Grace Johnecheck

Professor Louters

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Aggregation of GLUT1

Glucose transporter (GLUT) proteins bring the sugar glucose into cells. Cells then use glucose as an energy source. GLUT1 was the first glucose transporter protein discovered and has been found to be important in many different diseases, including cancer, Alzheimer's, and diabetes. Professor Louters' lab has previously found that GLUT1 increases the amount of glucose it lets into cells under different conditions without changing the amount of GLUT1 on the surface of the cells. Therefore, we hypothesize that GLUT1 is activated by aggregation, that is, by four GLUT1 proteins coming together in one complex, called a homotetramer. However, GLUT1 has never been shown to be in homotetramers in any cell type other than red blood cells. Therefore, the purpose of my project was to show that GLUT1 forms these homotetramers when activated in the human kidney cells and mouse fibroblast cells I was studying.

In order to work towards this goal, I used size exclusion chromatography and blue native gel electrophoresis. Both of these methods separate proteins based on size. Size exclusion chromatography requires using a column containing tiny porous beads. The smaller a protein is, the easier it is for it to get stuck in the pores of the beads. Therefore, smaller proteins take longer to come go through the column. In blue native gel electrophoresis, proteins are surrounded by a negatively charged compound (Coomassie G-250) and move towards the positive end of the gel when a voltage is applied to the gel. The gel has a gradient in the amount of crosslinking there is from the negative to positive end, that is, it is harder for large proteins to get through the gel as they move closer to the positive end. That is how it separates the proteins by size. I spent much of my time this summer attempting to perfect these techniques. Using both of these techniques, I was able to separate proteins by size and achieve a signal for GLUT1. There was potentially a shift in the size of GLUT1 in the size exclusion data depending on what cell line I was using.

One of the major challenges I faced in my work this summer was the method of extraction of GLUT1 from the membrane. Because GLUT1 is a membrane protein, it is insoluble in water. Therefore, detergents are necessary to separate GLUT1 from the membrane and make it soluble in water. However, the detergent must be able to separate GLUT1 from the membrane without breaking up the GLUT1 aggregates. I tested eleven different detergents using Bioluminescence Resonance Energy Transfer (BRET). For this technique, the cells contained GLUT1 with light-producing nanoluciferase enzyme attached. They also express GLUT1 with the fluorescent molecule mCherry attached to it. When the GLUT1 proteins are close together, such as when they are in a complex, the mCherry fluoresces red. Therefore, I was able to use this technique to group the different detergents into different classes based on how they affect this fluorescence signal in the cells and use this data to help determine which detergents may be most useful for my experiments.

During this summer, I learned how to perform specific biochemistry experiments. However, more importantly, I learned how to participate in the longer process of scientific research and became excited about the future contributions I could make to scientific research.