Elucidation of a Putative Enzyme-Catalyzed Methylation Reaction in Crustacean Neuropeptides

Dan Polasky, 2012

Previous research in the Stemmler and Dickinson labs at Bowdoin revealed a reaction modifying orcokinin-family neuropeptides during the process of extracting the neuropeptides from H. americanus (American lobster) eyestalk tissues in a methanol based solution. This reaction was hypothesized to be catalyzed by an enzyme, but due to the high concentration of methanol in extraction solvents and nature of the reaction, no biological enzyme of those known in the lobster would explain the reaction. This project built on previous work from an honors project for the Department of Chemistry in attempting to characterize and possibly identify the enzyme responsible for the reaction.

Three approaches were used in attempting to characterize the enzyme of interest responsible for modifying orcokinin neuropeptides during extractions. The first involved adding standards of peptides with sequences similar to the orcokinins, but with slight modifications near the reaction site, to solutions containing the unknown enzyme from the lobster eyestalks to probe the specificity of the enzyme towards different peptide substrates. It was found that the enzyme requires a specific –FG– motif near the c-terminus of the peptide, and will cleave between the F (phenylalanine) and G (glycine) residues whether they are two or three residues from the c-terminus. This implies that the enzyme exhibits endopeptidase behavior, as an exopeptidase (the alternative) would cleave always two residues (as in the original reaction) instead of a variable number of residues to a specific site (as was observed in this study).

The second approach involved adding inhibitors specific to different classes of enzymes in an attempt to categorize the type of protease responsible for the reaction. It was found that E-64, an irreversible cysteine protease inhibitor, completely halted enzymatic activity, while AEBSF and Pepstatin-A, serine and aspartic protease inhibitors respectively, had no significant impact on enzymatic activity. These results indicate that the enzyme responsible is a cysteine protease.

The third approach attempted to use the information gathered in the first two experiments to identify a specific enzyme from the lobster that could be the one responsible for the modification reaction of the orcokinin neuropeptides. To accomplish this, eyestalk extract solutions containing the enzyme of interest were digested with trypsin to break any large proteins, including the enzyme of interest, down into smaller peptides. The resulting solution of these peptides was analyzed by LC/MS/MS to sequence the peptides in solution. The sequences generated were then matched against a database of known proteins, including enzymes, based on genetic information from the lobster. However, only a small part of the lobster genome has been sequenced, and no match to the peptide sequences in solution was found at reasonable confidence levels for any cysteine protease-like proteins.

Faculty Mentor: Elizabeth Stemmler

Funded by a grant from the US Department of Energy and a James Stacy Coles Fellowship