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Crystal structure of the inhibitor-free form 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* that are transmitted by *Anopheles* mosquitoes. *Plasmodium 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, such as chloroquine, amodiaquine, and sulfadoxine-pyrimethamine, has stimulated searches for antimalarials with novel modes of action.

The non-mevalonate pathway of isoprenoid biosynthesis present in *Plasmodium 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.

To date several crystal structures of DXR from *Escherichia coli*, *Zymomonas mobilis*, *Mycobacterium tuberculosis*, and *Thermotoga maritima* have been reported. However, the crystal structure of *Plasmodium falciparum* DXR (PfDXR) itself has not yet been reported. Here we report the crystal structure of recombinant PfDXR.

Experimental

Crystallization

The expression and purification of PfDXR were performed as described [1]. Crystallization was carried out at 293 K by the hanging-drop vapour diffusion method. In the best case, a droplet was prepared by mixing equal volumes (2.0 + 2.0 µl) of the protein solution (5 mg/ml protein and 3 mM NADPH) and the reservoir solution (500 µl) containing 20%(w/v) PEG3350 and 0.3 M potassium chloride in 0.1 M Tris-HCl buffer at pH 8.0. Rhomboidal crystals with typical dimensions of about 0.1 x 0.1 x 0.1 mm^3 could be grown in 1 week [1].

X-ray data collection and structure determination

The crystals belong to a monoclinic space group *C*2 with cell dimensions of *a* = 168.89 Å, *b* = 59.65 Å, *c* = 86.58 Å, and β = 117.8 deg. Assuming two subunits (one dimer) per asymmetric unit, we obtained a V_M value of 2.03 $Å^3$/Da, corresponding to a solvent content of 39 %. The data collection was performed at 100 K using an ADSC Q270 CCD detector with the synchrotron radiation of BL17A (λ = 1.00 Å). The current best diffraction data from an inhibitor-free PfDXR crystal were collected up to 1.85 Å resolution.

The initial phase determination was carried out by the molecular replacement (MR) method using the coordinate set of *Escherichia coli* DXR dimer (PDB code: 1ONN) as a search model. The phase determination was carried out using the program AMoRe from the CCP4 suite. Crystallographic refinement at 1.85 Å resolution was performed using the program REFMAC5.

Results and Discussion

The overall structure of PfDXR is essentially similar to those of DXRs from other species. 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. Structural details of PfDXR will be published elsewhere.

Reference

[1] T. Umeda et al., Acta Crystallogr. F66, 330 (2010).

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