Metabolic Labeling of Zebrafish Embryonic Cell N-glycans Using Azido-Sugars

Alexander Spitznagle, Jillian Sylvia, Steven Weicksel & Christopher Reid

Biological & Biomedical Sciences, Bryant University, Smithfield, RI

Zebrafish embryonic cells are used in many facets of research such as maintenance of transient cell populations and control of chemical and mechanical cues received by cells, as well as their use as a model for environmental toxicology. A focus of our research is to use zebrafish as a model to investigate molecular changes that occur when vertebrates are exposed to pollutants (ie polyfluorinated alkylsulfonates (PFAS)). Protein glycosylation is a common post-translational modification involved in numerous biological functions such as cell to cell communication, protein trafficking, and cell adhesion. It is the process where sugars and carbohydrates (glycans) are covalently attached to proteins via an N- or O-glycosidic linkage. The objective of our research is to investigate the use of a bio-orthogonal metabolic labeling strategy using azido sugars to visualize changes in the cell N-glycosylation patterns in response to environmental stress. Our initial choice for synthesis of a metabolic label was N-acetylglucosamine as it is found in all N-glycans. Based on this we synthesized a 2-azidoacetamido-2-deoxy-D-glucopyranoside (GlcNAz) derivative. GlcNAz was synthesized from D-glucosamine hydrochloride via acetylation with chloroacetic anhydride. Next, the azide was introduced by addition of sodium azide in DMF at 50oC while stirring overnight. The resulting GlcNAz was peracetylated using acetic anhydride and pyridine to give 1,3,4,6-tetra-O-acetyl-2-azidoacetamido-2-deoxy-D-glucopyranoside (perOAcGlcNAz). The perOAcGlcNAz was purified by flash and reversed-phase chromatography. We tested the incorporation of GlcNAz into zebrafish cells in a titration experiment to investigate ideal sugar concentration for incorporation. A perOAcGlcNAz concentration range of 46µM to 138µM was screened. The cells were incubated for 3 days at 30°C until cell confluence was reached. Cells were harvested, lysed in phosphate buffered saline via sonication. Acetone precipitation of proteins was used to concentrate the samples. Glycans were then labeled with alkyne-(PEG4)-biotin in 1mM ascorbic acid and 1 mM copper (II) sulfate solution. The biotinylated glycans were detected in a dot blot with horseradish peroxidase labeled streptavidin. Preliminary results demonstrated weak labeling in cells incubated with 43 µM perOAcGlcNAz. Future experiments will involve flow cytometry and fluorescent microscopy to observe any changes within the cell.

This work was supported by RI NSF EPSCoR C-AIM