Examining the Bioactivity of Hemocyanin from the Lobster, *Homarus americanus*

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Marine antimicrobial peptides (AMPs) have been known to contribute to the innate immune system of certain organisms and have a recently discovered benefit to the skin that would be interesting to research further. These are typically used for the elimination of pathogenic microorganisms to prevent diseases and slow the progression of cancerous cells in organisms. In the past five years, marine AMPs have been used for a very different purpose. While most mammals have an innate and adaptive immune system, the lobster species *Homarus americanus* has only an innate, non-specific immunity. Marine AMPs, which are an important part of an organism's immune system, give the lobster the ability to fight off many different types of pathogens. Marine AMPs have a wide range of benefits, but their antiviral, antifungal, and antibacterial properties (Thomas & Antony, 2023) are most relevant to the newfound research. A new brand of skincare, Marin, uses marine glycoproteins, which have known skin health benefits such as increasing dermal and epidermal barrier health and stimulating collagen synthesis (Marin Skincare, 2024). The two most prevalent ingredients in Marin's products are water and hemocyanin, which is the main oxygen-binding glycoprotein found in the hemolymph of *Homarus americanus* (Justia, 2024).

Hemocyanin is a protein that my research was centered around this summer. Hemocyanin has a subunit structure where a copper-containing active site lies. Six of these subunits combine using intermolecular forces to form a hexamer, and four of the hexamers combine to form hemocyanin. The molecular weight of hemocyanin is around 70 kDa, but smaller AMPs can be cleaved by enzymes, and these smaller peptides may be bioactive. I hypothesized that the hemocyanin protein or hemocyanin-derived AMPs from lobsters would have antibacterial effects. I extracted hemolymph from a lobster and used centrifugation to separate the plasma from the hemocytes. I used molecular weight cutoff filters to separate hemocyanin from smaller putative AMPs, and I tested for bioactivity using *Escherichia coli.* Testing for bioactivity involved using the broth dilution method, where bacterial growth in the presence and absence of the test compounds was assessed. For my first three experiments, I included anticoagulant in all my solutions to keep the hemolymph from coagulating. After the tests yielded no bacterial growth even for the positive control, we realized anticoagulant was killing the *E. coli*. Further experiments eliminated anticoagulant Our results continued to show that hemocyanin does not have antibacterial effects on *E. coli,* as the two hemocyanin-containing samples and samples containing putative AMPs with bacteria still grew. Something that we learned from this experiment is that anticoagulant was killing *E. coli* for three of the previous tests that were run. Anticoagulant has trisodium citrate and citric acid, which, after doing some research, have known antibacterial effects on *E. coli.* This will be helpful to know for future research. Ways to prevent anticoagulant from killing *E.coli* include letting the hemolymph coagulate and using dialysis, which are two future directions for this experiment. Another direction for future research would be to test on immunosuppressed lobsters. I want to thank Elizabeth Stemmler for supervising my project, and I want to give a huge thank you to the Henry L. and Grace Doherty Charitable Foundation Coastal Studies Research Fellowship for funding my project!

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