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Crystallization and preliminary crystallographic analysis of orotidine 5'-monophosphate decarboxylase from the human malaria parasite *Plasmodium falciparum*

Orotidine 5'-monophosphate (OMP) decarboxylase (OMPDC; EC 4.1.1.23) catalyzes the final step in the *de novo* synthesis of uridine 5'-monophosphate (UMP) and defects in the enzyme are lethal in the malaria parasite *Plasmodium falciparum*. Active recombinant *P. falciparum* OMPDC (PfOMPDC) was crystallized by the seeding method in a hanging drop using PEG 3000 as a precipitant. A complete set of diffraction data from a native crystal was collected to 2.7 Å resolution at 100 K using synchrotron radiation at the Swiss Light Source. The crystal exhibits trigonal symmetry (space group *R*3), with hexagonal unit-cell parameters $a = b = 201.81$, $c = 44.03$ Å. With a dimer in the asymmetric unit, the solvent content is 46% ($V_M = 2.3$ Å³ Da⁻¹).

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 of malaria is available, but is complicated by both adverse effects and widespread resistance to most of the currently available antimalarial drugs (Attaran *et al.*, 2004; White, 2004). More efficacious and less toxic agents that 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 orotate by orotate phosphoribosyltransferase (OPRT; EC 2.4.2.10) to form orotidine 5'-monophosphate (OMP) and the subsequent decarboxylation of OMP to form UMP by OMP decarboxylase (OMPDC; EC 4.1.1.23). Both enzymes are encoded by two separate genes in most prokaryotes and the malaria parasite (Krungkrai *et al.*, 2003; Krungkrai, Aoki *et al.*, 2004), whereas their genes in most multicellular organisms, including humans, are fused into a single gene, resulting in the bifunctional UMP synthase (Livingstone & Jones, 1987; Suttle *et al.*, 1988; Suchi *et al.*, 1997). Our recent studies have demonstrated that the two enzymes exist as a heterotetrameric (OPRT)₂(OMPDC)₂ complex in two species of malaria parasites, *P. falciparum* and *P. berghei* (Krungkrai, Prapunwattana *et al.*, 2004; Krungkrai *et al.*, 2005). By multiple sequence-alignment analysis, the protein sequence of *P. falciparum* OMPDC was found to be less than 20% identical to both the human enzyme (Suttle *et al.*, 1988) and the four bacterial and yeast OMPDCs for which crystal structures are known, *i.e.* *Bacillus subtilis* (Appleby *et al.*, 2000), *Escherichia coli* (Harris *et al.*, 2000), *Methanobacterium thermoautotrophicum* (Wu *et al.*, 2000) and *Saccharomyces cerevisiae* (Miller *et al.*, 2000). The low sequence identities make it difficult to build a homology model for *P. falciparum* OMPDC. However, the low similarity between the enzymes of the host and the pathogen mean that OMPDC has promise as a drug target. In addition, OMPDC is known to be an unusually proficient catalyst (Miller & Wolfenden, 2002). These facts have prompted us to perform crystallization and X-ray diffraction analysis of the monofunctional *P. falciparum* OMPDC (PfOMPDC).

expressed in *E. coli*. Here, we report the conditions for expression, purification, crystallization and preliminary crystallographic analysis of the uncomplexed apo form of *Pf*OMPDC.

