BL-5A, BL-17A, AR-NW12A/2013G597

Crystal structure analysis of human drug metabolizing enzyme CYP2C9 complexed with medicinal compound Losartan

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1 Introduction

Human cytochrome P450 2C9 (CYP2C9) is responsible for the oxidative metabolism of 15-20% of the drugs that undergo phase I metabolism. Two known genetic variants CYP2C9*3(I359L) and *30(A477T) differ from the wild-type by a single amino acid substitution. Substrate-dependent reduced activities were reported for CYP2C9*3 and *30, which were observed at allele frequencies of about 3% and 0.2%, respectively, in Japanese population. Particularly, losartan oxidation activity of CYP2C9*3 and CYP2C9*30 were significantly decreased by 77% and 99%, respectively, compared with CYP2C9 expressed using baculovirus-insect cell system [1, 2]. Here, X-ray crystal structures of CYP2C9 wild-type and two genetic variants in complex with losartan were solved to reveal structural changes in protein and differences of molecular interactions between CYP2C9 and losartan.

2 Experiment

The N-terminal truncated CYP2C9, CYP2C9*3 and CYP2C9*30 enzymes with C-terminal His-tag were expressed in *Escherichia coli*. The purified proteins were mixed with losartan, and their complexes were crystallized by using precipitant solution (CYP2C9; 0.1M HEPES pH7.5, 20% (w/v) PEG-8000, CYP2C9*3; 0.1M BisTris pH6.5, 1.8M AS, 2%(w/v) MPEG-550, and CYP2C9*30; 0.1M TrisHCl pH8.5 1.5 M Ammonium Sulfate 12% Glycerol) at 291K. Statistics of collected data were listed in Table 1. The crystal structures were determined by molecular replacement using an ensemble of previously solved CYP2C9 structures.

3 Results and Discussion

The high-resolution crystal structures of CYP2C9 wild-type and genetic variants revealed binding of multiple losartan molecules. One losartan was bound for catalytic site, and the other losartan was located at a peripheral binding site on the surface of the protein. Figure 1 shows interactions of losartan in the catalytic site with CYP2C9. The interactions of CYP2C9*3 was significantly different from those of wild-type and CYP2C9*30. The oxidation site of losartan was positioned far from heme iron, indicating that non-productive binding form is predominant. The crystal structures of CYP2C9 will provide insights into the interaction of substrates and the role of single-nucleotide polymorphism.

Table 1: Statistics of collected diffraction data

Sample	Wild-type	2C9 *3	2C9 *30
Beam line	BL-17A	BL-5A	BL-17A
Resolution(Å)	2.10	2.50	2.10
Space group	*I*222	*I*222	*I*222
Cell constants a, b, c (Å)	74.6, 143.0, 161.4	74.9, 141.8, 160.7	75.0, 142.3, 161.8
Outer Shell (Å)	2.18-2.10	2.59-2.50	2.18-2.10
Reflections	352759	345222	367278
Unique ref.	50349[4957]	30420[3013]	50672[4974]
Completeness	99.3[98.6]	99.9[99.9]	99.2[98.7]
R-merge	8.5[62.7]	5.0[51.3]	7.6[54.5]
I/σI	33.9[2.2]	55.2[3.2]	36.7[2.9]
Redundancy	7.0[3.8]	11.3[6.8]	7.2[4.4]

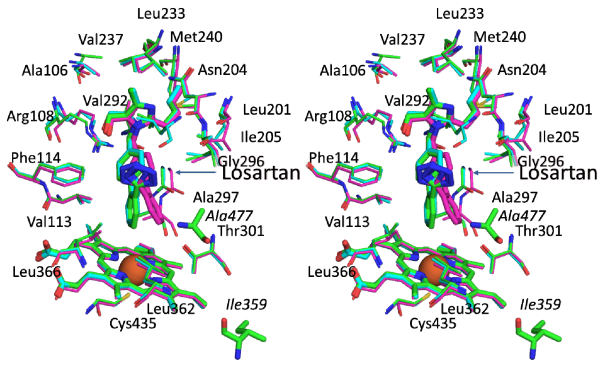

Fig. 1: Comparison of interactions for losartan complexes in the catalytic site (Green: wild-type, Magenta: CYP2C9*3, Cyan: CYP2C9*30).

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References

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