Biological Science

17A/2007G025

Crystal structure analyses of an isomerase from *Plasmodium falciparum*

Tomonobu Umeda, Nobutada TANAKA*, Yoshio KUSAKABE, Masaaki Ishihara, Keigo Inoue, Yuki Odanaka, Satoko Matsubayashi, Yasuyuki KITAGAWA, and Kazuo T. NAKAMURA
School of Pharmacy, Showa University,
1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142-8555, Japan

Introduction

Malaria is one of the world's most serious parasitic diseases. There are estimated 300-500 million cases and up to 2.7 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 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*, from *Zymomonas mobilis*, and from *Mycobacterium tuberculosis* have been reported. However, the crystal structure of *Plasmodium falciparum* DXR (PfDXR) itself has not yet been reported. Here we report the crystallization and preliminary X-ray crystallographic studies of recombinant PfDXR.

Experimental

Crystallization

The expression and purification of PfDXR will be published elsewhere. 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

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 a PfDXR crystal were collected up to 1.85 Å resolution.

Results and Discussion

The initial phase determination was carried out by the molecular replacement (MR) method using the coordinate set of *Escherichia coli* DXR (EcDXR) dimer (PDB code: 1ONN) as a search model. The results showed clear initial solutions (correlation coefficient of 0.369 and R-factor of 0.515 in the resolution range of 15.0 - 3.0 Å), and reasonable molecular arrangement of PfDXR dimer in an asymmetric unit. The MR solution was supported by the observation that the directions of the non-crystallographic twofold axes determined by the self-rotation function were consistent with the MR solution obtained. Automatic model building and refinement, and further iterative manual model building are currently in progress.

References

[1] T. Umeda et al., in preparation.

* ntanaka@pharm.showa-u.ac.jp