BL-17A/2021G068, 2023G041

X-ray structural study of CYP105A1 and inhibitor complex

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1 Introduction

CYP105A1 from *Streptomyces griseolus* catalyzes 2-setp hydroxylation reactions toward vitamin D_3 [1]. The X-ray crystal structures of CYP105A1 have been reported for substrate-free and product complex enzymes [2,3]. The crystal structures showed that many hydrophobic residues surround the ligand binding pocket, but three arginine residues are in the pocket. The ligand binding mode in CYP105A1 shows structural similarities with that in microsomal CYP2R1, which catalyzes the hydroxylation reaction toward vitamin D_3. The similarities in the binding mode suggested that the CYP family has a common recognition mechanism for VD_3. We performed X-ray structural analysis of CYP105A1 and azole-based inhibitor complex. Azole compounds are used as antifungal drugs and used for the treatment of breast cancer. We analyzed the interactions between CYP105A1 and the inhibitor to understand the molecular basis of the inhibitory effect toward CYP105A1.

2 Experiment

The overexpression and purification procedures of CYP105A1 were followed as previously reported [2,3]. The inhibitor (miconazole) was added to the purified enzyme in a 10-fold molar excess and incubated at 4°C for 1 day. The excess amount of the inhibitor was removed by filtration using an AmiconUltra 10 kDa molecular-weight cutoff filter. The crystallization experiment was performed by the hanging-drop vapor diffusion method at 10°C. Crystals were obtained using PEG2000MME as a precipitant. The diffraction data set was collected at the BL-17A beamline. The wavelength of X-rays was set to 0.98 Å. The diffraction intensities were integrated using the program *XDS* and scaled using the program *Aimless*. Structure refinement was performed using the program *Phenix*.

3 Results and Discussion

The X-ray crystal structure of the CYP105A1-miconazole complex was determined at 1.8 Å resolution (Table 1). The m*F*o-D*F*c omit map of miconazole shows that the imidazole group of miconazole is involved in the binding to the heme iron of CYP105A1 (Fig. 1). The Cys355 thiolate is the axial ligand to the heme iron and the nitrogen of the imidazole group of miconazole is at the sixth coordination position of the heme iron. The miconazole was bound with double conformations. The two dichlorophenyl groups showed different orientations, but the position of the imidazole groups showed high similarities in the two conformations. Although the commercial miconazole used for this study is the racemic mixture (*R* and *S*), both conformations of miconazole was observed as the *S* enantiomers. On the other hand, the *R* enantiomer of miconazole has been reported to reduce the growth of some yeast and fungi [4]. These results suggest that the enantiomer selectivity depends on the environment of the binding pocket of CYP enzymes.

Table 1: Diffraction data and refinement statistics

Diffraction data	
Resolution (Å)	50-1.8 (1.84-1.80)
Space group	$P2_12_12_1$
Unit cell *a*, *b*, *c* (Å)	52.3, 53.5, 140.1
Completeness (%)	99.9 (99.2)
*R*meas (%)	8.4 (53.8)
$I/\sigma(I)$	14.4 (3.3)
$CC_{1/2}$	0.998 (0.914)
Refinement	
Resolution (Å)	50-1.8
R_{work}/R_{free} (%)	18.5/23.8
R.m.s.d. bonds (Å)/angles (°)	0.007/0.835

Values in parentheses show the highest resolution shell.

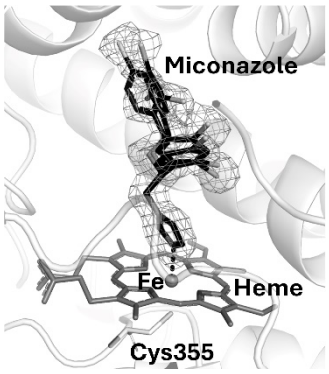

Fig. 1: m*F*o-D*F*c omit map of miconazole. The omit map contoured at 3.0 σ level is shown as gray mesh. The miconazole, heme, and Cys355 are shown as stick models. The distance between the heme iron and the nitrogen of the imidazole group of miconazole is 2.1 Å.

Acknowledgment

We thank the PF beamline staff for their help in the X-ray diffraction data collection.

References

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