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Crystallization and preliminary X-ray diffraction analysis of orotate phosphoribosyltransferase from the human malaria parasite *Plasmodium falciparum*

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Orotate phosphoribosyltransferase (OPRT) catalyzes the Mg^{2+}-dependent condensation of orotic acid (OA) with 5-α-D-phosphorylribose 1-diphosphate (PRPP) to yield diphosphate (PP_i) and the nucleotide orotidine 5′-monophosphate. OPRT from *Plasmodium falciparum* produced in *Escherichia coli* was crystallized by the sitting-drop vapour-diffusion method in complex with OA and PRPP in the presence of Mg^{2+}. The crystal exhibited tetragonal symmetry, belonging to space group $P4_1$ or $P4_3$, with unit-cell parameters $a = b = 49.15$, $c = 226.94$ Å. X-ray diffraction data were collected to 2.5 Å resolution at 100 K using a synchrotron-radiation source.

1. Introduction

There are an estimated 300–500 million cases of malaria and up to three million people die from this disease annually. *Plasmodium falciparum* is the causative agent of the most lethal and severe form of human malaria (Guerin *et al.*, 2002). Chemotherapy for malaria is available, but is complicated by both adverse effects and widespread resistance to most currently available antimalarial drugs (Attaran *et al.*, 2004; White, 2004). More efficacious and less toxic drugs which uniquely target the parasite are therefore required. The malaria parasite depends on *de novo* synthesis of pyrimidine nucleotides, whereas the human host has the ability to synthesize them by both *de novo* and salvage pathways (Krungkrai *et al.*, 1990; Jones, 1980; Weber, 1983). The final two steps of uridine 5-monophosphate (UMP) synthesis require the addition of ribose 5-phosphate from 5-phosphoribosyl 1-pyrophosphate (PRPP) to orotic acid (OA) by orotate phosphoribosyltransferase (OPRT; EC 2.4.2.10) to form orotidine 5-monophosphate (OMP) and the the subsequent decarboxylation of OMP to form UMP by OMP decarboxylase (OMPDC; EC 4.1.1.23). These enzymes are encoded by two separate genes in most prokaryotes and the malaria parasite (Krungkrai *et al.*, 2003; Krungkrai, Aoki *et al.*, 2004). Previous studies have demonstrated that the two enzymes exist as a heterotetrameric $(OPRT)_2(OMPDC)_2$ complex in two species of malaria parasite: *P. falciparum* and *P. berghei* (Krungkrai, Prapunwattana *et al.*, 2004; Krungkrai *et al.*, 2005). In contrast, in most multicellular organisms, including humans, these genes are fused into a single gene, resulting in the bifunctional UMP synthase (Livingstone & Jones, 1987; Suttle *et al.*, 1988; Suchi *et al.*, 1997).

Phosphoribosyltransferases (PRTs) are divided into two evolutionary families, types I and II, which are characterized by distinct folding architectures. OPRT belongs to the type I family. The *P. falciparum* OPRT (*Pf*OPRT) has only 28% sequence identity to the human enzyme according to *ClustalW* calculations (Krungkrai, Aoki *et al.*, 2004). Because of the low similarity between the two enzymes and the existence of the salvage pathway in humans, inhibition of OPRT would be harmless to the human body with respect to synthesis of pyrimidine nucleotides. Therefore, *Pf*OPRT has promise as an antimalarial drug target. To enable structure-based drug design of the enzyme, its three-dimensional structure is necessary. The crystal structures of three bacterial and yeast OPRTs are known:

Table 1
Crystal parameters and X-ray diffraction data-collection statistics.

Values in parentheses are for the highest resolution shell.

