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Crystallization and preliminary X-ray crystallographic study of phosphoglucose isomerase from *Plasmodium falciparum*

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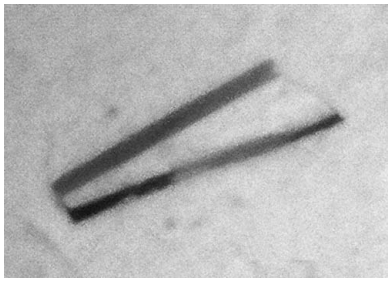

Phosphoglucose isomerase (PGI) is a key enzyme in glycolysis and glycogenesis that catalyses the interconversion of glucose 6-phosphate (G6P) and fructose 6-phosphate (F6P). For crystallographic studies, PGI from the human malaria parasite *Plasmodium falciparum* (PfPGI) was overproduced in *Escherichia coli*, purified and crystallized using the hanging-drop vapour-diffusion method. X-ray diffraction data to 1.5 Å resolution were collected from an orthorhombic crystal form belonging to space group $P2_12_12_1$ with unit-cell parameters a = 103.3, b = 104.1, c = 114.6 Å. Structural analysis by molecular replacement is in progress.

1. Introduction

Malaria is one of the world's most serious parasitic diseases. There are estimated to be 300–500 million cases and up to 2.7 million deaths from malaria each year. Human malaria is caused by infection with protozoan parasites of the genus *Plasmodium* that are transmitted by *Anopheles* mosquitoes. *Plasmodium falciparum* is the most lethal of the four species of *Plasmodium* (*P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae*) that infect humans. Chemotherapy for malaria is available, but the emergence of strains that are resistant to conventional drug therapy has stimulated searches for antimalarials with novel modes of action. Proteins important for survival of the parasite and that show differences in structure from the host homologue serve as potential drug targets. Such proteins can be classified as surface proteins, proteins involved in invasion and metabolic enzymes (Gayathri *et al.*, 2007).

Phosphoglucose isomerase (PGI; EC 5.3.1.9), also known as glucose 6-phosphate isomerase (GPI) or phosphohexose isomerase (PHI), is a key enzyme in glycolysis and gluconeogenesis. It catalyses the second step of glycolysis, namely the interconversion of glucose 6-phosphate (G6P) and fructose 6-phosphate (F6P). In recent years, several other protein factors have been confirmed to be identical to PGI: autocrine motility factor (AMF; Niinaka *et al.*, 1998), neuroleukin (NLK; Gurney *et al.*, 1986; Chaput *et al.*, 1988), maturation factor (MF; Xu *et al.*, 1996), antigens involved in rheumatoid arthritis (Matsumoto *et al.*, 1999) and sperm agglutination (Yakirevich & Naot, 2000), and a novel serine-protease inhibitor (MBSPI; Cao *et al.*, 2000). PGI not only acts as a housekeeping enzyme of sugar metabolism inside the cell, but also exhibits various properties of cytokines (AMF, NLK, MF) and several other protein factors outside the cell. The cytokine activity of PGI is specific to mammalian PGIs (Amraei & Nabi, 2002) and the enzymatic activity of PGI is not essential for either the receptor-binding function or the cytokine activity of mammalian PGIs (Tsutsumi *et al.*, 2003). The enzymatic activity of the PGI from *P. falciparum* (PfPGI) was found to be comparable to that of mammalian PGIs, but cytokine activity of PfPGI against mammalian cells was not detectable (Haga *et al.*, unpublished data). Since PGI is a housekeeping enzyme, its catalytic residues should be well conserved amongst PGIs from various species. However,

significant amino-acid sequence differences (insertions and deletions) are observed between the mammalian PGIs (558 residues) and PfPGI (579 residues). Thus, a detailed comparison of the three-dimensional structures of human and *Plasmodium* PGIs is important to investigate whether these differences can be applied to future structure-based drug design and to gain insight into the structural origin of the species specificity of the cytokine activity of PGI.

