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Crystal Structure of a Baeyer–Villiger Flavin-containing Monooxygenase from *Staphylococcus Aureus* MRSA Strain MU50

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Abstract

Flavin-containing Monooxygenase (FMO) catalyzed the oxygenation of broad spectrum of substrates. FMO can also serve as biocatalysts in the Baeyer–Villiger reaction in organic synthesis. Here we report the high-resolution crystal structure of a Baeyer–Villiger Flavin-containing Monooxygenase (BVFMO) from methicillin- and vancomycin-resistant *Staphylococcus aureus* strain MU50. The structure of *Staphylococcus aureus* FMO should facilitate further development of BVFMO as biocatalysts. A possible role of *Staphylococcus aureus* FMO in methicillin and vancomycin resistance is discussed.

Keywords

Baeyer–Villiger reaction; Vancomycin; Methicillin; FMO; Antibiotics Resistance

Introduction

Antibiotics resistant bacteria, which pose serious health risks to patients and imposes enormous economic burden, are grave concerns for the public. Methicillin-resistant *Staphylococcus aureus* (MRSA), bacteria that are resistant to many antibiotics, are the most

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Author's contribution

WH prepared the manuscript. QX refined the SAFMO structure. BW performed molecular docking with GOLD. AG commented on the manuscript.

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common opportunistic pathogens of humans. It causes a wide range of diseases, from mild skin infections to life-threatening diseases such as sepsis and pneumonia. In an effort to understand the molecular basis of pathogenicity, structural genomic centers such as JCSG determined crystal structures of proteins from *Staphylococcus aureus* MRSA Strain MU50, an MRSA strain with vancomycin and methicillin resistance. Here we reported a high-resolution crystal structure of a Baeyer–Villiger Flavin-Containing Monooxygenase (NCBI accession number NP_373108; UniProt accession number Q99R54) from *Staphylococcus aureus* strain MU50 at 2.40 Angstrom resolution.

Flavin-Containing Monooxygenases (FMO) are monooxygenases that utilize flavins as prosthetic groups. FMO in eukaryotes has been well characterized. Five tissue-specific FMO isoforms have been identified in humans[1]. While drug metabolism is predominantly associated with Cytochrome P450 (CYP) monooxygenases, FMO complement CYP in the detoxification of xenobiotics. Heptic FMO3 is responsible for breaking down nitrogen-containing compounds, such as trimethylamine (TMA), an odorous compound rich in the diet of mammals. In plants (Arabidopsis), FMO has been associated with systemic acquired resistance (SAR) against pathogens[2]. FMO are also found in bacteria, and are shown to be important in the utilization of carbon and/or nitrogen source. Trimethylamine monooxygenase (tmm), a bacterial FMO, breaks down methylated amines (MAs) and uses them as alternative nitrogen sources[3]. It was estimated that 20% of the bacteria in the surface ocean contain tmm, which played an unheralded yet important role in global carbon and nitrogen cycle.

Based on their sequences and structures, FMO can be classified into six (A-F) distinct flavoprotein monooxygenase subclasses[4]. Baeyer–Villiger Flavin-Containing Monooxygenases (BVFMO), a subfamily of Class B FMO, catalyzes the Baeyer–Villiger reaction. Baeyer–Villiger reactions have wide applications ranging from the synthesis of steroids, antibiotics, and pheromones. BVFMO can serve as biocatalysts, substituting traditional catalysts such as peroxyacid, in the Baeyer–Villiger reaction in organic synthesis. An alternative enzymatic substitute for peroxyacid is highly desirable, since the reaction with peroxyacid is prone to accident due to its explosive nature. As a result, BVFMO are highly treasured in synthetic chemistry.

The structure of *Staphylococcus aureus* FMO, abbreviated SAFMO herein, should aid further development of BVFMO as biocatalysts. We also explore the possible role of SAFMO in vancomycin and methicillin resistance.

Materials and Methods

The structure of SAFMO was solved by X-ray crystallography using the JCSG high-throughput structural biology pipeline. Crystals were grown in 1.6M $(NH_4)_2SO_4$, 0.1M MES, pH 6.0, using sitting drop vapor diffusion method at 4 degree Celsius. Equal volume (200 nL) of protein (16.8 mg/ml) and well solution were mixed and equilibrated against 50 uL of reservoir solutions. Diffraction data were collected at beamline 11-1 at SSRL (Stanford Synchrotron Radiation Lightsource), processed with mosflm, integrated with scala. A total of 8 Se sites were initially identified in SHELX and then refined in SHARP.

