Does bursicon control exoskeletal mineralization in the post-ecdysial blue crab, *Callinectes sapidus*?

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Abstract

In order for growth and development to occur, crustaceans must periodically shed their confining exoskeleton in a process called molting or ecdysis. The newly molted specimen is covered by a soft shell that must harden speedily. Shell-hardening in Crustacea consists of sclerotization and mineralization. While there is evidence that crustacean sclerotization is controlled by the neurohormone bursicon, it remains unknown as to which hormone regulates exoskeletal mineralization post-ecdysis. The blue crab bursicon or CasBurs is made up of α and β subunits (Chung et al., 2012). Templates for CasBurs α dsRNAs were generated based on cDNA sequences encoding these two subunits (EU67719, GenBank), and CasBurs dsRNA successfully synthesized. The synthesized dsRNA for CasBurs was injected to soft shell blue crabs 24 hours after molting to knock down the expression of bursicon gene through the RNAi mechanism. $ days later, the living specimens were sacrificed, and their carapace harvested and air dried before metal analysis took place. Analysis showed an average exoskeletal calcium level of 54.47 ± 42.89 mg Ca/g dry weight in the experimental group (N=2) and 99.63 ± 39.91 in the control group (N=3). Exoskeletal magnesium levels were 7.64 ± 3.26 mg Mg/g dry weight in the experimental group and 10.88 ± 5.28 mg Mg/ g dry weight in the control group. Average calcium and magnesium levels decreased 45.32 and 29.77 %, respectively. However, the results of the t-tests for calcium and magnesium were P= 0.31 and P=0.51, respectively. The results of this experiment indicate that possibility for bursicon to regulate the post-ecdysial mineralization in the blue crab. However, further experimentation needs to be performed with a larger sample size to provide a conclusive answer.

Introduction

Crustaceans shed their exoskeleton numerous times during their lives through the process of ecdysis, commonly known as molting. Ecdysis allows the crustacean to grow and develop, which is not possible unless the confining exoskeleton can be escaped. The molting cycle is comprised of 4 stages: postmolt, intermolt, premolt, and ecdysis. The process is regulated by molting hormones called ecdysteroids, which are secreted by the Y-organ. The Y-organ is regulated by the molt-inhibiting hormone (MIH), which is produced by the X-organ sinus gland complex in the eyestalk (Zou, 2020). The ecdysteroids are received by their receptor called the ecdysteroid receptor (EcR). Upon receptor dimerization with crustacean retinoid X receptor (RXR), this complex binds to its response element which mediates gene expression under the control of molting hormones (Durica and Hopkins, 1996; Chung et al., 1998). Once the old exoskeleton is shed, the crustacean is surrounded by a soft exoskeleton that makes the animal especially susceptible to predation and pathogens. The soft shell must rapidly harden to close this window of vulnerability.

Methodology

The blue crab bursicon, or CasBurs is made up of α and β subunits (Chung et al., 2012). Templates for CasBurs α dsRNAs have been generated based on cDNA sequences encoding these two subunits (EU67719, GenBank), and CasBurs dsRNA successfully synthesized. The synthesized dsRNA for CasBurs was injected to soft shell blue crabs to knock down the expression of bursicon gene through the RNAi mechanism. Late pre-molt blue crabs, or busters were individually placed in cages in aerated tanks filled with recirculating artificial seawater in a

greenhouse at an ambient temperature and a natural photoperiod, approximately 14 hr light/10 hr dark. Artificial seawater was made from Instant Ocean sea salt and adjusted to a salinity of 10 - 12 ppt. Crabs in late post-molt or 24 hours after ecdysis were used for dsRNA injection with a dosage of 0.02 µg/g or saline injection serving as the control. On day 4 after injection, survivors were sacrificed for collection of carapaces and subjected to metal analysis.

Results

The average calcium content in the exoskeleton of the experimental group was 54.47 ± 42.89 mg Ca/g dry weight, while it was 99.63 ± 39.91 in the untreated group. This was a 45.32 % decrease in exoskeletal calcium level following injection of dsRNA (P= 0.31). The average magnesium content in the exoskeleton of the experimental group was 7.64 ± 3.26 mg Mg/g dry weight, while it was 10.88 ± 5.28 mg Mg/ g dry weight in the control group. This was a 29.77% decrease in exoskeletal magnesium level following the injection of dsRNA (P= 0.51).

