NW12A, BL-5A, BL-17A/2011G129

Structure of cytochrome P450 RauA (CYP1050): an essential enzyme for aurachin antibiotic biosynthesis in *Rhodococcus erythropolis*

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1 Introduction

Aurachin alkaloids are rare antibiotic compound in nature, composed of quinolone ring and farnesyl chain. The backbone of aurachin resembles vitamin K_2 (menaquinone), the sole respiratory quinone for most eubacteria. Recent studies showed that aurachin functions as an inhibitor of menaquinon biosynthesis pathway as well as respiratory chain complex [1]. We have successfully cloned the genes encoding enzymes involving aurachin biosynthesis in *Rhodococcus erythropolis* JCM 6824 (*rauA-rauH*), and revealed that one cytochrome P450 (RauA) plays a crucial role in conferring strong antibiotic activity to aurachin skeleton. Gene disruption experiments and *in vitro* enzyme assay showed that RauA catalyzes the quinolone nitrogen hydroxylation, thereby producing the mature antibiotic aurachin alkaloid (aurachin RE) [2]. The P450s catalyzing nitrogen hydroxylation are uncommon, though it is known that a few eukaryotic P450s could catalyze aromatic amine hydroxylation. To investigate the structural mechanism of aurachin binding and quinolone-ring nitrogen hydroxylation of RauA, we have undertaken the crystallographic studies.

2 Experiment

P450 RauA was expressed by *Escherichia coli* BL21(DE3), and purified by Ni-affinity, anion-exchanging, and gel-filtration chromatography. Prior to crystallization, the purified sample was mixed with molar excess of substrate (biosynthetic intermediate of aurachin RE). Crystals of RauA were obtained by vapor-diffusion method at 20°C. The clustered polycrystals were reproducily obtained using the solution containing 0.04 M citric acid, 0.06 M bis-tris propane (pH 6.4), and 12-15% PEG3350. However, preliminary X-ray diffraction studies at BL-5A and BL-17A showed that those crystals exhinbited high degree of mosaicity and diffracted below 3.0 Å. In contrast, a good quality crystal was obtained using the solution containing 0.1 M MES pH 6.5 and 12% PEG20000, which diffracted to a resolution of 2.19 Å. The X-ray diffraction data were collected at AR NW12A at PF, using CCD detector (ADSC). Crystal belongs to space group $P2_1$ with unit-cell dimensions a = 41.5, b = 100.0, c = 52.3 Å, and β = 108.7°. The data were processed with programs iMosflm and SCALA. The structure was determined by the molecular replacement method with the program PHASER, using the atomic coordinates of P450 BioI (CYP107H1; PDB code, 3ejd) as a serch probe. The model refinement and manual corrections were performed with the program REFMAC5 and Coot.

3 Results and Discussion

The structure of RauA complexed with substrate was determined by the molecular replacement to a resolution of 2.19 Å. The final refined model consists of amino-acid residues 11-411, heme cofactor, non-hydroxylated aurachin RE (substrate), and 54 water molecules with a crystallographic R factor and R_{free} factor of 0.214 and 0.263, respectively. The data collection and refinement statistics are summarized in Table 1. Atomic coordinates and structure factors of RauA were deposited in PDB under accession code 3wec.

Table 1. Data collection and refinement statistics.

Data collection statistics	
Beamline	AR-NW12A
Wavelength (Å)	1.0000
Resolution (Å)	50–2.19 (2.31–2.19)
Unit-cell dimensions	
a, b, c (Å)	41.5, 100.0, 52.3
α, β, γ (°)	90.0, 108.7, 90.0
Space group	$P2_1$
Unique reflections	20,745
R_{merge}	0.056 (0.335)
Completeness (%)	99.5 (99.1)
Redundancy	3.6 (3.5)
Mean I/σ (I)	11.4 (3.0)
Refinement	
Resolution range (Å)	50–2.19
R_{work}	0.214
R_{free}	0.263
Total number of atoms	3,203
Average B-factor (Å^2)	55.7
r.m.s.d. bond distances (Å)	0.013
r.m.s.d. bond angles (°)	2.39

The overall structure exhibits the typical P450-fold consisting of 14 α-helices and 8 β-strands, and can be divided into two domains: larger α-helix rich domain and smaller β-strand rich domain (Fig. 1). Structure similarity search showed that the core structure of RauA is similar

to those of homologous bacterial P450s with main-chain rmsd of ~2.7 Å. Structure analysis also showed extra clear electron density map at the distal heme pocket, which was well fitted to the model of substrate (Fig. 1). The substrate was bound surrounded by the highly hydrophobic side-chains of amino-acid residues Phe68, Phe73, Leu77, Phe74, Phe88, Leu186, Ile188, Phe190, Met325, Met397 and Leu399. The farnesyl-chain moiety of the substrate forms U-shape topology, while the quinolone ring moiety is located roughly paralleled to the porphylin plane of the heme cofactor with stacking interaction (Fig. 2). Solvent molecule is clearly visible at the sixth coordinate ligand position of the heme iron (Fig. 1), suggesting that the heme iron is present in the low-spin resting state, even though the substrate was bound. The accommodation of both substrate and the solvent observed in the crystal structure of RauA is consistent with the results of substrate binding assay by UV-visible spectroscopy. It is thought that the solvent needs to move away for initiating the P450 catalytic cycle, so that there might be a hidden structural mechanism such as subtle local conformational change to expel the solvent. The structure of RauA reported in this study could serve as a model for molecular dynamics simulation and quantum computational chemistry needed for the explanation of ring nitrogen hydroxylation mechanism of RauA.

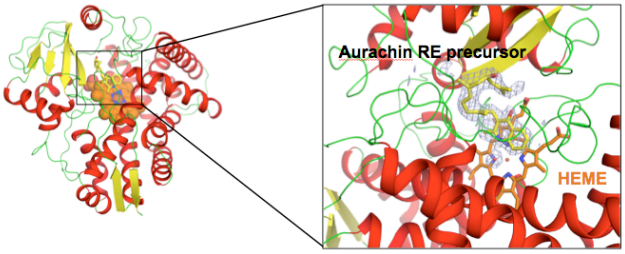

Fig. 1: Overall structure of RauA and the bound substrate. The unbiased Fo-Fc map for the substrate is also shown.

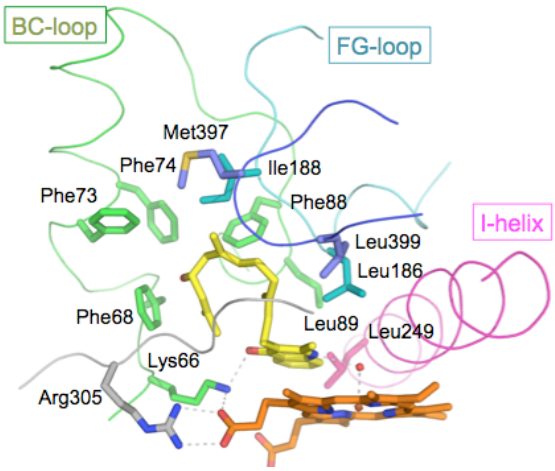

Fig. 2: Highly hydrophobic substrate binding pocket and the bound substrate (yellow).

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