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Crystal structures of 1-deoxy-D-xylulose 5-phosphate reductoisomerase from *Plasmodium falciparum* complexed with fosmidomycin analogs

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1 Introduction

Malaria is one of the world's most serious parasitic diseases. There are estimated 300-500 million cases and more than a million deaths from malaria each year. Human malaria is caused by infection with intracellular parasites of the genus *Plasmodium*. *P. falciparum* is the most lethal among the five species of *Plasmodium* that infect humans. The emergence of strains of malarial parasite resistant to conventional drug therapy has stimulated searches for antimalarials with novel modes of action. The non-mevalonate pathway of isoprenoid biosynthesis present in *P. falciparum* is known to be an effective target of antimalarial drugs. The second enzyme of the non-mevalonate pathway, 1-deoxy-D-xylulose 5-phosphate reductoisomerase (DXR or IspC, EC 1.1.1.267), catalyzes the NADPH and divalent cation (Mg^{2+} or Mn^{2+})-dependent transformation of 1-deoxy-D-xylulose 5-phosphate into 2-*C*-mthyl-D-erythritol 4-phosphate. We determined the crystal structures *P. falciparum* DXR (PfDXR) complexed with its inhibitor fosmidomycin [1, 2]. Here we report the crystal structures of PfDXR complexed with fosmidomycin analogs [3].

2 Experiment

Expression and purification of PfDXR were performed as previously described [1]. PfDXR inhibitors used for crystallographic analyses were α-aryl-substituted carba-analogs CBK3 and CLSKCB12, and an α-aryl-substituted oxa-analog KBK126. To obtain the quaternary (PfDXR-NADPH-Mg^{2+}-each of the Inhibitor) complexes, protein solution (10 mg/ml PfDXR, 50 mM Tris-HCl pH 7.8, and 2 mM DTT) was mixed with each of the inhibitor solution (50 mM Tris-HCl pH 7.8, 2 mM DTT, 6 mM NADPH, 4 mM $MgCl_2$, and 4 mM each of the inhibitor) at a volume ratio of 1:1. Crystallization was performed using the hanging-drop method, in which 2 μl of the quaternary complex solution was mixed with the same volume of reservoir solution (0.1 M Tris-HCl pH 7.0, 20%(w/v) PEG3000, and 0.2 M calcium acetate) and incubated at 293 K. The drops were suspended over 500 μl of reservoir solution in 24-well plates. Crystals grew to a final size of 20 x 20 x 150 mm^3 within 3-7 days.

The crystals were mounted in nylon loops and flash-cooled in a cold nitrogen-gas stream at 100 K just prior to data collection. Data were collected by the rotation method at 100 K using an ADSC Q315r CCD detector with synchrotron radiation (λ = 0.98 Å on BL17A). Initial phase determination was performed for the CLSKCB12 complex of PfDXR by molecular replacement (MR) using the coordinate set of the fosmidomycin complex of PfDXR (PDB code 3AU9). The refined CLSKCB12 complex model was then used as a template for the structure refinement of CBK3 and KBK126 complexes.

3 Results and Discussion

In this study, inhibition modes of CBK3, CLSKCB12, and KBK126 in complex with PfDXR, NADPH and Mg^{2+} were revealed by X-ray crystal structure analyses at 2.25-, 1.97-, and 2.35 Å resolutions, respectively. The overall structures of these complexes are essentially identical with previously published quaternary (fosmidomycin- or FR900098-bound) complexes of PfDXR [2] except for a flexible loop region (residues 291 to 299).

The α-aryl substituent has VDW contacts with active site residues: the side chains of Ser270, Cys338, and Pro358 belonging to the structural core of PfDXR subunit are well ordered, whereas Try296 and Met298 belonging to the flexible loop region showed higher temperature factors. In addition, an intra-molecular interaction is observed between the *N*-methyl group and the aromatic ring of CLSKCB12. A comparison of the binding mode of CLSKCB12 with that of fosmidomycin reveals that a closed conformation of Trp296 in the fosmidomycin complex crashes with the α-aryl substituent of CLSKCB12. As compared with the fosmidomycin complex, the flexible loop of the CLSKCB12 complex is rather disordered (poorer electron density and higher B-factors). Therefore, the cause of the tight binding of CLSKCB12 to PfDXR appears to be different from that of fosmidomycin to PfDXR [3].

References

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