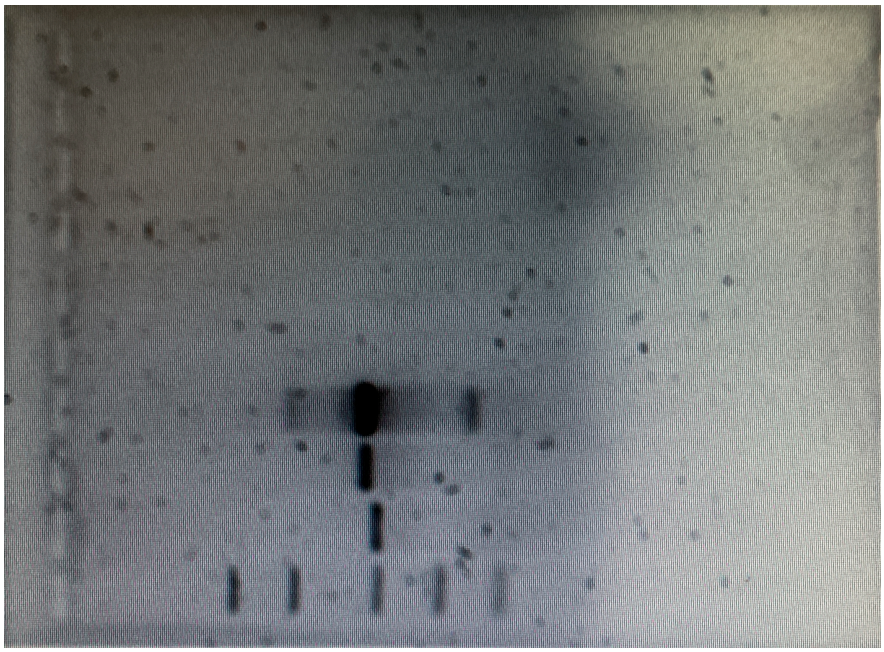

Fig. 1 Casburs α cDNA electrophoresis showing 400bp sample near predicted size alongside cDNA samples in lanes 3 and 4. The samples in lanes 3 and 4 were used to synthesize Casburs α dsRNA.

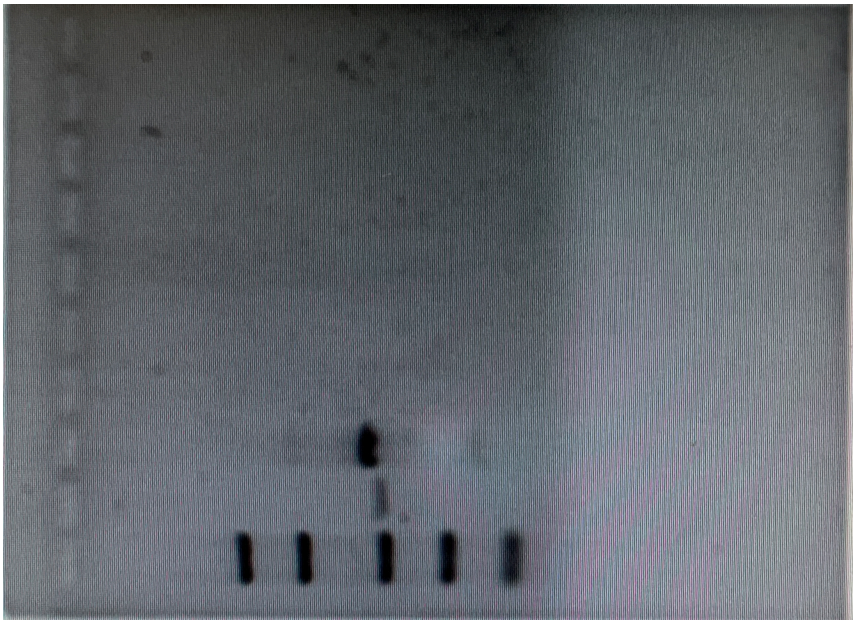

Fig. 2 Casburs α electrophoresis results showing Casburs α dsRNA sample alongside DNA sample near the predicted size of 400bp.