To date, crystal structures of PGI from human (Read *et al.*, 2001; Tanaka *et al.*, 2002; Cordeiro *et al.*, 2003; Davies *et al.*, 2003), pig (Davies & Muirhead, 2002), mouse (Solomons *et al.*, 2004; Tanaka *et al.*, 2006), rabbit (Jeffery *et al.*, 2000), *Bacillus stearothermophilus* (Sun *et al.*, 1999), *Pyrobaculum aerophilum* (Swan *et al.*, 2004), *Leishmania mexicana* (Cordeiro *et al.*, 2004), *Thermus thermophilus* (Yamamoto *et al.*, 2008) and *Trypanosoma brucei* (Arsenieva *et al.*, 2009) have been reported. However, the crystal structure of PfPGI has not yet been reported. Here, we report the crystallization of PfPGI in the presence of the carbohydrate inhibitor 6-phosphogluconic acid (6PGA). The crystals diffracted to at least 1.5 Å resolution and were suitable for X-ray structure analyses at high resolution.

2. Materials and methods

2.1. Overproduction and purification

The DNA encoding the full-length *P. falciparum* PGI (residues 1–579) was obtained by reverse-transcription PCR. Reverse transcription was carried out using SuperScriptII reverse transcriptase as described in the user's manual (Invitrogen) with the total RNA of *P. falciparum* (FCR-3) as the template. The target DNA was PCR-amplified from the reverse-transcription products using AccuPrime Pfx DNA polymerase (Invitrogen) with 5′-CGC**GGATCC**ATGAATATGGAGATTACAAAT-3′ and 5′-CGC**AAGCTT**ATTTGGACAAGTAATAATTTA-3′ as the forward and reverse primers, respectively. The PCR product was cloned into the pQE30 expression plasmid (Qiagen) with the *Bam*HI and *Hin*dIII cloning sites (bold). The construct was verified by sequencing.

Escherichia coli BL21 (DE3) cells (Novagen) harbouring the expression plasmid were grown in LB medium (3 l shake flask containing 1 l medium) at 310 K to an OD_{600} of 0.6. Overproduction of PfPGI was induced by 0.5 m*M* IPTG for 4 h at 310 K. After this period, the cells were harvested by centrifugation at 8000*g* for 15 min, suspended in buffer *A* (20 m*M* bis-tris–HCl pH 6.5, 50 m*M* NaCl) and disrupted by ultrasonication on ice for 4 × 30 s. The cell extract was obtained by centrifugation at 15 000*g* for 15 min and was applied onto a 5 ml HiTrap SP HP column (GE Healthcare) equilibrated with buffer *A*. The column was washed with 20 column volumes of wash buffer (0.3 *M* NaCl in buffer *A*). After washing, PfPGI was eluted with a linear gradient of 0.3–0.7 *M* NaCl in buffer *A*. The PfPGI was further purified by gel chromatography using a Superdex 200pg column (GE Healthcare) equilibrated with buffer *A*. The fractions containing PfPGI were pooled and concentrated to 10 mg ml^{-1} using a Centricon-50 (Millipore).

2.2. Crystallization

The protein solution was mixed with 10 m*M* 6-phosphogluconic acid (6PGA) dissolved in buffer *A* at a volume ratio of 1:1. Initial sparse-matrix crystal screening (Jancarik & Kim, 1991) was conducted using Crystal Screen I (Hampton Research) and Cryo I and II (Emerald BioSystems). Crystallization was carried out by the hanging-drop vapour-diffusion method, in which 1 µl protein solution (5 mg ml^{-1} protein and 5 m*M* 6PGA) was mixed with the same volume of reservoir solution and incubated at 293 K. The drops were suspended over 200 µl reservoir solution in 48-well plates.

2.3. X-ray data collection

Since the crystallization conditions for PfPGI included 38%(*v*/*v*) PEG 400 in the reservoir solution, X-ray data collection could be performed under cryogenic conditions without further addition of cryoprotectant. Crystals from the hanging drop were directly mounted in nylon loops and flash-cooled in a cold nitrogen-gas stream at 100 K just prior to data collection. Data collection was performed by the rotation method at 100 K using an ADSC Q315 CCD detector with synchrotron radiation [λ = 1.000 Å on beamline 5A at the Photon Factory (PF), Tsukuba, Japan]. The Laue group and unit-cell parameters were determined using the *DPS* program package (Rossmann & van Beek, 1999).

