A Mass Spectrometric Approach to the Identification of Pyrokinin Peptides in *Homarus americanus*.

Peter Tracy, 2014

The Pyrokinin family of neuropeptides are defined by the conserved sequence of amino acids, FXPRLamide, present at the c-terminus of the peptide (the end with the carboxyl group), with X representing a variable in the sequence. Pyrokinin peptides have been identified in a number of insect and crustacean species, generally two in each species, and have shown to have effects on the rhythmic systems such as the heart and gastric mill system. *Homarus americanus* has had one Pyrokinin peptide identified as FSPRLamide (Ma et al., 2008, *Gen. Comp. Endocr*), but this had no effects on the heart or gastric mill system of *Homarus* and is much smaller than all of the other Pyrokinin peptides identified in other species. Our hypothesis is that this is just a fragment of a Pyrokinin peptide in the *Homarus*, and that, just like the other crustacean species, there should be two located in *Homarus*. My goal is to identify the sequence of the Pyrokinin peptides of *Homarus americanus* so that, in another lab, it can be synthesized to test its effects on the *Homarus* nervous system.

We begin by gross and fine dissection of the lobsters, specifically of the eyestalk ganglia, where Immunohistochemical staining with an antibody for the sequence FSPRLamide had shown a strong response. The tissue was then homogenized in an extraction solvent which varies in different experimental designs. This extracted any peptides present in the tissue. This was followed by heat deactivation to inhibit degradation of endogenous compounds from enzyme activity. This was followed by a filtering process, using either a 4 micromolar ultracentrifuge filter to remove excess tissues, a 3 kDa molecular weight cut-off filter to remove large unwanted compounds present in the sample, neither, or both. The remaining compounds were run through a Q-TOF LC-MS mass spectrometer in 0.1% formic acid in water.

The mass spectrometer uses reverse phase liquid chromatography, separating the compounds in the sample by polarity, followed by electron-spray-ionization mass analysis in a time-of-flight mass analyzer, which gives a spectrum of mass-to-charge ratios about once a second of the compounds that pass through the instrument. Combined with this process in tandem mass spectrometry. Tandem mass spectrometry will isolate a precursor ion of a specific mass, accelerate the ion through a chamber of nitrogen gas to fragment the ion, and have the instrument create a mass spectrum of the mass-to-charge ratios of all of the fragments of that one compound. We chose software settings for the instrument’s decision making for its choice of precursor ions to take as many tandem mass spectra as possible and to increase the likelihood that any Pyrokinin peptides will be chosen. Peptides tend to fragment between amino acids, so if we attain a tandem mass spectrum of Pyrokinin peptide, we can use the mass differences in the most prominent fragment peaks to piece together the entire sequence of amino acids. Pyrokinin standards were run through the LC-MS to identify some characteristic fragment peaks for Pyrokinin peptides that we can search for in our data. Any Pyrokinin peptide must have the fragment with mass 384.27, which represents the PRLamide part of the conserved sequence.

While working on this project, Audrey Bergeron and I were able to obtain a tandem mass spectrum of a peptide whose mass and fragment masses proved the peptide’s sequence to be ADFAFSPRLamide. This is in fact a peptide of the Pyrokinin family, and is very similar to one of the Pyrokinin peptides found in *P. vannamei*, with just one difference in the amino acid sequence. In my analysis of the experimental setup of this project, I developed mass spectrometric methods of extracting what I call a full peak profile and qualitative view of the mass spectral data obtained from eyestalk ganglia. This is to be used in the further verification of previous findings and the discovery of a second pyrokinin for *Homarus americanus*. 
Tandem Mass Spectrum for \textbf{ADFAFSRPLamide}

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