Density modification was performed with Solomon in SHARP. A total of 290 out of 368 amino acids were traced and docked using ARP/wARP. Structure was refined with REFMAC5. Data collection and refinement statistics are listed in Table I. Conservation of SAFMO residues was assessed by ConSurf[5]. The electrostatics potential surfaces were calculated with PDB2PQR and APBS. Molecular dockings were performed with GOLD.

Results

Overall Structure

The structure of Flavin-containing Monooxygenase from *Staphylococcus aureus* strain MU50 was determined at 2.40 angstrom resolution. Atomic coordinates and experimental structure factors have been deposited into the Protein Data Bank (http://www.rcsb.org) with PDB ID 3D1C.

SAFMO, a mixed alpha/beta protein, is made of two domains (Figure 1a). Domain 1 is composed of residues from both the N- and C-terminus of the protein (1-144 and 269-368). Domain 2 is composed of remaining residues (145-268). Domain1 and 2 are responsible for NADPH and FAD binding, respectively.

The structure of SAFMO is most similar to that of *Stenotrophomonas maltophilia* flavin-containing monooxygenase (SMFMO) (PDB 4A9W), a Baeyer–Villiger monooxygenase, as identified by both FFAS[6] and DALI (Z-score 28.8) [7]. Structures of SAFMO and SMFMO can be aligned with MultiProt[8] with an RMSD of 1.63 Å over 246 aligned Cα atoms. Like SMFMO, SAFMO exists as a dimer in the crystal structure with an interface surface area of 1896 $Å^2$, as assessed by PISA[9].

Active Site

The SAFMO active site had FAD bound as cofactor. NADH/NADPH was predicted by IsoCleft[10] to be a ligand in SAFMO. Normally, NADPH, but not NADH, was suggested as electron-donating coenzyme in class B BVFMO. However, the structurally similar SMFMO promiscuously use both NADPH and NADH as cofactors. Structural alignment with similar FMO structure (PDB 3S5W) solved with FAD and NADP suggested a potential position of NADPH in the SAFMO structure (Figure 1a). Rossmann fold, one of the most represented protein folds, is associated with nucleotide binding. In SAFMO, there is a Rossmann motif GX(X)GXXG located in the loop between beta 1 and helix A that binds to and stabilized FAD. A second Rossmann motif GXXXG[11], found between beta 7 and helix H, is next to the putative NADPH binding site (Figure 1a).

Discussion

The structure of SAFMO reported here resembles *Stenotrophomonas maltophilia* flavin-containing monooxygenase, suggestive of similar function. Baeyer–Villiger reaction, in which the aldehydes are oxidized to carboxylic acids and ketones are oxidized to esters, is fundamental in organic synthesis. Baeyer–Villiger reaction is normally carried out in the presence of peroxyacid, which is highly unstable and poses serious safety risks. Importantly, the classical Baeyer–Villiger oxidation lacks the highly desirable high chemo-, regio-, and

enantioselectivity and substrate specificity requisite for organic synthesis[12]. BVMO is able to catalyze the Baeyer–Villiger oxidation with high stereoselectivity and faster turnover rate than conventional catalysts. As a result, BVMO is promising as biocatalyst in organic synthesis, and is a highly sought-after subject of protein engineering.

In the crystal structure of phenylacetone monooxygenase (PAMO)(PDB 1W4X), a Baeyer–Villiger monooxygenase, Arg337 (which lay above the flavin ring) was suggested to be crucial in stabilizing the negatively charged flavin-peroxide and Criegee intermediates[13]. It is also thought to be responsible for NADPH specificity. However, no such positively charged residues are found in the corresponding positions in neither SAFMO nor SMFMO (Figure 2a). The absence of likewise positively charged residues in SAFMO and SMFMO likely explain the loss of NADPH specificity. Comparison of FAD indicates that the AMP orientations are similar in all BVMO. However, there is a 22.4 degree rotational shift of the flavin ring in the phenylacetone monooxygenase structure (Figure 2a).