Crystal system	Tetragonal
Space group	$P4_1$ or $P4_3$
Unit-cell parameters (Å)	$a = b = 49.15$, $c = 226.94$
Resolution range (Å)	50–2.5 (2.59–2.50)
No. of molecules in asymmetric unit	2
V_M (Å^3 Da^{-1})	2.08
V_{solv} (%)	41
No. of measured reflections	244322
No. of unique reflections	15318
R_{merge}† (%)	9.7 (25.0)
Completeness (%)	82.2 (74.3)
Average $I/\sigma(I)$	9.4 (2.0)

† $R_{merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ is the value of the ith measurement of the intensity of a reflection, $\langle I(hkl)\rangle$ is the mean value of the intensity of that reflection and the summation is over all measurements.

those from *Salmonella typhimurium* (Scapin *et al.*, 1994, 1995), *Escherichia coli* (Henriksen *et al.*, 1996), *Streptococcus mutans* (Liu *et al.*, 2010) and *Saccharomyces cerevisiae* (González-Segura *et al.*, 2007). Here, we report the crystallization and preliminary X-ray diffraction study of *Pf*OPRT in complex with OA and PRPP.

2. Experimental

2.1. Protein expression and purification

Recombinant *Pf*OPRT was expressed in *E. coli* BL21 (DE3) pLysS (Novagen) as described previously (Krungkrai, Aoki *et al.*, 2004). The *E. coli* cells were disrupted using a sonicator or a French press in buffer *A* (50 m*M* NaH_2PO_4 pH 8.0, 300 m*M* NaCl, 10 m*M* imidazole) containing a protease-inhibitor cocktail (Roche). The sample was applied onto a HisTrap HP column (GE Healthcare) and eluted with a linear gradient of 10–250 m*M* imidazole in buffer *A*. The fractions containing *Pf*OPRT were pooled and applied onto a HiLoad 16/60 Superdex 75 column (GE Healthcare), which was developed with buffer *B* (20 m*M* Tris–HCl pH 8.0, 300 m*M* NaCl, 1 m*M* dithiothreitol). The purified protein was concentrated to 20 mg ml^{-1} using a Vivaspin (Sartorius) and stored at 193 K until the crystallization experiments. Up to 3.7 mg purified *Pf*OPRT was obtained from 9 l *E. coli* cell culture.

2.2. Crystallization of *Pf*OPRT

Crystals of *Pf*OPRT with maximum dimensions of 0.05 × 0.02 × 0.01 mm appeared using the sitting-drop vapour-diffusion method in an Intelli-Plate 96 (Art Robbins Instruments) at 293 K within one month (Fig. 1). The drop consisted of 0.5 µl protein solution (8 mg ml^{-1} *Pf*OPRT, 20 m*M* Tris–HCl pH 8.0, 300 m*M* NaCl, 10 m*M* $MgCl_2$, 200 µ*M* OA, 500 µ*M* PRPP) and 0.5 µl reservoir solution (60 m*M* sodium cacodylate trihydrate pH 6.8, 31.5% polyethylene glycol 300, 3% trimethylamine *N*-oxide dihydrate) and was equilibrated against 60 µl reservoir solution in the deep well of an Intelli-Plate 96.

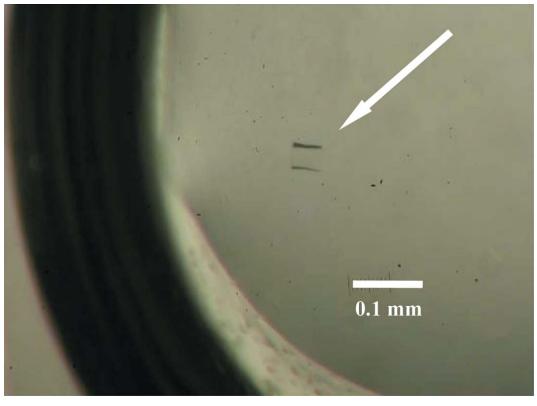

Figure 1
Crystal of *Pf*OPRT. Crystals were obtained in a sitting drop.

3. Data collection and analysis

X-ray diffraction data were measured on beamline BL17A at the Photon Factory (Tsukuba, Japan). A crystal fished out from a crystallization drop was flash-cooled in a nitrogen-gas stream at 100 K

