Top-Down and Bottom-up Peptidomics for the Identification of Crustacean Neuropeptides and Precursor-Related Peptides Predicted By Transcriptomics

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The central nervous system (CNS) of the crustacean is one of the simplest and most practical models to study. *Homarus americanus* is an ideal organism because of the ability of the lobster ganglia to function outside of the body when stored in saline. Understanding the underlying factors of physiological outputs lies in the ability to identify what neuropeptides are responsible for them. Neuropeptides and their modifications give insight into how physiological outputs correlate among species. Therefore, if neuropeptides can be identified and connected to their function, the information could be used to understand more complex nervous system networks.

The work of this study specialized in the pure identification of neuropeptides and their precursor related peptides (PRPs) in *Homarus americanus* lobster brain tissue. Lobster brains were dissected out, heat-treated in water, homogenized, and filtered by molecular weight for analysis by liquid chromatography-mass spectrometry (LC-MS). LC-MS allowed for identification of peptides by mass to charge ratio through ionization. After the ionization of peptides, tandem mass spectrometry created fragments of these peptides. These fragments were pieced back together to efficiently and effectively identify presence of peptides and their post translational modifications. With initial methods, smaller peptides were being identified repeatedly (top-down approach), but larger neuropeptides became much more difficult to identify. In order to identify peptides with 20+ amino acids within the sequence, peptides were reduced, alkylated, and digested with trypsin (bottom-up approach). The digestion of these peptides allowed for cleavage at arginine and lysine in order to be able to piece the cleaved tryptic fragments together for full identification.

The Christie lab, based at the University of Hawaii, does research in predicting peptides through transcriptomics and bioinformatics. From this information, these predicted peptides are searched through an in-house database. Interesting findings from combining the top-down and bottom-up approaches include finding post translational modifications on residual PRPs. Therefore leading us to believe that these PRPs contain some bioactive function. This potential marker for bioactivity in these peptides will be continuously investigated throughout the academic year. Ultimately, these peptide identifications can be used to further investigations on physiological implications of neuropeptides and their modifications. In turn, we hope this research can translate into understanding how these identified neuropeptides affect species from lobsters with simple nervous systems to other organisms with much more complex ones.

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