2. Experimental

2.1. Protein expression and purification

The recombinant OMPDC protein was prepared by cloning and expression of the gene encoding *Pf*OMPDC in *E. coli*. The oligonucleotide primers were 5'-CGG **GAT CCA TGG GTT TTA AGG TAA AAT TA**-3' and 5'-CCA **TCG ATT TAC GAT TCC ATA TTT TGC TTT AA**-3', which encompass *Bam*HI and *Cla*I restriction sites (in bold), respectively. The polymerase chain reaction was carried out using *P. falciparum* cDNA as a template and *Pfu* DNA polymerase (Promega) at 368 K for 5 min, followed by 40 cycles of 368 K for 1 min, 328 K for 1 min and 341 K for 3 min and finally an additional 341 K for 10 min. The PCR-derived fragment of ~980 bp complete *Pf*OMPDC was inserted into the expression vector pTrcHis-TOPO (Invitrogen) linearized with *Bam*HI and *Cla*I. *E. coli* TOP10 (Invitrogen) cells were transformed with the recombinant plasmid and induced with 1 mM isopropyl β -D-thiogalactopyranoside at 291 K for 18–20 h and the cell paste was then harvested by centrifugation at 8000g for 10 min. The recombinant enzyme was expressed as a soluble protein and purified using an Ni^{2+} -nitrilotriacetic acid-agarose affinity column (Qiagen) with 50 mM Tris-HCl pH 8.0, 300 mM NaCl, 250 mM imidazole and 10% glycerol as the elution buffer. The recombinant protein was further purified by AKTA fast protein liquid chromatography using a Superdex-75 HiLoad 26/60 column (Amersham Biosciences) equilibrated with 50 mM Tris-HCl

pH 8.0, 300 mM NaCl, 5 mM dithiothreitol. The enzyme activity was eluted as a single symmetrical peak at a position corresponding to 76 kDa. SDS-PAGE analysis (Laemmli, 1970) showed a homogenous preparation with a molecular weight of 38 kDa, suggesting that the active form of the *Pf*OMPDC is a dimer. The OMPDC activity was monitored by the decrease in the absorbance of OMP at 285 nm according to a previously described method (Yablonski *et al.*, 1996). The purified recombinant enzyme had a specific activity of approximately $10 \mu\text{mol min}^{-1}$ per milligram of protein, corresponding to a 300-fold purification and 30% yield. Up to 30 mg of the pure recombinant protein was obtained from 10 l *E. coli* cell culture. The purified protein was concentrated by Centricon ultrafiltration to 10 mg ml^{-1} and the homogeneity was confirmed by dynamic light scattering using a DynaPro-MS/X (Protein Solutions Inc.).

2.2. Crystallization of *Pf*OMPDC

Purified *Pf*OMPDC in 50 mM Tris-HCl pH 8.0 buffer containing 300 mM NaCl and 5 mM dithiothreitol was concentrated to 10 mg ml^{-1} . Crystallization of *Pf*OMPDC was first performed using the sitting-drop vapour-diffusion method at 293 K with the semi-automatic crystallization robot SHOZO (Adachi *et al.*, 2005). The crystallization conditions were initially screened by a sparse-matrix

(a)

(b)

Figure 1

Crystals of OMPDC from *P. falciparum*. Thin crystals were obtained in the first crystallization trial in a sitting drop (a); however, the quality of the crystals was improved by using the seeding method in a hanging drop (b).

Figure 2

X-ray diffraction image from a *OMPDC* crystal. The frame edge in the close-up is 2.7 Å.

sampling method (Jancarik & Kim, 1991) using Crystal Screens I and II (Hampton Research) and Wizard Screens I and II (Emerald BioStructures) in 96-well sitting-drop plates (Corning) at 293 K. The drop size was 1 μ l protein solution (10 mg ml⁻¹) plus 1 μ l of one of the precipitating reagents. The reservoir contained 0.2 ml of the same reagent. From the 200 crystallization conditions, needle-shaped crystals with maximum dimensions of 0.03 $\times$ 0.03 $\times$ 1.2 mm appeared in the drops using Crystal Screen II solutions No. 30 [10% PEG 6K, 0.1 M HEPES pH 7.5, 5% 2-methyl-2,4-pentenediol (MPD)] and No. 37 (10% PEG 8K, 0.1 M HEPES pH 7.5, 8% ethylene glycol) and Wizard Screen I solution No. 41 (30% PEG 3K, 0.1 M CHES pH 9.5) within 2 d at 293 K (Fig. 1). While the crystals that appeared from Crystal Screen II solutions No. 30 and No. 37 were fragile, those from Wizard Screen II solution No. 41 were suitable for X-ray crystallographic studies after size improvement by the seeding method using 13%(w/v) and 18%(w/v) PEG 3K solutions for the sitting drop and the mother liquor, respectively.

