CALIFORNIA STATE SCIENCE FAIR
2011 PROJECT SUMMARY

Name(s)

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Project Number

S0501

Project Title

Therapeutic Natural Products: Effect of H36A Mutation on Structure and Function of StfQ

Abstract

Objectives/Goals

Polyketide chains are natural products with antitumor, antibiotic, and immunosuppressive properties. The Aromatase/Cyclase (ARO/CYC) is a PKS domain that cyclizes and aromatizes the first ring of the polyketide. StfQ, a specific bacterial ARO/CYC, is integral to the biosynthesis of the antitumor Steffimycin natural compound, which is toxic in mammalian cultures. Understanding the structure and function of StfQ will allow us to bioengineer a safer Steffimycin analogue for future natural antitumor treatments.

Methods/Materials

Transformation and protein expression: H36A plasmid construct, Nova Blue E. Coli cells, LB broth, Kanamycin, Spectrometer (to measure concentration), Incubation and low temp shakers, Gene sequencing services

Purification and concentration: High speed centrifuges, Centrifugal tubes, Ice bucket to store temperature-sensitive StfQ protein (when in use and not stored in 4C room), Lysis buffer (Tris-HCl, imidazole, NaCl), Sonicator, Imidazole and lysis buffer washes, Ni iMAC column and Ni resin, Magnetic stirrer, PD-10 column, and chosen storage buffer (varies with crystallization experimentation), SDS/PAGE gel, Bradford

Crystallization: Pre-crystallization test kit, Sitting drop and hanging drop trays, chosen well solutions, glass slips and tape

Polyketide Product Detection: HPLC (High Performance Liquid Chromatography) machine; Assay reactants minPKS: SKM, ACP, and Malonyl-CoA; native StfQ: SKM, ACP, native StfQ, and Malonyl-CoA; H36A StfQ: SKM, ACP, H36A StfQ, and Malonyl-CoA.

Results

H36A StfQ was screened in 1,152 different crystallization conditions. The Classics I Screening contained one condition (Classics #15) which did crystallize H36A StfQ: 0.02 M CaCl2, 0.1 M Na Acetate pH 4.6, 30% (v/v) MPD. It was found that H36A StfQ crystallized in the hanging drop trays.

HPLC (High Performance Liquid Chromatography) results show that the H36A mutation stops StfQ from functioning, since the native StfQ product NonaSEK4 is not present in the H36A StfQ reaction assay.

Conclusions/Discussion

Polyketide product detection results show that the specific mutation H36A obstructs all StfQ activity. Furthermore, different crystal morphologies of Native StfQ and H36A StfQ, as well as different crystallization conditions, hint at the altered StfQ structure caused by the H36A mutation.

Summary Statement

I perform a specific point mutation to study its effects on the structure and function of StfQ, an enzyme integral to the biosynthesis of the bacterial, natural antitumor Steffimycin compound.

Help Received

Participant in American Cancer Society/Beckman Coulter Youth Science Research Fellowship; used lab equipment at the University of California, Irvine, under the supervision of Dr. Shiou-Chuan Tsai, and mentors Grace Caldera and Stephanie Aguilar