Initial characterization of a carbonic anhydrase repressor from the eyestalks of the euryhaline green crab, *Carcinus maenas*

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The enzyme carbonic anhydrase (CA) is induced approximately six fold in the posterior, ion regulating gills of the green crab after transfer from high (33 ppt) to low (10 ppt) salinity[1]. This induction has been shown to be under transcriptional regulation; CA mRNA increases at 24 hr after transfer to low salinity, and the initial increase in protein-specific CA activity occurs at 48 hr post-transfer[2]. Furthermore, this induction appears to be under inhibitory control by a repressor substance found in the major endocrine complex of the crab, the eyestalk. Removal of this complex by eyestalk ablation (ESA) potentiates CA induction, and injection of eyestalk extract from crabs acclimated to high salinity inhibits CA induction in intact crabs transferred to low salinity[3]. The putative CA repressor is present at high salinity and is down-regulated in crabs acclimated to low salinity[4].

The localization of this repressor, and its inhibitory mode of action, suggest that it could be a member of the crustacean hyperglycemic hormone (CHH) family of regulatory peptides. This group includes molt inhibiting hormone, mandibular gland inhibiting hormone, and vitellogenesis inhibiting hormone, among others. These compounds are all peptides in the range of about 85 amino acids, and they all are inhibitory (repressors) in nature. A series of experiments were initiated to characterize the CA repressor, the first being to determine if it was a peptide.

Green crabs were acclimated to 33 ppt and subjected to ESA, low salinity exposure, and injections of eyestalk extracts. This was carried out over a 4-day time course. At that time, the crabs were killed, and anterior (G3) and posterior (G7) gills were dissected out and assayed for CA activity. Crabs acclimated to 33 ppt had low and uniform levels of CA activity in anterior (dark bars) and posterior (light bars) gills (Fig. 1, 33). In the first group of crabs, four-day exposure to 11 ppt resulted in an approximate 3 fold induction of CA in the posterior, ion transporting gills but not in the anterior, respiratory gills (Fig. 1, 33-11). The relatively low level of induction may have occurred as a result of these animals having been kept in the lab for over 4 weeks. ESA potentiated this induction by about 30%, again, in the posterior gills only (Fig. 1, ESA).

In intact crabs transferred from 33 to 11 ppt there was a 3-fold induction of CA activity in G7 over 4 days. In individuals given daily injections of eyestalk extracts from a separate group of crabs acclimated to 33 ppt, CA induction was inhibited by approximately 40% (Fig. 1, inj). This experiment was then repeated, using eyestalk extracts that had either been boiled for 15 min or boiled in 0.1 N HCl for 15 min and then neutralized with 0.1 N NaOH. Both treatments abolished the inhibitory effects of the eyestalk extract (Fig. 1, B 15, HCl 15).

A second group of green crabs were used in a similar experiment. CA activity in this group was induced by about 8 fold after a 4-day exposure to 11 ppt salinity (Fig. 1, 33-11B). When treated with daily injections of eyestalk extract, normal CA induction was inhibited by 35% (Fig. 1, inj B). Boiling the eyestalk extract for 2.5 min, or boiling the extract in 0.1N HCl for 2.5 min and neutralizing with 0.1N NaOH had no effect on the inhibitory potency of the extract (Fig. 1, B 2.5, HCl 2.5).

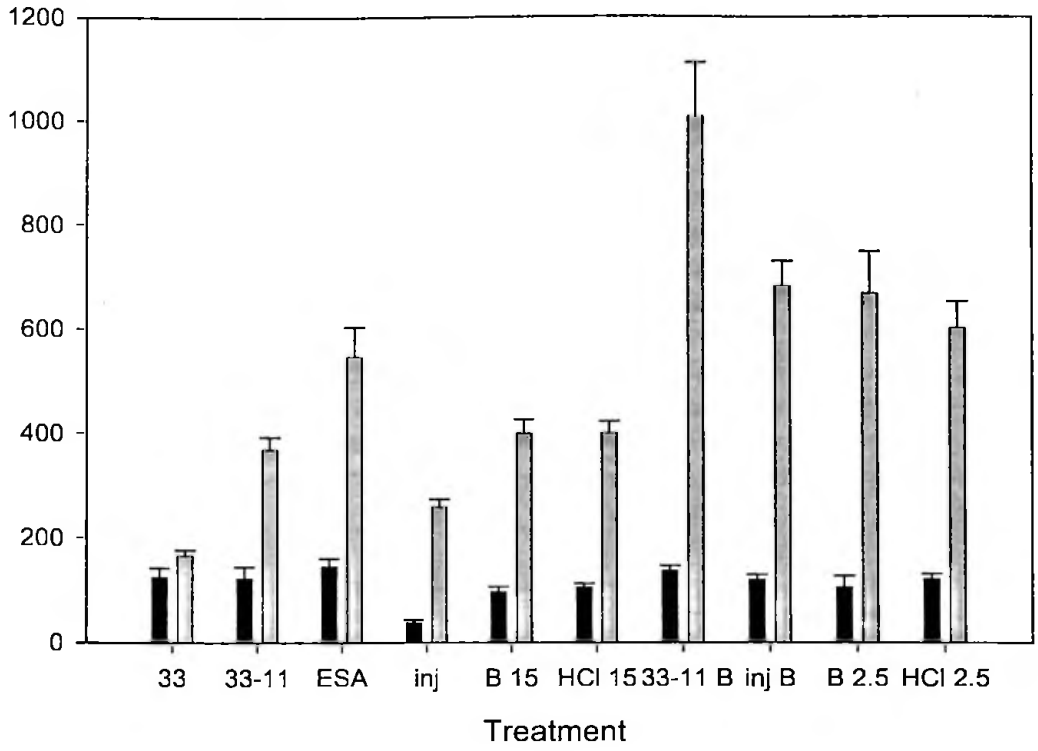

Figure 1. CA activity in anterior (G3 - dark bars) and posterior (G7 - light bars) gills of *C. maenas* acclimated to 33 ppt salinity and given a series of treatments. Mean ± SEM (N = 6-8). See text for details of experimental treatments.

These results show that the putative CA repressor is heat and acid stable for short periods of time and is therefore most likely not a large protein. It is, however, heat and acid labile after 15 min of treatment, a response that is consistent with that of a small peptide and similar to that seen by some members of the CHH family[5]. These initial results support the general hypothesis that the CA repressor is a peptide; however, definitive results will come only after treatment with a variety of proteases.

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