Partial nucleotide sequence and expression of plasma membrane Ca-ATPase in the hypodermis of the blue crab, *Callinectes sapidus*

Robert Roer[1] and David Towle[2]
[1]Department of Biological Sciences
University of North Carolina at Wilmington
Wilmington, NC 28403
[2]Mount Desert Island Biological Laboratory
Salisbury Cove, ME 04672

Being bound by an exoskeleton, crustaceans must molt in order to grow. The exoskeleton of the brachyuran crabs is hardened, in many areas, by both sclerotization and impregnation with calcium carbonate[6,7]. During the preparation for an ensuing molt, mineral is resorbed from the old, calcified exoskeleton by the underlying hypodermis. Following the molt, mineral is deposited by the hypodermis in the new exoskeleton[4]. In addition to the temporal changes in mineralization, there are spatial differences as well. The exoskeleton of the branchial chamber, gills and arthrodial membrane of the joints does not calcify.

Physiological[5,10,11], biochemical[3], molecular[12,13], histological[3,7] and immunocytochemical[12] data indicate that a Ca-ATPase is involved in the hypodermal transport of calcium out from the exoskeleton during premolt resorption and into the exoskeleton during postmolt deposition. The precise timing of the induction of Ca-ATPase expression in the hypodermis during the pre- and postmolt stages is not known. Moroever, the differences in expression pattern between those tissues that mineralize (e.g. the dorsal carapace) compared to those that do not (e.g. the arthrodial membrane) has not been studied. The latter question is particularly important in understanding the control of the mineralization.

In order to address these questions, blue crabs were collected prior to the molt (stages D_2 and D_3), immediately upon molting (0 h), and at 1, 2, 3, 4, 6, 8, 12, 24 and 48 h postmolt. Pieces of dorsal carapace and arthrodial membrane were excised, the hypodermis was separated from the exoskeleton with forceps, and the hypodermis stored in RNA Later (Ambion) and stored at -20°C. Total RNA was extracted using the spin-column RNeasy Protect Mini Kit (Qiagen), with the following modifications to increase yield and quality of RNA. Tissue was homogenized in 1ml TRIzol (Invitrogen). RNA was eluted from the column in 30 µl nuclease-free water (Ambion), and the eluate was passed through the column a second time to increase the yield. mRNA was reverse-transcribed to make first-stand cDNA (SuperScript II kit, Invitrogen).

The following specific forward and reverse primers were constructed for the plasma membrane Ca-ATPase (PMCA) and for arginine kinase (AK, as a constitutively expressed control[4]):

PMCA F3: TTG AAC CGA TGG CGT GTA AT
PMCA R4: TGA TGT CTG AGG CTT CTT TTG

AK F51: CGC TGA GTC TAA GAA GGG ATT
AK R31: GAT ACC GTC CTG CAT CTC CTT

The PCR product was run on an agarose gel, stained with ethidium bromide, photographed, and the appropriate band was cut from the gel and extracted using the QiaQuick kit and protocol (Qiagen). The gel-purified cDNA was quantified by agarose gel electrophoresis and sequenced on an ABI Prism 3100 sequencer at the Marine DNA Sequence Center at MDIBL.

The mRNA was contaminated with genomic DNA. The segment of the genomic DNA that amplified along with the cDNA bore one intron, resulting in two PCR products. It was, therefore, not possible to run quantitative PCR. However, the results of a semi-quantitative comparison showed low levels of expression of Ca-ATPase, relative to arginine kinase, until 4 h postmolt in both dorsal carapace and arthrodial membrane (Fig. 1). Between 4 h and 48 h postmolt, there is a marked upregulation in the expression of the Ca-ATPase mRNA.

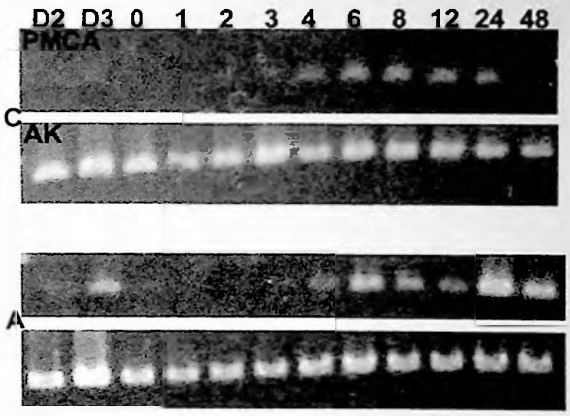

Fig. 1. Semi-quantitative analysis of the expression of Ca-ATPase mRNA (PMCA) compared to arginine kinase (AK) in the hypodermis of dorsal carapace (C) and arthrodial membrane (A), before the molt (D_2, D_3) and from 0 to 48 h postmolt.

