CARBONIC ANHYDRASE IN THE HYPODERMIS OF THE SHORE CRAB, *CARCINUS MAENAS*, AND ITS ROLE IN THE POST-MOLT CALCIFICATION OF THE CUTICLE

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The exoskeleton of crustaceans is composed primarily of chitin and protein, mineralized with calcium carbonate to form a rigid cuticle over the entire body. The cells responsible for mineralization are the epithelial cells of the hypodermis which have long membranous villi extending into the layers of the cuticle through narrow pore canals. The mineralization of the new cuticle involves the deposition of calcium carbonate crystals which form as a result of the high concentrations of calcium and bicarbonate ions in the extracellular space around the villi. Calcium ions are pumped from the cells by Ca^{++}-ATPase, whereas bicarbonate is produced by intracellular reactions involving carbonic anhydrase (CA). The addition of acetazolamide (a CA inhibitor) blocks mineralization, suggesting a significant role for CA in the process. Carbonic anhydrase activity in the hypodermis of the blue crab, *Callinectes sapidus*, fluctuates during the molt cycle, with maximal activity during the post-molt, mineralization stages (Henry, R.P. and Kormanik, G.A., J. Crustacean Biol. 5:234-241, 1985). We hypothesized that similar changes occur in the hypodermis of the shore crab, *Carcinus maenas*, with increased levels of CA mRNA during the post-molt phase.

Crabs in various stages of the molt cycle were caught along the shore near MDIBL. Total RNA was isolated from hypodermis using RNA isolation kits (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 (MDIBL Sequencing Facility). The cDNA fragment identified from hypodermis seems to be identical to the cDNA fragment obtained from *Carcinus* gill (see Gehnrich, S.C., et al. in this volume of the Bulletin for sequence data).

Based upon the sequence of *Carcinus* CA cDNA, a pair of specific primers was synthesized and used for semi-quantitative PCR. cDNA from hypodermis of "soft" (postmolt) and "hard" (intermolt) crabs was amplified in the presence of biotinylated-dUTP, and after separation on agarose gels and Southern blotting, PCR products were quantified using streptavidin-conjugated alkaline phosphatase (New England BioLab Phototope System).

Semi-quantitative PCR of hypodermis cDNA revealed that levels of CA mRNA in the post-molt crab were not higher than the intermolt crab, when normalized to levels of actin mRNA. These data, therefore, do not support the hypothesis for increased CA gene transcription during the post-molt phase.

Fig 1. Levels of actin and carbonic anhydrase gene expression in *Carcinus* hypodermis measured by semi-quantitative PCR.
Lane 1: DNA standards
Lane 2: post-molt hypodermis
Lane 3: intermolt hypodermis

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