ALTERATIONS IN CARBONIC ANHYDRASE GENE EXPRESSION DURING LOW SALINITY ADAPTATION IN THE SHORE CRAB *CARCINUS MAENAS*

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The posterior gills of the euryhaline shore crab, *Carcinus maenas*, are an important site of ion uptake in low salinity environments. In the gill epithelial cells, the hydration of carbon dioxide (catalyzed by carbonic anhydrase) produces intracellular bicarbonate and protons which are thought to be exchanged for extracellular chloride and sodium (Henry, R.P., Ann. Rev. Physiol 58:523-538, 1996). Carbonic anhydrase (CA) activity has been shown to increase in the posterior gills of these crabs during adaptation to low salinities (Henry, R.P., et al., Bull. MDIBL 38:55, 1999), possibly as a result of increased gene transcription. This study presents the first molecular identification and characterization of carbonic anhydrase in a euryhaline crustacean.

Crabs were caught along the shore near MDIBL, and maintained in recirculating seawater tanks at 35 ppt salinity. Some crabs were acclimated to low salinity (10 ppt) over a period of at least two weeks. Total RNA was isolated from anterior and posterior gills using an RNA isolation kit (Qiagen) and single-stranded cDNA was synthesized using reverse transcriptase (Life Technologies) and an oligo-dT primer. PCR was carried out using degenerate primers based upon conserved regions of known CA sequences. Following electrophoresis, products of the expected size (~300bp) were extracted from agarose gels, re-amplified, and sequenced at MDIBL's Marine DNA Sequencing Center. A search of the GenBank database was done to determine sequence identity and amino acid homologies. Based upon this cDNA fragment (which represents approximately _ of the full-length cDNA), *Carcinus* CA has approximately 40% homology with vertebrate CAs, and has the greatest homology with type III CA isoforms (Fig. 1).

Fig 1. Alignment of *Carcinus* gill carbonic anhydrase fragment with mouse type-III carbonic anhydrase.

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Carcinus CA    WGSDSSRGSEHTIDGVRYPMELHMVHYKGSYGTLGEAVKRRDGLAVL
Mouse CA-III   WGSSDDHGSEHTVDGVKYAAELHLVHWNPRYNTFGEALKQPDGIAVV
               ***...:*****:***:*. ***:**::  *.*:***:*: **:**:

Carcinus CA    GVMLEVSSSDNPALTPLATALLNVTDAEMYAEISAMYPLKAF-LPRNIE
Mouse CA-III   GILLKIGR-EKGEFQILLDALDKIKTKGKEAPFTHFDPSCLFPACRDYW
               *::*::.  ::  :  *  ** ::.     * :: : *   *   *:
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Based upon the sequence of the crab gill CA cDNA, a pair of crab-specific primers was synthesized and used for semi-quantitative PCR. cDNA from gills of crabs acclimated to either high or low salinity was amplified in the presence of biotinylated-dUTP. Preliminary tests were done to verify the quantity of cDNA and cycle number which gave a linear response to template

availability. After separation on agarose gels and Southern blotting, PCR products were quantified using streptavidin-conjugated alkaline phosphatase (New England Biolab Phototope System).

After adaptation to low salinity, CA mRNA content was elevated (compared to actin) in the posterior gills, whereas the anterior gills showed no change in CA mRNA levels (Fig. 2). These results support the likely role of gill CA in ion regulation in the shore crab, and provide evidence for the genetic "up-regulation"of CA activity during adaptation to low salinity. Future experiments will attempt to identify the regulatory mechanisms involved in CA gene expression.

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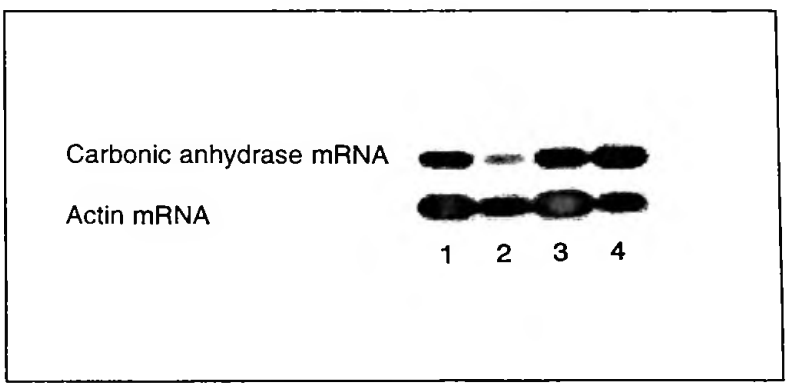

Fig. 2. Levels of carbonic anhydrase and actin gene expression in *Carcinus* gill measured by semi-quantitative PCR.

Lane 1: Anterior gill; high salinity
Lane 2: Posterior gill; high salinity
Lane 3: Anterior gill; low salinity
Lane 4: Posterior gill; low salinity