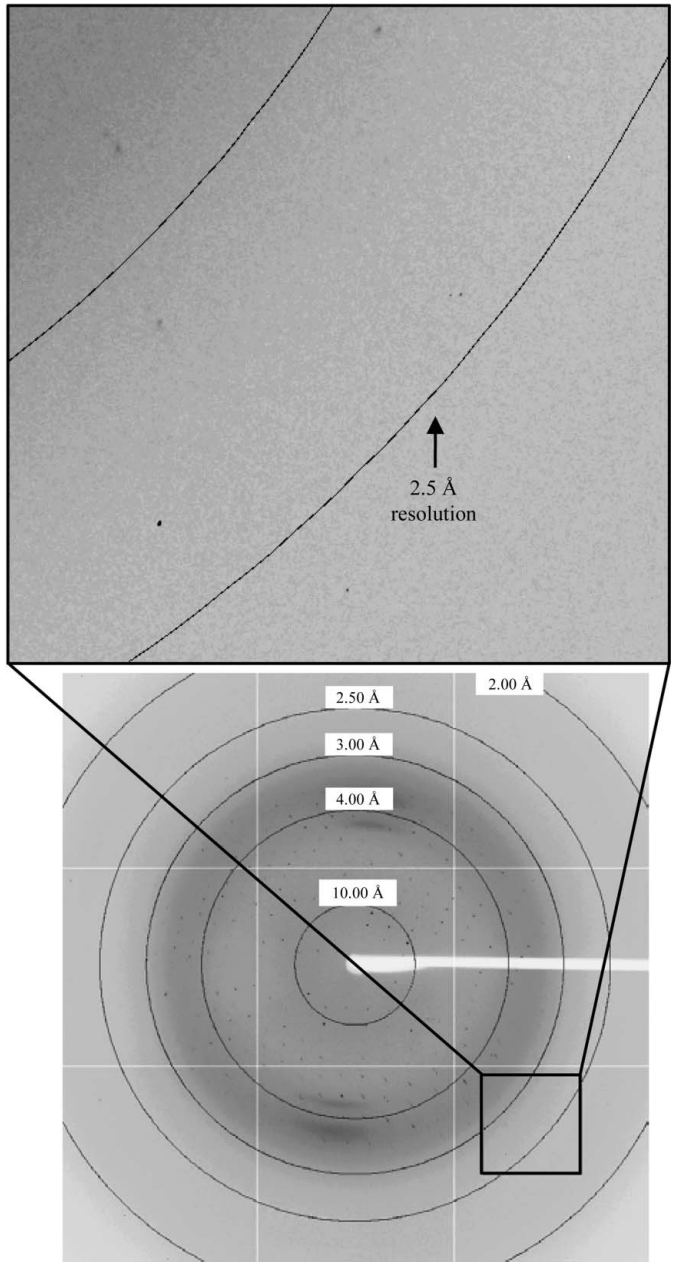

Figure 2
X-ray diffraction image from a *Pf*OPRT crystal. The frame edge in the enlargement is 2.5 Å.

without soaking in additional cryoprotectant solution. The diffraction patterns (Fig. 2) were recorded on a Quantum 315 CCD detector (ADSC). The wavelength, crystal-to-detector distance, crystal oscillation angle per image and beam exposure time were set to 0.98 Å, 319.1 mm, 0.8° and 12 s, respectively. A complete data set was collected from 150 images covering 120° in total.

The data set was processed using the *HKL*-2000 program suite (Otwinowski & Minor, 1997). The crystal of *Pf*OPRT was tetragonal, belonging to space group $P4_1$ or $P4_3$, with unit-cell parameters $a = b = 49.15$, $c = 226.94$ Å. From the 244 322 accepted observations to 2.5 Å resolution, 15 138 unique reflections were obtained. Assuming two monomers of the *Pf*OPRT in the asymmetric unit, the crystal volume per enzyme mass (V_M) and solvent content were calculated to be 2.08 $Å^3$ Da^{-1} and 41%, respectively. The low completeness of the diffraction data (82.2%) was caused by the crystal anisotropy. A summary of the data statistics is presented in Table 1.

Molecular-replacement calculations with the programs *MOLREP* (Vagin & Teplyakov, 2010) and *BALBES* (Long *et al.*, 2008) from the *CCP*4 program package (Winn *et al.*, 2011) are currently in progress using the structure of *S. cerevisiae* OPRT (PDB entry 2ps1; González-Segura *et al.*, 2007) as a search model. The model enzyme shares 22% sequence identity with *Pf*OPRT. The solved atomic structure should provide insight into how *Pf*OPRT interacts with OA and PRPP in the presence of Mg^{2+}.

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