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Presentation Title: Deducing the Mechanisms of E2-Conjugating Enzymes: Synthesis of a Di-Ubiquitin Probe through Incorporation of an Unnatural Amino Acid
Research focus: Biochemsitry
School: Georgia State University
Student Level: Undergraduate Presentation
Type: Poster Presentation

Abstract

Ubiquitin is a small protein with a molecular weight of only 76kDa, that is used for post-translational modifications (PTM) on target proteins. These PTM by ubiquitin control many cellular processes in Eukaryotes, including protein trafficking, DNA repair, protein degradation, and signal transduction. Ubiquitin is covalently linked through its C-terminal glycine residue to specific lysine residues on target proteins. Ubiquitin can be bound to one of multiple lysine residues on the target protein and can bind as a single ubiquitin (mono-UB) or as a poly-ubiquitin chain. Ubiquitin is bound to the target protein in an E1-E2-E3 cascade. E1 activates ubiquitin by adding an AMP group. Thiol-containing E2's provide an active site while E3's catalyze the transfer to the target protein. Ubiquitin has seven polyubiquitination sites which include K6, K11, K27, K29, K33, K48, and K63.

This laboratory synthesizes a small molecular di-ubiquitin probe that can be utilized to map the E1-E2-E3 signaling cascade, specifically aiming to capture the transition state of ubiquitin transfer through E2 conjugating enzymes. This feat is accomplished through synthesis of an unnatural amino acid similar to lysine and incorporates this amino acid into ubiquitin at either the K11, K48, or K63 positions. This unnatural amino acid is incorporated into ubiquitin via arabinose induction and the unnatural amino acid is then deprotected via pH control and linked to the donor ubiquitin through intein-mediated chemical ligation.

By mapping the E1-E2-E3 signaling cascade, a greater understanding of ubiquitin PTM can be gained. This knowledge can be used to garner a greater understanding of the underlying mechanism and pathophysiology of neurodegenerative diseases linked to anomalies in ubiquitin PTM such as Parkinson's disease and Alzheimer's disease. The di-ubiquitin probe has been successfully synthesized with K11, K48, and K63 linkages, with these linkages confirmed through tandem mass spectroscopy.

Currently, this laboratory is working on creating a tri-protein complex by conjugating the di-ubiquitin probe with various E2 and E3 enzymes, including Ube2s, Nedd4, and HUWE1. In the future, the di-ubiquitin probes will be used to perform affinity pull-downs on live tissue samples from Alzheimer's patients. We are also preparing to send the di-ubiquitin E2 and E3 conjugates to a structural biology laboratory to obtain more insight into theses transition states.