CALIFORNIA STATE SCIENCE FAIR
2013 PROJECT SUMMARY

Name(s)

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Project Number

33147

Project Title

Improving Binding Affinity of the Calbindin-D9k Protein to Develop Efficient Calcium Biosensors

Abstract

Objectives/Goals

Calcium (Ca2+) plays a critical signaling role at the cellular level in our body to regulate important biological processes such as muscle cell contraction, cell division and growth, and transmission of neural signals. Ca2+ concentration varies widely at the organelle level within the cell. Organelles coordinate to generate a variety of dynamic Ca2+ signals in response to external stimuli. Analyzing these dynamic changes in Ca2+ concentration at the subcellular level is critical to understanding causes of diseases like cancer and Alzheimer's. Fluorescent protein Ca2+ biosensors are an effective probe to measure. However, current fluorescent protein Ca2+ biosensors use Calmodulin, a Ca2+ binding protein that is present in almost all eukaryotic cells, thus interfering with normal cell functions.

Methods/Materials

This project uses an alternative Ca2+ binding protein called Calbindin-D9k (CaBP-D9k) to develop Ca2+ biosensors that will not interfere with normal cell function. To develop Ca2+ biosensors, which can detect the wide range of Ca2+ concentrations at the organelle level, I investigated how to control Ca2+ binding affinity of CaBP-D9K by identifying and mutating its Ca2+-binding sites using site directed mutagenesis.

Results

The CaBP-D9k cDNA was successfully cloned into the pE-SUMOstar expression vector. The CaBP-D9k protein was expressed and run through a Nickel Protein Purification Column. While it was difficult to completely isolate the protein and optimize the expression, after experimenting with various parameters including time and temperature, it was determined that the ideal conditions for protein expression were 20 hours and 20 degrees. I optimized the protein expression of CaBP-D9k to increase the percent yield from less than 5% to greater than 50%. The purified protein will be characterized using luminescence spectroscopy and mutated using site-directed mutagenesis to change binding affinity.

Conclusions/Discussion

This experiment will help alter the binding affinity to allow fluorescent protein biosensors based on CaBP-D9k proteins to detect differences in concentration at the organelle level. This can be used to understand the relation between Ca2+ concentration and diseases like cancer and Alzheimer's.

Summary Statement

This project develops a novel calcium-biosensor by improving binding affinity of Calbindin-D9k protein to detect changes in intracellular calcium concentration without interfering with normal cell functions.

Help Received

Used lab equipment at San Jose State University under the supervision of Dr. Elaine Collins and graduate student, Mallory Kato.