Identifying and Determining the Cell Localization of Antimicrobial Peptides in the American Lobster, *Homarus americanus*, Using Mass Spectrometry

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The rapid overuse of antibiotics for humans, agriculture, and livestock has led to the rise of antimicrobial resistance in harmful bacteria like *E. coli*. Bacteria that gain resistance through random mutations can become the dominant strain, which would make antimicrobial resistance the leading cause of death by 2050 (Gani et. al, 2025). The American lobster (*Homarus americanus)* relies on an innate immune system, which uses nonspecific defense mechanisms like antimicrobial peptides to fight infection without using memory-based antibodies that make up an adaptive immune system. Antimicrobial peptides or AMPs are small, positively charged molecules that can penetrate bacterial cell membranes to kill the bacteria. AMPs are produced within lobster immune cells or hemocytes, and they are stored within granules inside the hemocytes. When triggered by an invading microbe, hemocytes can release their granule contents into the hemolymph, or circulatory fluid, to combat infection. There are three different types of hemocyte cells in the hemolymph: the hyalinocytes, which have the largest nucleus to cytoplasm ratio and few to no granules; the granulocytes, which have a small nucleus to cytoplasm ratio and are rich in granules; and the semi-granulocytes which have a moderate number of granules (Hose et. al, 1990). The Stemmler lab has identified four crustin-family AMPs and one defensin-family AMP using mass spectral data of the hemocytes. However, it has not been determined whether these AMPs are present in only certain hemocyte cell types (Vu et. al, 2018)

Over the summer, my goal was to separate the hemocytes by cell type using a density gradient. When centrifuged, the hyalinocytes, which have the fewest protein rich granules, make up the top least dense layer, followed by the semi-granulocytes in the middle layer. The granulocytes would be spun to the bottom. One issue that arose was the aggregation of cells during centrifugation. When the cells bumped into each other, they would stick together in aggregates, disrupting the density layers. After comparing the impact of adding different disaggregation agents, I found that polyethylene glycol (8 kDa; 1%) worked well to sterically hinder the cells from aggregation. I also adjusted the pH of the cell collection anticoagulant to 7.4, which reduced protein precipitation and was compatible with the Percoll used to produce the density gradient. Additionally, I prerinsed the tubes containing the hemocytes with a solution of bovine serum albumin (BSA) to deactivate surfaces. These changes resulted in clear separation of the hemocytes.

After extracting the peptides from the separated cell layers using 8 M urea and purifying the peptide-containing fraction using solid phase extraction, I mass analyzed the three samples with an LCMS instrument. The analysis showed that the granulocytes only had AMP type proteins while the hyalinocytes had a wider array of proteins including insulin binding proteins and an astakine believed to regulate the circadian rhythm. In the future, using a protein-digest approach with proteomics to confirm the results of the intact protein analysis would be helpful in validating our data. Also, cutting out specific protein bands in a gel run could allow us to focus on confirming certain AMPs in the different cell types.

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References

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