Hormonal Control of the Making of Crab Shell, a Biological concrete

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Abstract

This endeavor sought to determine the hormonal mechanism driving post-ecdysial shell calcification in crustaceans. Carbonic anhydrase (CA) catalyzes the carbon dioxide hydration reaction, which generates bicarbonate ions essential for calcium carbonate formation. Using metal analysis, enzymatic assay, tissue culture, and real-time PCR, my lab has found that epidermal CA activity is correlated to exoskeletal calcium content and that both epidermal CA activity and CA gene expression are inducible by the molting hormone in the blue crab *Callinectes sapidus*. These results have led to the new paradigm of post-ecdysial shell mineralization being controlled by the molting hormone. Intriguingly, while molting hormone injection elevated epidermal CA activity, the exogenous molting hormone was found to suppress shell calcification, implicating that sclerotization and mineralization of the new shell must be coordinated and enhanced deposition of calcium carbonate as a result of increased epidermal CA activity following hormonal treatment would be inhibited to avoid the formation of a structurally defective exoskeleton.

Background

• Crab shell, a biological concrete, is made up of an organic matrix of chitin and proteins ("steel rods") mineralized by inorganics, primarily carbonate salts ("cement").

• After molting, crustaceans are covered by a soft shell, which must harden rapidly. Shell hardening consists of sclerotization ("tightening of steel rods") and mineralization ("addition of cement").

Crustacean exoskeleton (A biological concrete)
- Chitin (glucosamine polymer)
- Proteins (such as collagens)
- Inorganics (such as $CaCO_3$)

Scientific Question to Be Addressed

While it well known that sclerotization is controlled by the neurohormone bursicon [1], the hormonal mechanism driving mineralization remains unresolved. This project attempted to address the question of which hormone controls mineralization in crustaceans , using the blue crab, *Callinectes sapidus,* as the model crustacean.

Shell hardening
- Sclerotization or Tanning of organic matrix ("Tightening of steel rods") — Controlled by bursicon
- Mineralization ("Cement addition") — Controlled by ????

Hypothesis

Given the similarity between the patterns of changes in molting hormone concentration and epidermal carbonic anhydrase (CA) activity during the molting cycle [2], it was hypothesized that the molting hormone regulates mineralization through controlling epidermal CA due to the following reactions.

$$CO_2 + H_2O \xleftrightarrow{\text{Carbonic Anhydrase}} H^+ + HCO_3^-$$

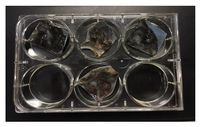
$$HCO_3^- + Ca^{2+} \longleftrightarrow H^+ + CaCO_3$$

Methodologies

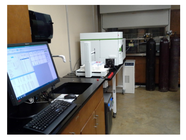
1. The "buster" blue crabs purchased from local softshell crab makers were used to produce postmolt crabs in the laboratory.

"Busters" in cages

A molting crab



Epidermal tissue culture



ICP-OES for metal analysis

2. Calcium content in the shell was analyzed using ICP-OES.
3. Epidermal CA activity was assayed according to Calhoun and Zou [2].
4. The in vitro molting hormone 20-hydroxyecdsone (20-HE) treatment of epidermal tissues followed Booth and Zou [3], while for in vivo treatment 20-HE was injected to post-ecdysial crabs.
5. The expression of epidermal CA genes (CA mRNA) was quantified using quantitative real-time PCR.
6. Epidermal CA gene expression of knocked down using RNAi (through injection of dsRNA to softshell crabs).

Results and Discussion

1. Significant correlation between epidermal CA activity and calcium content (Fig. 1) suggests that CA mediates calcium incorporation into the exoskeleton.

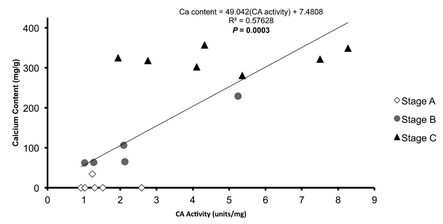


Figure 1. Correlation between epidermal CA activity and exoskeletal calcium content in post-ecdysial blue crabs.

2. Significant in vitro induction of epidermal CA mRNA (Fig. 2) and significant in vivo induction of epidermal CA activity (Fig. 3) suggest that **epidermal CA is controlled by the molting hormone**.

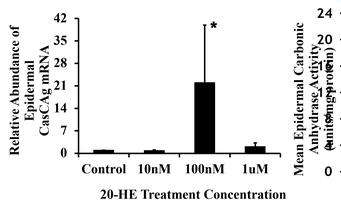


Figure 2. In vitro induction of epidermal CA gene (CasCAg mRNA) by 20-HE. N = 5, * P < 0.05 relative to control.

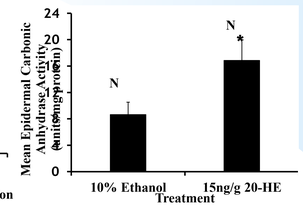


Figure 3. In vivo induction of epidermal CA activity by 20-HE. * P < 0.05.

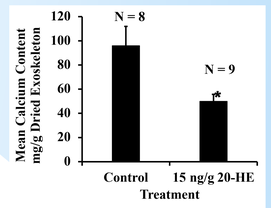


Figure 4. Effect of 20-HE injection on shell calcium content. * P < 0.05.

3. Interestingly, injection of the exogenous molting hormone inhibited shell calcium content (Fig. 4).

This result implicates that sclerotization and mineralization of the new shell must be coordinated and enhanced deposition of calcium carbonate as a result of increased epidermal CA activity following hormonal treatment would be inhibited to avoid the formation of a structurally defective exoskeleton.

4. Preliminary RNAi results

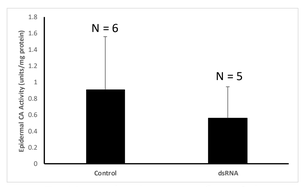


Figure 5. Knock-down of epidermal CA activity by 10 µg *CasCAg* dsRNA/crab.

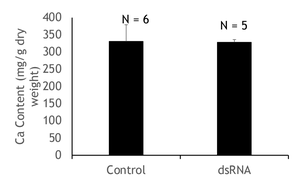


Figure 6. Effect of CasCAg dsRNA injection on exoskeletal Ca content.

Conclusion

Epidermal CA is controlled by the molting hormone, suggesting that the molting hormone is involved in the regulation of post-ecdysial shell mineralization in Crustacea.

Future Investigation

Further RNAi experiments will be conducted to ascertain the role of epidermal CA in post-ecdysial deposition of calcium to the shell.

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References

[1] Chung, J.S., Katayama, H., Dircksen, H., 2012. New functions of arthropod bursicon: inducing deposition and thickening of new cuticle and hemocyte granulation in the blue crab, *Callinectes sapidus*. PLoS One 7(9), e46299.

[2] Calhoun, S., Zou, E. (2016) Epidermal carbonic anhydrase activity and exoskeletal metal content during the molting cycle of the blue crab, *Callinectes sapidus*. J. Exp. Zool. 325A:200–208.

[3] Booth, A., Zou, E. (2016) Impact of molt-disrupting BDE-47 on epidermal ecdysteroid signaling in the blue crab, *Callinectes sapidus*, in vitro. Aquatic Toxicology 177:373-379.