

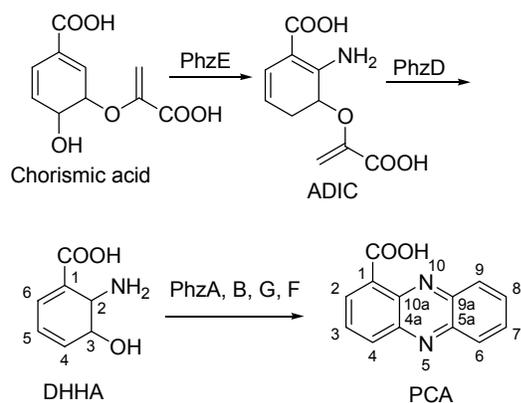
Enzymes in the Pathway for Production of Aromatic Hydrocarbons

Aromatic hydrocarbons are difficult to produce synthetically. The study of how these compounds are produced enzymatically in bacteria can greatly benefit the chemical and pharmaceutical industry. There are currently two focus areas in our study. One is on the enzyme chorismate mutase which is at the heart of the pathway at one of the key branch points. The second involves phenazines which are some of the products of one branch of the pathway and are difficult to synthesize but are important potential drug targets. Phenazines are important since several species of the bacterium *Pseudomonas*, including the human pathogen *P. aeruginosa*, produce phenazines as secondary metabolites.

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Dozens of naturally occurring phenazines have been described, all of which share the characteristic tricyclic heteroaromatic ring system. Phenazines are redox-active compounds that participate in reactions yielding superoxide and peroxide ions, and hydroxyl radicals. These toxic molecules are thought to control the growth of other microorganisms. But they also provide *Pseudomonas* with a competitive growth advantage, and may enhance the ability of these pathogens to colonize human and other tissues. Two operons in *Pseudomonas aeruginosa*, each containing seven genes, are involved in the biosynthesis of phenazine (*phzA-G*). A similar, single operon has been described in *P. fluorescens* 2-79. Each of these operons encode all of the genes required to produce phenazine-1-carboxylic acid (PCA) from chorismate.

Pathway for the Conversion of Chorismic Acid to Phenazine-1-Carboxylate



The schematic diagram shows the steps and the enzymes involved in the conversion of chorismic acid through intermediates aminodeoxyisochorismate (ADIC) and *trans*-2,3-dihydro-3-hydroxyanthranilic acid (DHHA) to phenazine-1-carboxylate (PCA).

The structure of the enzyme chorismate mutase is known to be an α -helical dimer in *Escherichia coli* and to be a trimer with an α/β structure in *Bacillus subtilis*. The chorismate mutases from *Mycobacterium tuberculosis* and *Yersinia pestis* are predicted to also be all α -helical dimers but the monomers are nearly twice as long as the monomer in *E. coli*. Elucidation of the structure of this class of chorismate mutase will help us relate the variation in the structures of these enzymes, which all have the same function and which could reveal some relationships at the gene level.

The work this year has yielded a much-improved understanding of the phenazine pathway. Specifically, we have solved the structure of the product of gene *phzF* and have pursued both biochemical and crystallographic methods to determine its role, substrate, and product. Previously, we had solved the structures of *phzD* and *phzG* of this pathway. This year we cloned and purified the chorismate mutase from *Yersinia pestis* and have a selenomethionine version of this enzyme. Thus, we are close to solving a structure of the longer α -helical chorismate mutase. Solving this structure could allow us to use it to solve the related chorismate mutase from *Mycobacterium tuberculosis*. The determination of these structures and the elucidation of the biochemical pathways makes it possible to alter and utilize these materials and pathways to make useful chemical products or to produce drug inhibitors which halt the production of pathogens.

Publication:

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We plan to continue our studies of the enzymes along the chorismate pathway. In particular, we are continuing to look at other enzymes in the phenazine pathway in *Pseudomonas* in order to more fully elucidate the mechanisms involved in the production of these biologically active products. We are also looking at enzymes which are homologues of these enzymes from other related pathways to try to understand the details of how the enzymes perform their specialized tasks.