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X-ray structural analysis of the ESI complex comprising CYP105A1, a substrate, and a noncompetitive inhibitor

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1 Introduction

Competitive and noncompetitive inhibitors have been found for cytochrome P450 (CYP) enzymes. Noncompetitive inhibitors are believed to form an enzyme-substrate-inhibitor (ESI) complex. To date, there has been no experimental structural evidence of an ESI complex formed by CYP enzymes, whereas over nine hundred CYP structures have been deposited in the Protein Data Bank. Our enzyme inhibition assays showed that lanoconazole acts as a noncompetitive inhibitor of CYP105A1, which catalyzes hydroxylation reactions toward non-steroidal anti-inflammatory drugs [1]. We performed X-ray structural analysis of CYP105A1-diclofenac-lanoconazole complex to understand the molecular mechanism of the noncompetitive inhibition [2].

2 Experiment

Purification of CYP105A1 R84A was performed by Ni-affinity, anion exchange, and size-exclusion chromatography steps. CYP105A1, diclofenac, and lanoconazole were mixed in a molar ratio of 1:5:5. The crystallization experiment was performed by the hanging-drop vapor diffusion method at 10°C. Crystals were obtained using polyethylene glycol 2000 monomethyl ether 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 structure of the ESI complex comprising CYP105A1 R84A, diclofenac, and lanoconazole was determined at 2.1 Å resolution (Table 1). The mF_o-DF_c omit map indicates that diclofenac and lanoconazole both bind in the binding pocket (Fig. 1). The imidazole group of lanoconazole is bound to the heme iron. Diclofenac is located distant from the heme group, and the bound diclofenac closely contacts with the lanoconazole by forming Cl-π interactions between their chlorophenyl groups. The diclofenac position is also stabilized by the salt-bridge formed with the Arg73 side-chain. Noncompetitive inhibitors are generally believed to bind to allosteric sites remote from the active site. The ESI complex observed in this work could form because the size of the binding pocket is optimal for the entrance both of diclofenac and lanoconazole. Our structural analysis indicates that lanoconazole is not a typically defined noncompetitive inhibitor but it apparently acts as a noncompetitive inhibitor of CYP105A1.

Table 1: Diffraction data and refinement statistics

Diffraction data	
Resolution (Å)	50-2.1 (2.16-2.10)
Space group	$P2_12_12_1$
Unit cell *a*, *b*, *c* (Å)	52.6, 53.6, 141.2
Completeness (%)	100.0 (99.9)
R_{meas} (%)	12.6 (89.3)
$I/\sigma(I)$	8.8 (1.8)
$CC_{1/2}$	0.998 (0.815)
Refinement	
Resolution (Å)	50-2.1
R_{work}/R_{free} (%)	21.2/25.5
R.m.s.d. bonds (Å)/angles (°)	0.003/0.568

Values in parentheses show the highest resolution shell.

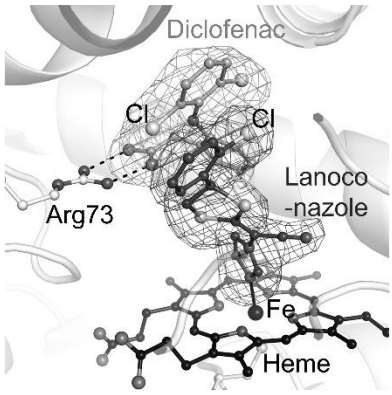

Fig. 1: mF_o-DF_c omit map of diclofenac and lanoconazole. The omit map contoured at 3.0 level is shown as dark gray (diclofenac) and light gray (lanoconazole).

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References

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