The translation of the portion of the *Callinectes* Ca-ATPase sequence that was amplified revealed a high degree of amino acid identity with that of the enzyme from the crayfish, *Procambarus clarkii*[2] (Fig. 2).

The upregulation of Ca-ATPase during early postmolt supports previous data implicating this enzyme in the postecdysial mineralization of the pre-exuvially deposited epi- and exocuticle and the post-exuvially deposited endocuticle[4]. Interestingly, there was at least as much Ca-ATPase expressed in the non-calcifying arthrodial hypodermis as there was in the calcifying dorsal tissue. This suggests that the lack of calcification in the arthrodial membrane is not due to the inability of the hypodermis to transport calcium, but to differences in the nature of the organic matrix comprising the two cuticle types[1,8,9].

This work was supported by a MDIBL New Investigator Award (RDR) and by grant (IBN 01-14597) from the National Science Foundation (RDR).

```
callinectes_    ------------------------------------------------------------
procambarus_    MGDDANSSIEFHPRPNQRRDGNQAGGFGCSLMELRSLMELRGLEAVVKIQEDYGDVEGLC

callinectes_    ------------------------------------------------------------
procambarus_    RRLKTSPTEGLADNTNDLEKRRQIYGQNFIPPKKPKTFLQLVWEALQDVTLIILEIAAIV

callinectes_    ------------------------------------------------------------
procambarus_    SLGLSFYRPPGETGGGAAAGGAEDEGEAEAGWIEGAAILLSVVCVVLVTAFNDWSKEKQF

callinectes_    ------------------------------------------------------------
procambarus_    RGLQSRIEQEQKFTVVRNGQVLQIPVAELVVGDIAQVKYGDLLPADGVLIQGNDLKIDER

callinectes_    ------------------------------------------------------------
procambarus_    SLTGESDHVRKSADKDPMLLSGTHVMEGSGRMVVTAVGVNSQTGIIFTLLGAGAEEEEVE

callinectes_    ------------------------------------------------------------
procambarus_    AKKRKKEAKKQRKKQKKGDSGEELIDANPKKQDGEMESNQIKAKKQDGAAAMEMQPLKSA

callinectes_    ------------------------------------------------------------
procambarus_    EGGEADEEEEKKVNTPKKEKSVLQGKLTKLAVQIGKAGLVMSAITVIILVLYFGIETFVV

callinectes_    ------------------------------------------------------------
procambarus_    EGRPWTPVYIQYFVKFFIIGVTVLVVAVPEGLPLAVTISLAYSVKKMMKDNNLVRHLDAC

callinectes_    ------------------------------------------------------------
procambarus_    ETMGNATAICSDKTGTLTTNRMTVVQSYIGDEHYKEIPDPGSLPPKILDLLVNAISINSA

callinectes_    ------------------------------------------------------------
procambarus_    YTTKILPPDKEGDLPRQVGNKTECALLGFVLDLKRDYQPIRDQIPEEKLYKVYTFNSVRK

callinectes_    ----------------------------SFIHGKDGKLESFSKSMQDRLVREVIEPMACN
procambarus_    SMSTVVPMRDGGFRIYSKGASEIVLKKCSQILNRDGELRSFRPRDKDDMVRKVIEPMACD
                                            * * .:**:*.**    :* :**:*******:

callinectes_    GLRTISIAYRDFVRGKAEINQVHFENEPHWDDEDHIINNLTCLCVLGIEDPVRPEVPDAI
procambarus_    GLRTICIAYRDFVRGCAEINQVHFENEPNWDNENNIMSDLTCLAVVGIEDPVRPEVPDAI
                *****.********* ************:**:*::*:.:****.*:***************

callinectes_    HKCQRAGITVRMVTGDNINTARSIASKCGILKPGDNSLILEGKEFNRRVRDSTGKIQQHL
procambarus_    QKCQRAGITVRMVTGANINTARAIASKCGIIQPGEDFLCLEGKEFNRRIRDESGCIEQER
                :*************** ******:********::**:: * **********:**.:* *:*.

callinectes_    VDKVWVNLRVLARSSPTDKYTLVKGIIESKVSANREVVAVTGDGTNDGPALKMADVGFAM
procambarus_    IDKVWPKLRVLARSSPTDKHTLVKGIIDSTTNDQRQVVAVTGDGTNDGPALKKADVGFAM
                :**** :************:********:*... :*:***************** *******

callinectes_    GIAGTDVAKEASDIILLDDNFNSIVKAVMWGR----------------------------
procambarus_    GIAGTDVAKEASDIILTDDNFTSIVKAVMWGRNVYDSISKFLQFQLTVNVVAVIVAFTGA
                **************** ****.**********

callinectes_    ------------------------------------------------------------
procambarus_    CITQDSPLKAVQMLWVNLIMDTFASLALATEPPTESLLLRKPYGRTKPLISRTMMKNILG

callinectes_    ------------------------------------------------------------
procambarus_    HAVYQLLIIFTLLFVGEGFFDIDSGRNAPLHSPPSEHYTIIFNTFVMMQLFNEINARKIH

callinectes_    ------------------------------------------------------------
procambarus_    GERNVFDGIFSNPIFCTIVLGTFGIQIVIVQFGGKPFSCTPLPAEQWLWCLFVGAGELVW

callinectes_    ------------------------------------------------------------
procambarus_    GQVMATIPTSQLKSLKGAGHEHRKDEMNAEDLNEGQEEIDHAERELRRGQILWFRGLNRI

callinectes_    --------------------------------------------------
procambarus_    QTQIEVVNAFKSGSSVQGAVRRPSSILSQNQDVTNVSTPSHASSGMPLAL
```