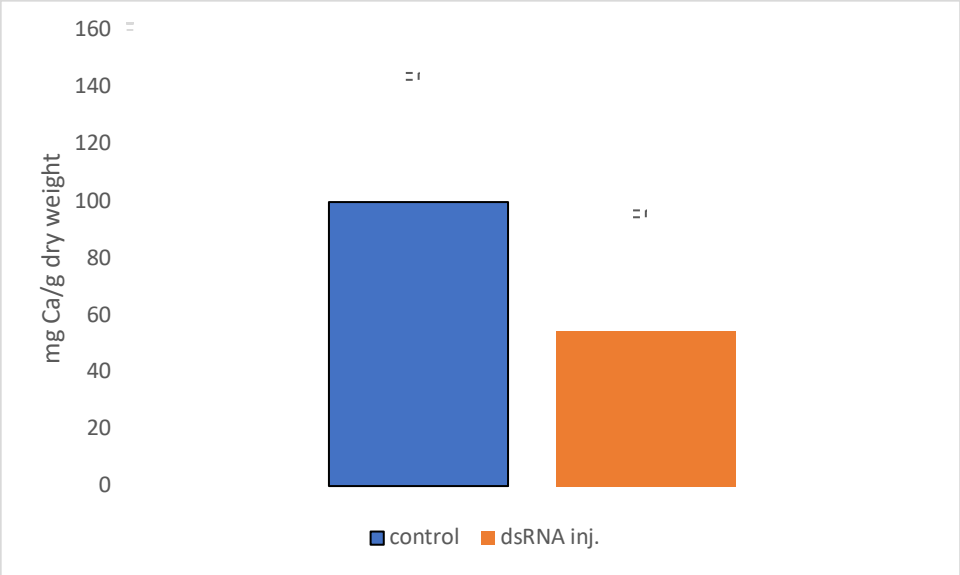

Fig. 1 Average (± standard deviation) calcium content in exoskeleton of control (N=3) and dsRNA injected groups (N=2).

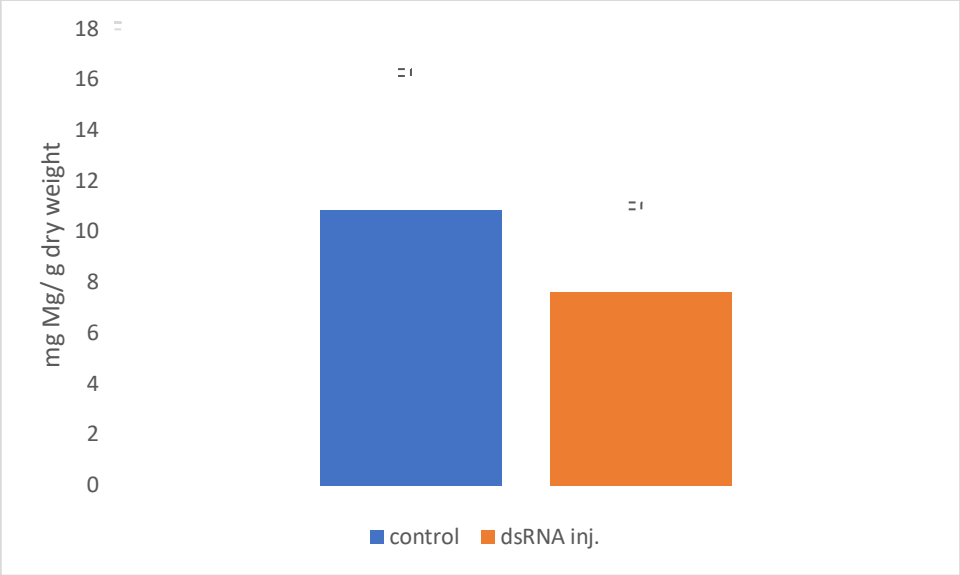

Fig. 2 Average (± standard deviation) magnesium content in exoskeleton of control (N=3) and dsRNA injected groups (N=2)

Discussion

When comparing the levels of mineralization between the experimental and control groups of blue crab, some degree of inhibition of the mineralization process appears to be evident. A two tailed t-test was performed for the levels of both calcium and magnesium. For calcium and magnesium, the p-values were 0.31 and 0.51, respectively indicating that these results do not hold statistical significance. This is likely due to the low number of specimens (5) that were able to survive for four days after the injection process. Because the results of this experiment point to bursicon regulating mineralization, further experimentation should be done in attempt to demonstrate this phenomenon with statistically significant results. Prospective experimentation

should include larger sample size and possibly injection of a higher quantity of Casburs α dsRNA.

Acknowledgement

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