Not surprisingly, residues surrounding the active site cavity where FAD is bound are highly conserved (Figure 1b), including aromatic residues Phe42 and Trp45 that support the flavin ring, as well as Cys317 which sits above the flavin ring. Unexpectedly, the C-terminus of SAFMO is also highly conserved. The C-terminus (S365, C366, T367, C368) of SAFMO forms a protruding knob, which dovetailed into the other monomer in the SAFMO dimer (Figure 1b). This C-terminal protrusion is unique for SAFMO amongst BVMO. Phe56, also highly conserved, at the dimer interface is wedged into the other monomer. Phe56 and the C-terminus tail collectively likely play an important role in stabilizing the SAFMO dimer.

All BVMO have uneven electrostatic charge distribution (Figure 1b). The active site cavity is highly positively charged, which is especially pronounced in the phenylacetone monooxygenase structure (PDB 1W4X). The remaining BVMO surfaces are mostly negatively charged, which may help direct the substrate into the positively charged active site.

Inactivation of antibiotics by monooxygenase-mediated modification of antibiotics has been reported. TetX has been described as a tetracycline-degrading, flavin-dependent, monooxygenase [14, 15]. This raised the intriguing possibility that SAFMO, also a flavin-dependent monooxygenase, may play a similar role in conferring antibiotics resistance in *Staphylococcus aureus* by degrading vancomycin and/or methicillin. Molecular docking simulations suggest that vancomycin and methicillin can be accommodated in the SAFMO active site (Figure S3). This hypothesis, if confirmed, will lead to invaluable novel drug target against MRSA *Staphylococcus aureus*.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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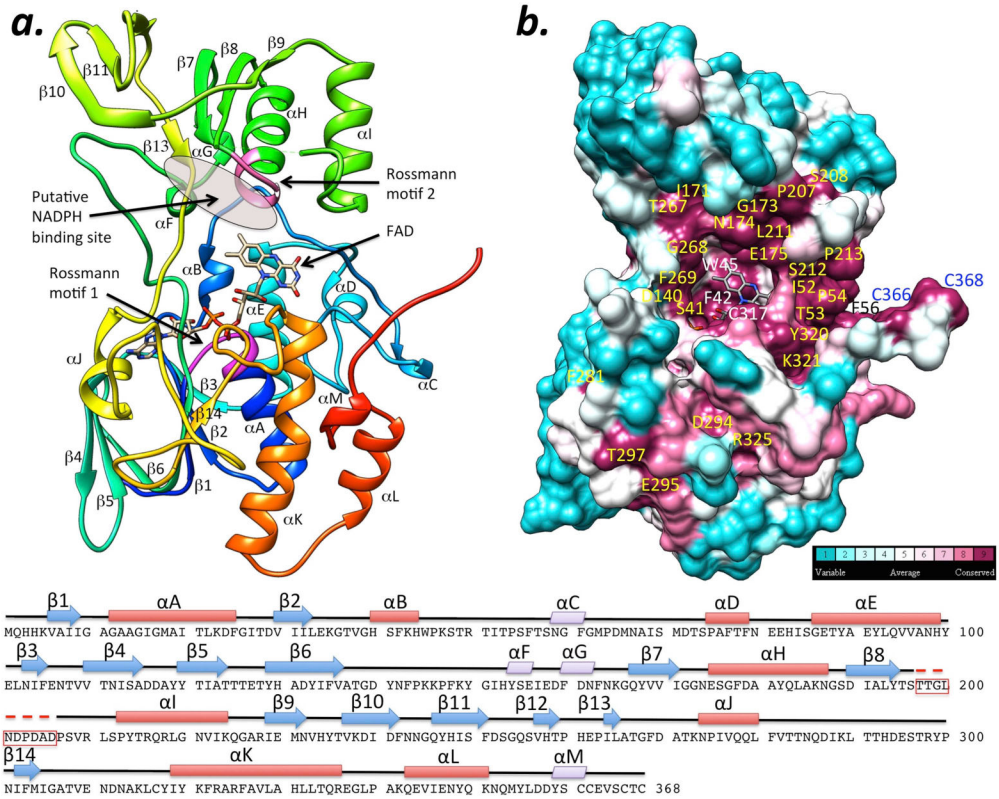

Figure 1.

Structure of SAFMO. ***a***. The structure is shown in cartoon style and colored in rainbow format (N-terminus blue and C-terminus red). A Rossmann motif GX(X)GXXG (colored magenta), located in the loop between beta 1 and helix A, binds to and stabilized FAD. A second Rossmann motif GXXXG (colored pink)[5], found between beta 7 and helix H, is the putative NADPH binding site based on structural alignment with similar FMO structure (PDB 3S5W) solved with FAD and NADP. ***b***. Conservation of residues projected onto the three dimensional structure of *Staphylococcus aureus* flavin-containing monooxygenase. The conservation of residues is assessed by ConSurf.

