Development of a Microscale Chemical Technique to Selectively Reduce and Alkylate Disulfide Bonds in the AST-C peptide found in the Crab *Cancer borealis*

Abishag Suresh, Class of 2012

Pericardial organs, a neuroendocrine organ in crustaceans, were extracted from crabs and analyzed with Matrix Assisted Laser Desorption/Ionization-Fourier Transform Mass Spectrometry (MALDI-FTMS). It was found that were high concentrations of both the AST-C and NPY-Like peptides here. Tandem mass spectrometry can be used to find the amino acid sequence of peptides through fragmentation. However, when peptides, such as AST-C, contain disulfide bonds, these bonds prevent fragmentation from occurring and the complete sequence cannot be determined. Several techniques have been developed to overcome this by reducing and alkylating the disulfide bond. This effectively allows fragmentation to occur.

AST-C peptide, which was extracted from the pericardial organs, was reduced with Tris-2-carboxyethylphosphine (TCEP, 0.01 M, 20 µl, 37 degree Celsius water bath, 1 hour) and then tagged using Iodoacetamide (IAA) in NaOH (20 µl, 0.01 M IAA in 0.1 M NaOH, in the dark for 1 hour) an alkylating agent. The sample was put onto a probe face and analyzed.

The mass spectra show that the reduction and alkylation work as long as a sufficient amount of AST-C peptide is present and the final reaction sample is strongly alkaline (Figure 1). It was found that preparation of the sample and both reactions need to be done on the same day for best results.

![Mass Spectra](image)

**Figure 1.** Mass Spectra of AST-C: a) AST-C neuropeptide peak b) Reduced AST-C peptide c) Alkylated AST-C peptide. Note: the m/z values represent the mass to charge ratio specific to each peptide

Faculty Mentors: Elizabeth Stemmler, Patsy Dickinson

Funded by the Merck Fellowship