# Structural Genomics of CYP Complement from *Streptomyces*: Application for Generating New Antibiotics

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## Introduction

The genus *Streptomyces* produces approximately two-thirds of naturally occurring antibiotics as well as a wide array of the secondary metabolites. Polyketides form one of the largest and most diverse group of these natural products. They include numerous medical compounds (e.g., antibacterial, antifungal, anticancer, and immunosuppressant agents). Although polyketide molecules have diverse structures, the biosynthetic pathways share common features. The antibiotic diversity of polyketides is generated during their biosynthesis by several means, including post-polyketide modification performed mainly by group transferases and oxidoreductases, a very broad group of enzymes that includes cytochrome P450 monooxygenases (CYPs). CYP activities include the site-specific oxidation of macrolide antibiotic precursors, which introduces regiochemical diversity into the macrolide ring system, thereby significantly affecting antibiotic activity. The efficient manipulation of *Streptomyces* CYPs to generate new antibiotics will require the identification and/or engineering of monooxygenases with activities toward a diverse array of chemical substrates.

To better understand the relationship between the structure and the function of CYPs from secondary metabolic pathways of the industrially important *Streptomyces*, we initiated the genetic, biochemical, and structural evaluation of all 18 CYPs from *Streptomyces coelicolor* A3(2). X-ray structures for three *S. coelicolor* A3(2) CYPs from two different CYP families, CYP154 and CYP158, were recently determined [1, 2].

## Methods and Materials

Methods and materials are described in Refs. 1 and 2. Data were collected at SER-CAT beamline 22-ID and SBC beamline 19-ID at the APS.

## Results

The first result relates to the 1.92-Å crystal structure of *S. coelicolor* A3(2) CYP154C1, a new monooxygenase that functionalizes macrolide ring systems. Although the endogenous function and biological role of *S. coelicolor A3(2)* CYPs remain unknown, the sequence of a number of them is significantly similar to that of P450 monooxygenases from other microorganisms involved in regiospecific oxidation of macrolide antibiotics. CYP154C1 has multiple catalytic activities *in vitro*. The enzyme converts 12-member and 14-member ring macrolide intermediates YC-17 and narbomycin to the antibiotics methymycin, neomethymycin, and pikromycin. The most significant feature of the CYP154C1 structure is a large, open cleft between the α-helical and β-rich domains, which extends from the distal protein surface directly to the heme (Fig. 1). An even larger cleft has recently been reported for the mammalian xenobiotic metabolizing cytochrome P450 2B4. Such an open conformation of the substrate binding site allows more space above the heme and confers more freedom to accommodate substrates of variable size. This is consistent with the biological function of monooxygenases with broad substrate specificity and xenobiotic functionalizing enzymes.

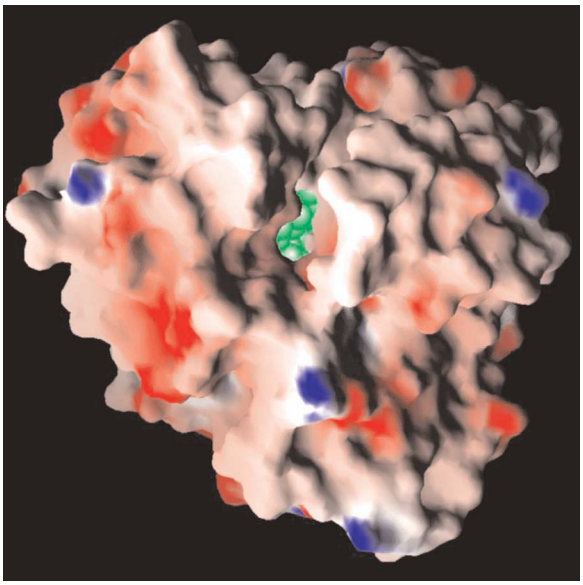

*FIG. 1. Molecular surface of CYP154C1 colored according to electrostatic potential. Heme partially seen through cleft is green.*

The second result comes from a comparison of the

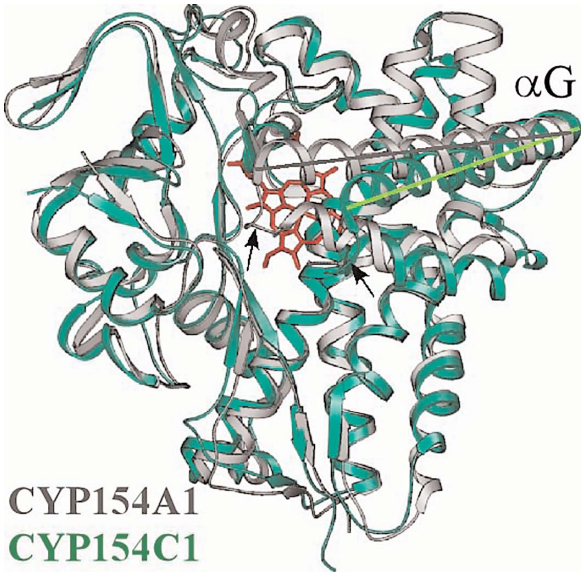


*FIG. 2. Comparison of the CYP154A1 and CYP154C1 structures. Solid lines show orientation of the G helix in both structures*

