

Bacterial P450 Cytochromes as tools for designing novel antimicrobial agents.

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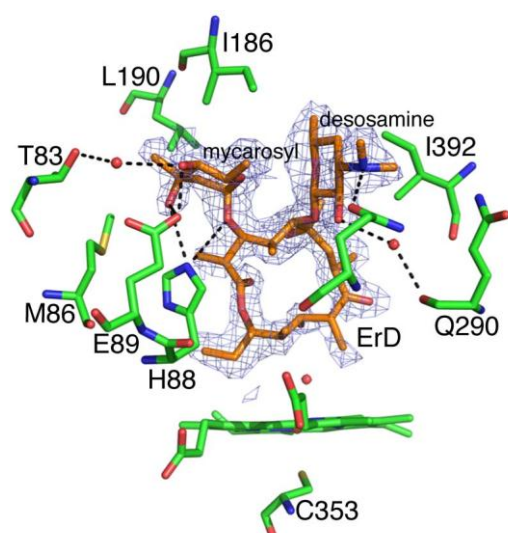
Abstract

Cytochrome P450s are heme-containing proteins that catalyze the oxidative metabolism of a wide array of compounds (i.e. antibiotics, lipids, steroids). Because of their unique oxygen chemistry and their key role in drug and xenobiotic metabolism, particular attention has been devoted to their mechanism of substrate recognition. In this project, we propose to analyze and engineer the structure of two P450 involved in antibiotic biosynthesis.

The P450 from *S. erythraea* called EryK catalyses one of the final steps of erythromycin A (ErA) biosynthesis. EryK acts as a hydroxylase on the C12 of the macrolactone ring of the metabolic intermediate erythromycin D (ErD). The high specificity of EryK toward its physiological substrate causes the accumulation of the shunt metabolite ErB, which differs from ErD only for the presence of a methyl group (1,2). Our goal is to engineer EryK making it able to convert ErB in ErA, still maintaining its activity on ErD. Furthermore, we plan to introduce mutations to alter its substrate specificity to produce novel erythromycin variants or to act on different substrates.

The P450 monooxygenase OleP is involved in oleandomycin biosynthesis, catalyzing the epoxidation of C8 of the oleandomycin lactone ring. However, there are significant uncertainties about the sequence of events during the biosynthetic pathway, including the timing of the oxidative steps, which lead to the introduction of an epoxy group by OleP (3).

Our aim is to determine the structure of this enzyme and characterize its peculiar functional properties, with the perspective to adapt it towards acting on different substrates.



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Legend: The active site of the P450 Cytochrome EryK, bound to its substrate, ErD from Ref 1.

GROUP COMPONENTS

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