Identification of Carbonic Anhydrase Isoforms in the Gills of the Shore Crab, and Changes in their Expression During Acclimation to Low Salinity

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The euryhaline shore crab, *Carcinus maenas*, regulates levels of sodium and chloride in its body fluids despite fluctuations in environmental salinity. To maintain these ion concentrations in dilute seawater (10 ppt), the crabs exchange intracellular HCO_3^- and H^+ for environmental Na^+ and Cl^-. This ion transport occurs primarily across the epithelium of the posterior gills (Henry, R.P., Ann. Rev. Physiol. 58:523-538, 1996), where the intracellular HCO_3^- and H^+ are generated by the activity of carbonic anhydrase (CA). It has been shown that the level of CA activity increases in the gills of crabs during acclimation to low salinity, and that this increase involves the expression of more than one isoform of CA (Bottcher, K. and Siebers, D., J. Exp. Zool. 265:397-409, 1993; Henry, R.P., J. Exp. Zool. 245:1-8, 1988). Although CA isoforms have been well characterized in vertebrates, relatively little is known about CA isoforms in invertebrates.

We have identified and partially sequenced mRNAs for two distinct isoforms of CA from *Carcinus* gill (Fig. 1). The two isoforms have approximately 50% homology, and share many of the conserved nucleotides found in CA from other species (Gehnrich et al., MDIBL Bulletin, 39:28-29, 2000; Hewett-Emmett, D. and Tashian, R.E., The Carbonic Anhydrases, 1991).

Fig. 1. Alignment of partial cDNA sequences of carbonic anhydrase isoforms from *Carcinus maenas*.

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Carcinus CA-2    ---CAGTTGCATTTTCACTGGGGGAAGACCAACGAGAGG
Carcinus CA-1    GCTCAGTTCCACTTCCATTGGGGCTCAGATTCTTCTCGT
                    ***** ** ** ** *****                *

Carcinus CA-2    GGATCCGAACACACAATAGATGGCGCTTGTTACCCGGCC
Carcinus CA-1    GGCTCTGAGCACACTATTGATGGAGTCAGGTACCCAATG
                 ** ** ** ***** ** ***** *   * *****

Carcinus CA-2    GAGCTTCATTTGGTGCACTGGAACAAGACTAAGTTCTCC
Carcinus CA-1    GAGCTTCAYATGGTTCACT---ACAAGGGTTCGTACGGT
                 ********  **** ****   *****  *  ** *

Carcinus CA-2    AGCTTCGCCCAGGCCGCTGCTTCTGAGGGAGGTCTGGCT
Carcinus CA-1    ACTCTGGGCGAGGCGGTGAAGAGGAGGGACGGTCTGGCA
                 *   * * * **** *          **  ********

Carcinus CA-2    GTCTTGGGCATGTTCCTGGCGGTGG--GACGGGAGCA-C
Carcinus CA-1    GTACTGGGTGTGATGCTYGAGGTGTCCAAYAGTGACAAY
                 **  ****  ** * ** * ****    *  *   **

Carcinus CA-2    CCAGAGATGGCTAAGATCTGTAACCTCCTTCCCTTCATC
Carcinus CA-1    CCTGCTCTTACTCCCCTCGCTACTGCCCTCCTCAACGTG
                 ** *   *  **    **  **    *** * *  * *

Carcinus CA-2    AATCATAAAGGGCAGGCGATCAGTATGACGGGCGTTGTG
Carcinus CA-1    ACGGATGCTGAGATGTACGCTGAGATCTCCGCCATGTAC
                 * * **   * *  *         **  * * * *

Carcinus CA-2    CATCCTGAAACCTTCCTACC---CAAGAACGGTTCCTACTACACT-
Carcinus CA-1    CCACTCAAGGCTTTCCTCCCACGTAACATCGAGAAGTTCTACCGTT
                 *  *   *  * ***** **    ** * **     * ****  *
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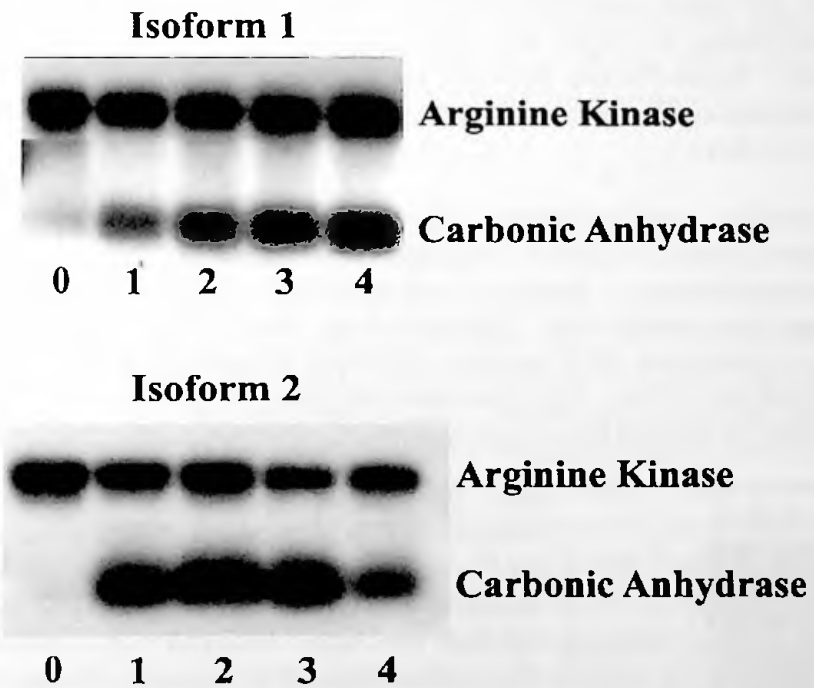
During the 4-day acclimation period, total CA activity in the posterior gills increased approximately 5-fold. We found that the levels of the two carbonic anhydrase mRNAs also increased during acclimation (Fig. 2), with isoform-1 being expressed at significantly higher levels than isoform-2 (PCR products for isoform-1 were evident after only 20 cycles of amplification, whereas 26 cycles were required for isoform-2 products).

Fig. 2. Levels of mRNA from two isoforms of carbonic anhydrase in posterior gills during acclimation to low salinity. Gills were removed 0, 1, 2, 3, and 4 days after crabs were transferred to 10ppt seawater. mRNA was reverse transcribed and amplified by PCR using isoform-specific primers in the presence of biotinylated d-UTP. PCR products were separated by electrophoresis, blotted onto nylon membranes, and quantified with a streptavidin-conjugated luminescent system. Arginine kinase activity does not change during acclimation, and its mRNA served as the control.



These results support a role for carbonic anhydrase in the adaptation of euryhaline crabs to low salinity. Although there is evidence for both cytoplasmic and membrane-bound CA in crab gill (Bottcher, K. and Siebers, D., J. Exp. Zool. 265:397-409, 1993; Henry, R.P., J. Exp. Zool. 245:1-8, 1988), our partial sequence data do not make it possible, at this time, to determine the cellular localization of the isoforms.

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