Bioactivity of a Putative Antimicrobial Peptide from the lobster, *Homarus americanus:* Isomerization of synthetic Hoa-D1 peptide in Preparation for Antimicrobial Activity Testing

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Antibiotic resistance is a growing global concern. As bacteria develop resistance to once-effective treatments, the need for new types of antibiotics becomes increasingly urgent. Interestingly, researchers have begun looking to marine crustaceans, including lobsters, for answers. Unlike humans, who rely on both innate and adaptive immune systems, lobsters only have an innate immune system, which has allowed them to survive for millions of years. A key part of their defense involves small proteins called antimicrobial peptides (AMPs), which help them fight off foreign invaders.

Previously, the Stemmler lab identified and characterized one such peptide from the American lobster that they named *Homarus* Defensin 1 (*Hoa-D1).* The Stemmler lab attempted to purify the peptide and tested its bioactivity against *E.coli.* Although the peptide showed some bioactivity, the sample used was not completely pure, making it unclear whether *Hoa-D1* alone was responsible for the observed bioactivity. To solve this problem, the Stemmler lab had the peptide chemically synthesized to ensure purity.

Surprisingly, the synthesized peptide turned out to inhabit a different conformation compared to the native peptide. This change in confirmation (the 3D shape of a molecule) is determined, in part, by disulfide bonds that connect six cysteines (amino acids) in the molecule, and the change can have a big impact on whether a protein works as intended. The goal of my summer research was to refold the synthetic *Hoa-D1* peptide so that its conformation matched that of the natural peptide, enabling accurate future bioactivity testing.

To begin, I first studied an enzyme called protein disulfide isomerase (PDI), which plays a key role in helping proteins fold correctly inside human cells. PDI works by rearranging disulfide bonds– covalent links between sulfur-containing amino acids called cysteines. These bonds help stabilize a protein's structure. In the synthetic *Hoa-D1,* the disulfide bonds were arranged differently, resulting in the wrong folding pattern.

Following a 1994 experiment by Darby and colleagues, I set up a small-scale isomerization reaction. This involved a buffer mixture, the synthetic peptide, the PDI enzyme, and a mix of reduced and oxidized glutathione, which helps drive the reaction forward. I monitored the peptide through high-performance liquid chromatography (HPLC) and fluorescence detection–methods that tell us how the peptide is folded based on how long it takes to pass through a column. After we confirmed that the small-scale experiment was working and that some of the peptide was folding correctly, I scaled up the reaction to isomerize 1 mg of synthetic *Hoa-D1.* The result was a mixture–some correctly folded version of the peptide, and some in other incorrect configurations. We used HPLC again–this time to separate and collect only the fractions with the desired confirmation.

The next steps will be to dry those purified fractions and begin testing them for bioactivity. If *Hoa-D1* in its native confirmation shows strong antibacterial properties, it could offer new insights into fighting antibiotic-resistant bacteria.

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References:

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