CALIFORNIA STATE SCIENCE FAIR
2011 PROJECT SUMMARY

Name(s)

Eesha Khare

Project Number

31348

Project Title

A Novel Method Using Chemically Engineered CYP101 Enzyme and Light to Hydroxylate Camphor

Abstract

Objectives/Goals

P-450 is a family of enzymes involved primarily in drug metabolism and bioremediation. These enzymes catalyze a variety of reactions and are a promising alternative to chemical synthesis. Commercial use is limited by substrate specificity, low activity, poor stability, and the need for expensive cofactors. The role of P-450cam C is to catalyze the hydroxylation of camphor, making it polar and easily excreted. In vivo, cam C works with cam A and cam B, which regenerate the cofactor NADPH required for electron transport. In vitro, cam C requires additional cofactors because it does not have cam A and cam B for regeneration. In vitro, this standard reductive process produces a reactive oxygen species, which has the potential of damaging the enzyme during catalytic cycle, limiting its usefulness. The goal is to find an alternative catalysis pathway, which bypasses the production of a radical oxygen species and does not use NADPH. This will stabilize the enzyme and eliminate the need of cofactors, allowing for the P450cam C enzyme to be used for the breakdown of many toxic substances in commercial businesses more effectively.

Methods/Materials

In our novel method, an oxidative process, instead the reductive process was used. No cofactors, cam A, or cam B were used and all surface cysteines of the protein were mutated to attach a Ruthenium label for faster electron transfer. Light and water was used to process the electron transport required to carry out the reaction.

Results

The mutated cam C cDNA was cloned into the pCAL plasmid. The cam C variant protein was expressed from E. coli expression cells. It was difficult to optimize the protein expression to obtain a high percent yield. After troubleshooting various parameters including expression media, time, temperature, protein stability, and concentration of media supplements, I was finally able to improve the cam C protein expression from a yield of less than 1% to 50%. The 0.5 mM concentration of IPTG proved best because it allowed for the cells to produce proteins at a reasonable pace.

Conclusions/Discussion

This experiment will show an oxidative pathway of catalysis through the elimination of cofactors and increased protein stability. It can be useful to other scientists or businesses interested in bioremediation, alternative chemical synthesis, and detoxifying liver toxins.

Summary Statement

This project uses an oxidative pathway instead of a reductive pathway in the catalysis of the hydroxylation of camphor by the P-450cam C gene thus making it more stable and eliminating the need of cofactors.

Help Received

Used lab equipment at San Jose State University under the supervision of Dr. Elaine D. Collins.