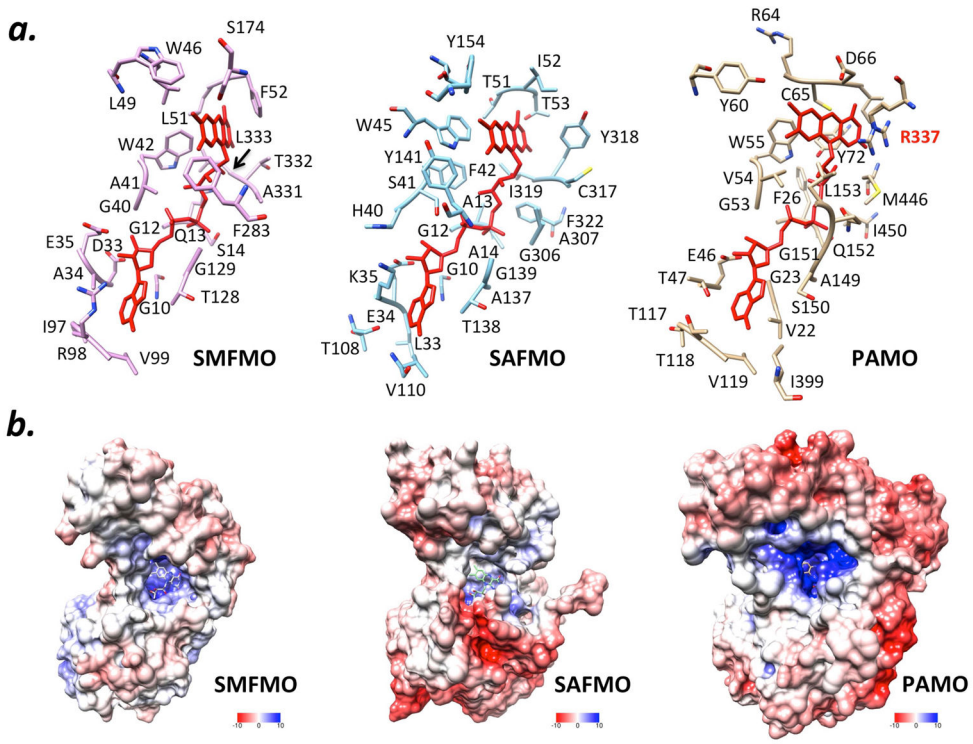

Figure 2.

Comparison of active site residues surrounding FAD and electrostatics potential surfaces in three BVFMO. ***a.*** active site of *Stenotrophomonas maltophilia* flavin-containing monooxygenase (SMFMO)(PDB 4A9W), *Staphylococcus aureus* flavin-containing monooxygenase (SAFMO)(PDB 3D1C), phenylacetone monooxygenase (PAMO)(PDB 1W4X). ***b.*** electrostatics potential surface of SMFMO, SAFMO, PAMO.

Table I

Data collection and refinement statistics of 3D1C.

	λ_1 MAD-Se	λ_2 MAD-Se
Data collection		
Space group	P 4_3 2_1 2	P 4_3 2_1 2
Cell dimensions		
a, *b*, *c* (Å)	109.25, 109.25, 87.06	109.25, 109.25, 87.06
α, β, γ (°)	90.00, 90.00, 90.00	90.00, 90.00, 90.00
Wavelength (Å)	0.97920	0.91837
Resolution (Å)	28.89-2.40 (2.46-2.40) *	28.87-2.40 (2.46-2.40) *
R_{sym} or R_{merge}	0.09 (0.68)	0.09 (0.61)
$I/\sigma I$	7.1 (1.1)	7.6 (1.3)
Completeness (%)	99.9 (100)	99.9 (100)
Redundancy	4.8 (4.9)	4.8 (4.9)
Refinement		
Resolution (Å)	28.89-2.40 (2.46–2.40)	
No. reflections	21137	
R_{work}/R_{free}	0.181 (0.25)/0.23 (0.27)	
No. atoms		
Protein	2773	
Ligand/ion	84	
Water	115	
B-factors		
Protein	46.72	
Ligand/ion	48.03	
Water	44.70	
R.m.s. deviations		
Bond lengths (Å)	0.01	
Bond angles (°)	1.51	

* Values in parentheses are for highest-resolution shell.