1.85-Å structure of CYP154A1 from *S. coelicolor* A3(2) with the closely related CYP154C1 and CYPs from antibiotic biosynthetic pathways. Despite the 42% identity between CYP154A1 and CYP154C1, CYP154A1 reveals an active site that is inaccessible from the molecular surface and an absence of catalytic activities observed for CYP154C1 (Fig. 2). Systematic variations in the amino acid patterns and the length of the surface HI loop correlate with the degree of rotation of the F and G helices relative to the active site in CYP154A1 and related CYPs, CYP154C1, OxyB, and EryF, presumably regulating the degree of active site accessibility and its dimensions. Heme in CYP154A1 is in a 180° flipped orientation compared with most other structurally determined CYPs.

The third result relates to the crystal structure of CYP158A2 from *S. coelicolor*. As is the case for other *S. coelicolor* A3(2) CYPs, the function of CYP158A2 is not yet clearly established. It is thought that CYP158A2 is required for the synthesis of flaviolin and/or its dimerized form. These molecules have antibiotic activity and make up a gray *Streptomyces* pigment. The crystal structure of CYP158A2 is determined at a resolution of 1.5 Å by the multiwavelength anomalous diffraction (MAD) technique, with both Fe and Hg used as anomalous scatterers. The structure reveals another example of CYP with widely open access to the heme from the protein surface (Fig. 3). Open conformation arises from the dissociation of contacts between residue in the B′ and G helices and displacement of the helices F and G.

## Discussion

Current structural studies of *Streptomyces* CYPs have resulted in the determination of the first 3-D structure for CYP with activity toward polyketides of diverse structures: CYP154C1. This has advanced our understanding the molecular basis for the specificity of oxidative tailoring in macrolide antibiotic biosynthesis. In addition, the crystal structure of another CYP assigned to the same CYP family — CYP154A1 — was determined. CYP154A1 possesses none of the catalytic activities observed *in vitro* for CYP154C1 and has prominent structural differences from CYP154C1. Variations in the length of the HI loop correlate with the degree of rotation of the F and G helices relative to the active site in CYP154A1-related CYPs, presumably regulating active site accessibility and dimensions.

## Acknowledgments

We thank D. Lamb and S. Kelly for providing us with expression vectors, D. Sherman and H. Bach for performing tests for functional activity, personnel at SER-CAT beamline 22-ID at the APS for expert assistance and support during data collection, M. Arase for excellent technical assistance, and T. Poulos at the University of California, Irvine, for helpful discussions and critical reading of the manuscripts. This work was supported by National Institutes of Health Grant Nos.

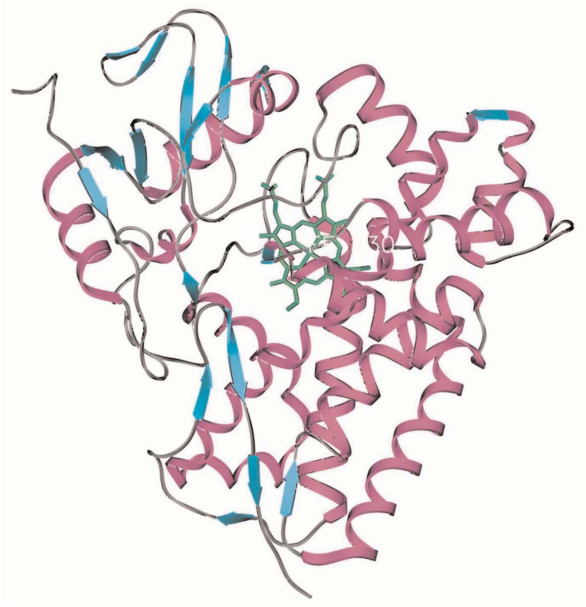

*FIG. 3. Ribbon representation of the CYP158A2 structure.*

GM37942 and ES00267 to M.R. Waterman and Grant No. P30 ES00267 to L.M. Podust. Use of the APS was supported by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences, under Contract No.W-31-109-ENG-38.

## References

[1] L.M. Podust, H. Bach, Y. Kim, D.C. Lamb, M. Arase, D.H. Sherman, S.L. Kelly, and M.R. Waterman, "Comparison of the 1.85 Å structure of CYP154A1 from *Streptomyces coelicolor* A(3)2 with closely related CYP154C1 and CYPs from antibiotic biosynthetic pathways," Protein Science (in press, 2003).

[2] L.M. Podust, Y. Kim, M. Arase, B.A. Neely, B.J. Beck, H. Bach, D.H. Sherman, D.C. Lamb, S.L. Kelly, and M.R. Waterman, "The 1.92-Å structure of *Streptomyces coelicolor* A3(2) CYP154C1. A new monooxygenase that functionalizes macrolide ring systems," J. Biol. Chem. **278**, 12214-12221 (2003).