17A, NW12A/2007G025

Crystal structures of the inhibitor-bound quaternary complexes of 1-deoxy-D-xylulose 5-phosphate reductoisomerase from *Plasmodium falciparum*

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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 four 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, 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. Here we report the crystal structures of *P. falciparum* DXR (PfDXR) complexed with inhibitors.

Experimental

Crystallization

Expression and purification of PfDXR were carried out as described previously [1]. To obtain the quaternary (PfDXR-NADPH-Mg^{2+}-inhibitor) complex crystals, the protein solution (10 mg/ml PfDXR, 50 mM Tris-HCl pH 7.8 and 2 mM DTT) was mixed with inhibitor solution (50 mM Tris-HCl pH 7.8, 2 mM DTT, 6 mM NADPH, 4 mM $MgCl_2$, and 4 mM fosmidomycin or FR900098) at a volume ratio of 1:1. Crystallization was carried out by the hanging-drop method, in which 2 µl of the protein-inhibitor complex solution was mixed with the same volume of reservoir solution (0.1 M Tris-HCl pH 8.0, 20 (w/v)% PEG8000, and 0.3 M Calcium acetate) and incubated at 293 K. Rod-shaped crystals with typical dimensions of about 0.02 x 0.02 x 0.1 mm^3 could be grown in 1 week.

X-ray data collection and structure determination

Data collections were performed by the rotation method at 100 K using an ADSC Q210r CCD detector with synchrotron radiation (λ = 1.000 Å on beamline NW12A of the PF-AR). The current best diffraction data for fosmidomycin and FR900098 complexes were collected up to 1.90 and 2.15 Å resolutions, respectively.

The initial phase determination for the fosmidomycin complex was carried out by the molecular replacement method using the coordinate set of the inhibitor-free ternary complex of PfDXR as a search model. Then the refined fosmidomycin complex model was used for the template of structure refinement of the FR900098 complex by D-Fourier method.

Results and Discussion

The overall structure of PfDXR is essentially similar to those of DXRs from other species [2]. The subunit of PfDXR consists of two large domains, linker region, and a C-terminal domain. One of the large domains is responsible for NADPH binding, and the other provides the groups necessary for catalysis. A comparison of the crystal structure of the inhibitor-free PfDXR and those of the inhibitor-bound PfDXR showed that the large cleft between the NADPH-binding and catalytic domains were closed upon inhibitor binding. Disordered loop region in the inhibitor-free form is well defined in the inhibitor-bound quaternary complexes. Fosmidomycin and FR900098 bind to PfDXR in a similar manner where each of the inhibitor is buried in the active site and is shielded from the solvent environment. We expect present structures to be a quite useful guide for design of more effective antimalarial compounds.

References

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[2] T. Umdea *et al.*, Sci. Rep. **1**, MS#9 (2011).

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