3. Data collection and processing

X-ray diffraction data were collected from a single crystal of *Pf*OMPDC on the X06SA beamline at the Swiss Light Source (Paul Scherrer Institute, Villigen, Switzerland). Prior to data collection, the crystal of *Pf*OMPDC was soaked in a cryoprotectant solution consisting of 18%(w/v) PEG 3K, 0.1 M CHES pH 9.5, 8% MPD. The crystal of *Pf*OMPDC was then mounted in a standard nylon loop in a stream of cold nitrogen gas at 100 K. The diffraction patterns were recorded on a MAR CCD detector (MAR USA) at cryogenic temperature (100 K). The wavelength, crystal-to-detector distance, oscillation range and exposure time were 0.978 Å, 250 mm, 1.0° and 1 s, respectively. A complete data set was collected from 200 images covering 200° in total (Fig. 2).

The diffraction intensity data were processed and scaled using *MOSFLM* (Leslie, 1992). The crystal of *Pf*OMPDC is trigonal,

Table 1

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

Values in parentheses are for the highest resolution shell (2.85–2.70 Å).

Crystal system	Trigonal with hexagonal unit cell
Space group	<i>R</i> 3
Unit-cell parameters (Å, °)	$a = b = 201.81$, $c = 44.03$, $\alpha = \beta = 90.0$, $\gamma = 120.0$
Resolution range (Å)	50.0–2.70
No. of molecules in ASU	2
V_M (Å ³ Da ⁻¹)	2.3
V_{solv} (%)	46
No. of measured reflections	451733
No. of unique reflections	18313
$R_{merge}^\dagger$ (%)	5.9 (27.5)
Completeness (%)	99.6 (100.0)
Average $I/\sigma(I)$	12.4 (4.0)

$^\dagger R_{merge} = \sum |I(k) - \bar{I}| / \sum I(k)$, where $I(k)$ is value of the k th measurement of the intensity of a reflection, $\bar{I}$ is the mean value of the intensity of that reflection and the summation is over all measurements.

belonging to space group *R*3, with unit-cell parameters $a = b = 201.81$, $c = 44.03$ Å. From the 451 733 accepted observations to 2.7 Å resolution, 18 313 unique reflections were obtained. The statistics of the diffraction data are shown in Table 1.

The self-rotation function calculated with the program *POLARRFN* from the *CCP4* program package (Collaborative Computational Project, Number 4, 1994) suggested that two *Pf*OMPDC molecules correlated by a non-crystallographic twofold axes were present in the asymmetric unit (Fig. 3), giving a Matthews coefficient of 2.3 Å³ Da⁻¹ and a solvent content of 46%.

Preliminary molecular-replacement calculations were performed with the program *AMoRe* (Navaza, 2001) from the *CCP4* program package (Collaborative Computational Project, Number 4, 1994) using the structure of OMPDC from *B. subtilis* (PDB code 1dbt) as the search model (Appleby *et al.*, 2000). Since the identity of the amino-acid sequences between *Pf*OMPDC and *B. subtilis* OMPDC is only 16%, MAD data collection using SeMet-derivative crystals was attempted. During the phase calculation using the anomalous dispersion signal, the crystal structure of OMPDC from *P. vivax* was deposited in the PDB (PDB code 2ffc). The deposited structure is that of a complex with UMP, demonstrating a product-binding form. The space group of the crystal is *P*₂₁₂₁₂, in contrast to the crystal reported here, which belongs to space group *R*3. The molecular-replacement method using this structure and a structural comparison between the apo and holo forms are in progress.

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Figure 3

Stereographic projections of the self-rotation function calculated in the $\kappa = 180^\circ$ section. The resolution of the data used was 15.0–2.7 Å. The X-ray data were reduced in a trigonal crystal system using a hexagonal cell. 12 peaks were clearly obtained in the calculated self-rotation map, suggesting that a non-crystallographic twofold axis exists in the asymmetric unit.

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