CALIFORNIA STATE SCIENCE FAIR
2003 PROJECT SUMMARY

Name(s)
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Project Number
S0402

Project Title
Crystallization and X-Ray Structure Analysis of the Novel Plant Protein 279

Abstract

Objectives/Goals
The recently sequenced genome of the plant Arabidopsis has revealed five proteins homologous to Chalcone Isomerase, a critical enzyme involved in plant biosynthesis. One of these, protein 279, interacts with fibrillin, a protein pivotal in the fruit ripening process. We set out to purify, crystallize and solve the tertiary structure of 279 by X-ray crystallography in order to help unravel its mechanism of action in plant biosynthetic pathways.

Methods/Materials
Histidine-tagged 279 was expressed in E. Coli using Polymerase Chain Reactions. The cells were lysed, centrifuged and the His-tagged 279 was purified using Nickel NTA and gel-filtration columns. The His-tag was cleaved using thrombin dialysis and the pure 279 was concentrated using a Centriprep. The purified 279 was set up for crystallization in trays using the hanging drop technique under 24 different conditions of polyethylene glycol (PEG) concentration, salt and pH (5.5-8.5) per plate.

Results
Of the hundreds of conditions set up, 279 crystallized under the conditions of 21% PEG 3350 and 0.3M potassium chloride at pH 8.5, 21% PEG 10k and .05M sodium cacodylate at pH 7, 21% PEG 5k and 0.3M potassium nitrate at pH 7 and 25% PEG 20k and 0.05M ammonium formate at pH 7. Preliminary X-Ray crystallographic analysis revealed that 279 crystallizes into an orthorhombic lattice.

Conclusions/Discussion
This research revealed that 279 crystallizes and hence functions under conditions of pH 7 and above

Summary Statement
The crystallization and analysis through X-Ray diffraction of the novel plant protein 279

Help Received
Used lab equipment at the Salk Institute under the supervision of Dr. Joe Noel and Marianne Bowman.