3. Results and discussion

3.1. Overproduction, purification and crystallization

PfPGI was successfully cloned, overproduced and purified to homogeneity. SDS–PAGE of the purified enzyme revealed a single

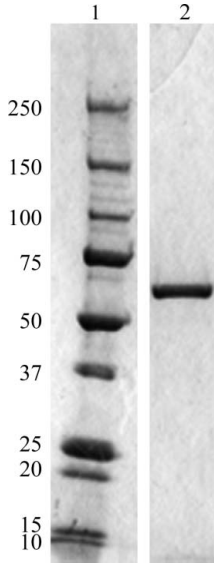

Figure 1
SDS–PAGE (5–20% gradient gel) of the purified PfPGI. Lane 1, molecular-mass markers (kDa); lane 2, after gel-filtration chromatography.

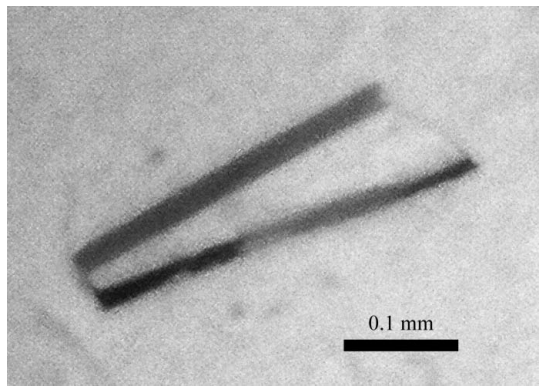

Figure 2
An orthorhombic crystal of PfPGI.

65 kDa protein band on Coomassie Brilliant Blue staining (Fig. 1). Initial crystal screening produced several microcrystals within one week. Micro-plate crystals grew from condition Nos. 6, 13, 24 and 38 of Cryo I [No. 6, 40%(*v*/*v*) PEG 600 and 0.2 *M* calcium acetate in 0.1 *M* sodium cacodylate–HCl pH 6.5; No. 13, 30%(*v*/*v*) PEG 200 and 5%(*m*/*v*) PEG 3000 in 0.1 *M* MES–NaOH pH 6.0; No. 24, 40%(*v*/*v*) PEG 400 and 0.2 *M* sodium chloride in 0.1 *M* sodium/potassium phosphate pH 6.2; No. 38, 40%(*v*/*v*) PEG 400 and 0.2 *M* lithium sulfate in 0.1 *M* Tris–HCl pH 8.5] and No. 4 of Cryo II [40%(*v*/*v*) PEG 400 and 0.2 *M* calcium acetate in 0.1 *M* HEPES–NaOH pH 7.5]. Trials to improve the crystallization conditions were performed by varying the pH, the buffer system and the concentration of crystallizing agent. To obtain crystals suitable for X-ray analysis, a droplet was prepared by mixing equal volumes (2 µl + 2 µl) of the working solution described above and reservoir solution [38%(*v*/*v*) PEG 400 and 0.2 *M* calcium acetate in 0.1 *M* sodium cacodylate–HCl pH 6.5] and was suspended over 500 µl reservoir solution in 24-well plates. Plate-shaped crystals with typical dimensions of approximately 0.3 × 0.1 × 0.03 mm grew within one week (Fig. 2).

3.2. Data collection

The Laue group of the PfPGI crystals was found to be *mmm*, with unit-cell parameters $a = 103.3$, $b = 104.1$, $c = 114.6$ Å. Only reflections with $h = 2n$, $k = 2n$ and $l = 2n$ were observed along the [*h*00], [0*k*0] and [00*l*] axes, respectively, indicating the orthorhombic space group $P2_12_12_1$. Assuming the presence of two subunits (one dimer) per asymmetric unit led to an empirically acceptable V_M value of 2.29 Å^3 Da^{-1}, corresponding to a solvent content of 46.3% (Matthews, 1968). The current best diffraction data from a PfPGI crystal were collected to 1.5 Å resolution (Fig. 3). Data-collection statistics are summarized in Table 1.

