Online Proceedings

Poster Session 1

MGH 258, Easel 180

11:00 AM to 1:00 PM

Production of Highly Active EGCase I From *E. coli*

Jonathan Evan Tam, Senior, Biochemistry, Psychology
Mentor: Miklos Guttman, medicinal chemistry

Glycans are ubiquitous in biology and play a crucial role in cell to cell signaling, immunity, and disease. The A, B, and O blood groups are differentiated by different glycan structures and linkage patterns presented on either glycoproteins or glycolipids. To date, the exact glycan structures that differentiate blood types remain unknown. A clear understanding of these structures would increase the success of transfusion procedures and reduce the risk of complications. Our lab is currently examining glycans from glycoproteins isolated from red blood cell membranes, however we do not have the enzymes needed to remove glycans from glycolipids. ECGase I is an enzyme that was recently shown to effectively cleave glycans from a wide range of glycolipids. We have cloned the gene for ECGase I into a GST fusion vector for recombinant bacterial expression. With this we have been able to generate high yields of purified ECGase I. The EGCase I produced was more active than commercially available product. The ability to grow EGCase I enables large scale investigation of glycolipids involved in the ABO blood group system.

Poster Session 1

Balcony, Easel 107

11:00 AM to 1:00 PM

Defending Against Protein Aggregation: How HspB1 Affects Tau and Its Disease-Associated Mutants

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UW Honors Program
Mentor: Hannah Baughman, Medicinal Chemistry
Mentor: Abhinav Nath, Medicinal Chemistry

In neurons, microtubules are critical for cellular and developmental functions including neurite outgrowth and maintaining stable wiring of the nervous system. Tau, an intrinsically disordered protein, plays important roles in microtubule assembly and stabilization. However, tau can dissociate from microtubules and form amyloid fibrils in a set of neurodegenerative diseases termed tauopathies, which includes Alzheimer's disease and frontotemporal dementia. Mutations in the tau sequence cause the disease FTDP-17T, providing evidence that alterations in tau alone can cause tauopathies. Most mutations occur in or near the microtubule-binding domain of tau, which suggests that mutant protein may be deficient in its capacity to stabilize neuronal microtubules, more prone to aggregate, or both. In particular, the disease-associated mutation P301L appears to enhance tau's propensity to aggregate into fibers and alter its pathological activity. Molecular chaperones are responsible for maintaining protein solubility, promoting proper folding, and preventing atypical aggregation. The chaperone HspB1 delays wild-type tau fibril formation by weakly interacting with early species in the aggregation process. It is able to recognize aggregation-prone motifs within the microtubule binding repeat region of wild-type tau. Therefore, we used fluorescence spectroscopy to test the affinity and activity of HspB1 against wild-type and mutant models of the tau microtubule binding region, to see whether changes in the tau sequence alter interaction with the chaperone. In addition, tau oligomers are known to disturb lipid bilayers, a harmful interaction that could lead to potential cell death. Using a vesicle permeabilization assay, we tested the interactions between artificial lipid membrane with our tau constructs, and whether HspB1 can counteract these harmful interactions. This will extend our understanding of the characteristics and behaviors of disease-associated mutations of tau and the ways in which chaperone target aggregation-prone tau species.