The Observation of Antimicrobial Peptide Concentration in the American Lobster, *Homarus americanus*

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This summer I worked in Elizabeth Stemmler’s laboratory studying concentration changes to antimicrobial peptides (AMP) in our blue lobsters (*Homarus americanus*). Peptides and proteins are biological molecules composed of amino acid chains. AMPs are shorter chains of amino acids that have antimicrobial properties[1]. AMPs are of interest because crustaceans lack adaptive immune systems that produce antibodies yet have developed a natural defense mechanism using their innate immune system. In response to immune system challenges, lobsters generate AMPs that are then stored in their cells (granulocytes and semi-granulocytes). When lobsters' immune systems are challenged, they become more susceptible to disease. Consequently, via an immune response called exocytosis, AMPs are released from the cells into their circulatory fluid (hemolymph) which functions to kill bacteria by penetrating bacterial membranes. Due to increased use of antibiotics in medicine, there has been a rise of antibiotic-resistant pathogens. This resistance limits treatment options available emphasizing the need for alternative antimicrobial sources, of which crustacean AMPs are a potential new source of[2].

The Stemmler laboratory has previously identified and quantified the lobster AMP, *Hoa*-D1, and established a concentration baseline for adult lobsters[3]. However, AMP concentration levels in the blue lobsters during a challenge experiment had yet to be assessed. I planned and conducted a time course challenge experiment where I injected my control lobster with saline and experimental lobster with Lipopolysaccharide (LPS) in saline. LPS is a component of the outer membrane of *e coli* bacteria that has been found in previous literature to trigger an immune response in crustaceans[4]. I hypothesized that the *Hoa*-D1 concentration decreases due to exocytosis in response to a LPS injection. When collecting my samples I drew samples (0.5 mL) of hemolymph, then utilized a centrifuge to isolate the hemocytes from the hemolymph. To prepare my samples for analysis I extracted *Hoa*-D1 from the hemocytes using 8 M urea and purified it through solid-phase-extraction. Oxytocin was added to the samples as an internal standard. *Hoa*-D1 was then quantified using High Performance Liquid Chromatography (HPLC). The samples of hemolymph I collected show the Hoa-D1 concentration at different timepoints prior to and after the injections. The control lobster’s Hoa-D1 concentrations were found to increase immediately after the saline injection. They then lessened at the next time point, and by the last draw time point it rebounded and was abundant. The experimental lobster’s Hoa-D1 concentrations were found to be the highest prior to the LPS injection. The AMP drastically decreased after the challenge and continued to decrease until the last draw. In the future it would be necessary to replicate this challenge time course experiment to validate our findings. It would also be helpful to examine the plasma collected after the hemocytes are separated by the centrifuge to confirm that Hoa-D1 is released into the hemolymph through exocytosis. This research has allowed us to learn about how Hoa-D1 concentrations are impacted by an immune system challenge. As AMPs may play an important role in antibiotic innovation it is critical to learn when hemolymph collection would be most effective.

References

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