Fig. 2. Comparison of the deduced amino acid sequence of the partial transcript of *Callinectes sapidus* Ca-ATPase with that from *Procambarus clarkii.*

1. **Coblentz, F.E., T.H. Shafer and R.D. Roer.** Cuticular proteins from the blue crab alter *in vitro* calcium carbonate mineralization. *Comp. Biochem. Physiol.* 121B: 349-360, 1998.
2. **Gao, Y. and M.G. Wheatly.** Characterization and expression of plasma membrane Ca^{+2} ATPase (PMCA3) in the crayfish *Procambarus clarkii* antennal gland during molting. *J. Exp. Biol.* 207: 2992-3002, 2004.
3. **Greenaway, P., R.M. Dillaman and R.D. Roer.** Quercitin-dependent ATPase activity in the hypodermal tissue of *Callinectes sapidus* during the moult cycle. *Comp. Biochem. Physiol.* 111A: 303-312, 1995.
4. **Kotlyar, S., D. Weihrauch, R.S. Paulsen and D.W. Towle.** Expression of arginine kinase enzymatic activity and mRNA in gills of the euryhaline crabs *Carcinus maenas* and *Callinectes sapidus*. *J. Exp. Biol.* 203: 2395-2404, 2000.
5. **Roer, R.D.** Mechanisms of resorption and deposition of calcium in the carapace of the crab *Carcinus maenas*. *J. Exp. Biol.* 88: 205-218, 1980.
6. **Roer, R.D. and R.M. Dillaman.** The structure and calcification of the crustacean cuticle. *Am. Zoologist* 24: 893-909, 1984.
7. **Roer, R.D. and R.M. Dillaman.** Molt-related change in integumental structure and function. In: *The Crustacean Integument - Morphology and Biochemistry*, edited by M. Horst and J.A. Freeman. Boca Raton, FL: CRC Press, 1993, p. 1-37.
8. **Shafer, T.H., R.D. Roer, C.G. Miller and R.M. Dillaman.** Postecdysial changes in the protein and glycoprotein composition of the cuticle of the blue crab, *Callinectes sapidus*. *J. Crust. Biol.* 14: 210-219, 1994.
9. **Shafer, T.H., R.D. Roer, C. Midgett-Luther and T.A. Brookins.** Postecdysial cuticle alteration in the blue crab, *Callinectes sapidus*: Synchronous changes in glycoproteins and mineral nucleation. *J. Exp. Zool.* 271: 171-182, 1995.
10. **Wheatly, M.G.** An overview of calcium balance in crustaceans. *Physiol. Zool.* 69: 351-382, 1996.
11. **Wheatly, M.** Calcium homeostasis in Crustacea: The evolving role of branchial, renal, digestive and hypodermal epithelia. *J. Exp. Zool.* 283: 620-640, 1999.
12. **Wheatly, M., Z. Zhang, J.R. Weil, J.V. Rogers and L.M. Stiner.** Novel subcellular and molecular tools to study $Ca2^{+}$ transport mechanisms during the elusive moulting stages of crustaceans: Flow cytometry and polyclonal antibodies. *J. Exp. Biol.* 204: 959-966, 2001.
13. **Ziegler, A., D. Weihrauch, D.W. Towle and M. Hagedorn.** Expression of Ca^{2+}-ATPase and Na^{+}/Ca^{2+}-exchanger is upregulated during epithelial Ca^{2+} transport in the hypodermal cells of the isopod *Porcellio scaber*. *Cell Calcium* 32: 131-141, 2002.