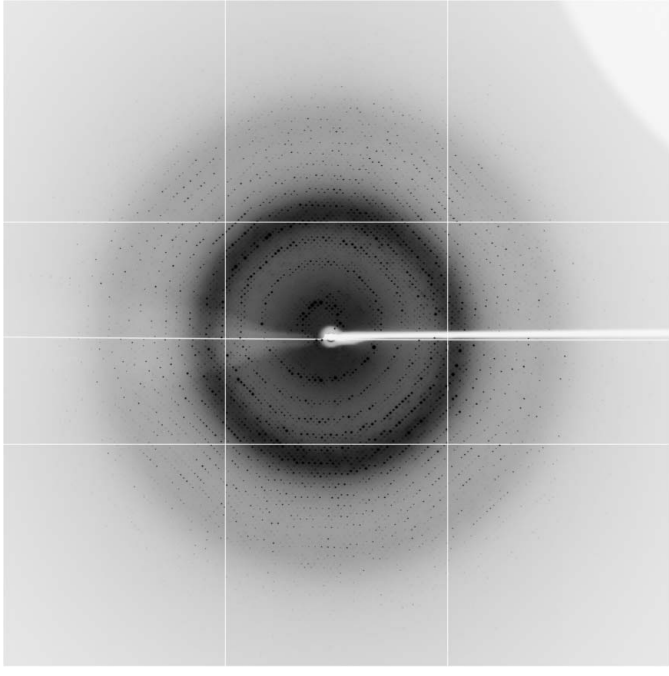

Figure 3
X-ray diffraction image from a PfPGI crystal. The edge of the detector corresponds to a resolution of 1.5 Å.

Table 1
Data-collection statistics for PfPGI.

Values in parentheses are for the outer shell.

Space group	$P2_12_12_1$
Unit-cell parameters (Å)	$a = 103.3$, $b = 104.1$, $c = 114.6$
No. of subunits per asymmetric unit	2 [one dimer]
Solvent content (%)	46.3
X-ray source	PF BL5A
Detector	ADSC Q315
Wavelength (Å)	1.000
Resolution range (Å)	50–1.5 (1.58–1.50)
No. of observed reflections	933398
No. of unique reflections	195801
Multiplicity	4.8 (4.7)
Mean $I/\sigma(I)$	7.6 (2.9)
B factor (Wilson plot) (Å^2)	16.4
$R_{\rm merge}$† (%)	5.7 (26.2)
Completeness (%)	99.3 (96.5)

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ is the *i*th measurement and $\langle I(hkl)\rangle$ is the weighted mean of all measurements of $I(hkl)$.

3.3. Initial phase determination

Initial phase determination was performed by molecular replacement (MR) with the coordinates of the dimeric molecule of the mouse PGI–6PGA complex (PDB code 2cxr; Tanaka *et al.*, 2006), which shares approximately 36% amino-acid sequence identity with PfPGI, as a search model. The bound inhibitor and water molecules were removed from the search model. Cross-rotation and translation functions were calculated using the program *MOLREP* (Vagin & Teplyakov, 1997) from the *CCP*4 suite (Collaborative Computational Project, Number 4, 1994). The results showed a clear solution [correlation coefficient of 0.313 (the first noise solution was 0.215) and an *R* factor of 0.519 (the first noise solution was 0.554) in the resolution range 40.0–3.0 Å] and a reasonable molecular arrangement of PfPGI in the 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 (data not shown) were consistent with the MR solution obtained. Automatic model building and refinement using the programs *ARP*/*wARP* (Lamzin & Wilson, 1993) and *REFMAC*5 (Murshudov *et al.*, 1997) and further iterative manual model building and refinement with the programs *XtalView* (McRee, 1999) and *REFMAC*5 are currently in progress. In parallel with refinement, we are preparing crystals of PfPGI in complex with glucose 6-phosphate or fructose 6-phosphate (the substrate or product) in order to study their mode of interaction with the enzyme.

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