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Crystallization and preliminary crystallographic study of the phosphoglucose isomerase from *Bacillus subtilis*

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Crystallization and preliminary crystallographic analysis of the phosphoglucose isomerase from a *Bacillus subtilis* native strain were carried out. The crystals belonged to the monoclinic space group *C*2, with unit-cell parameters a = 145.7, b = 136.0, c = 109.1 Å, β = 119.4°. The diffraction quality of the crystal was significantly improved from 2.4 Å to greater than 1.9 Å resolution by using the *in situ* flash-annealing method. A 98% complete data set with an overall $R_{\rm merge}$ of 4.6% was collected using an R-AXIS IV^{++} image-plate system and a copper rotating-anode X-ray generator. The crystals contained four molecules per asymmetric unit and the predicted solvent content and the Matthews coefficient ($V_{\rm M}$) were 46.8% and 2.3 Å^3 Da^{-1}, respectively. Structure determination by the molecular-replacement method provided a reasonable solution for model building.

1. Introduction

The glycolytic enzyme phosphoglucose isomerase (PGI; EC 5.3.1.9) interconverts glucose 6-phosphate and fructose 6-phosphate, which is an essential reaction in the glycolysis and gluconeogenesis pathways (Schray *et al.*, 1973; Jeffery, 1999). It is highly conserved in bacteria and eukaryotes. In addition to its cytosolic enzymatic function, studies have shown that PGI is also involved in a variety of extracellular functions that are closely related to those of neuroleukin (NLK), which promotes the survival of peripheral and central neurons *in vitro* and causes B cells to mature for Ig synthesis (Gurney, 1987; Gurney *et al.*, 1986), maturation factor (MF), which mediates the differentiation of human myeloid leukaemia cells (Xu *et al.*, 1996), and autocrine motility factor (AMF), which stimulates cancer-cell metastasis (Watanabe *et al.*, 1996).

In recent years, a wealth of structural information on PGI and its complexes from eukaryotes ranging from protozoan parasites to humans (Cordeiro *et al.*, 2004; Solomons *et al.*, 2004; Jeffery *et al.*, 2000; Davies & Muirhead, 2002; Read *et al.*, 2001) and a thermophilic *Bacillus stearothermophilus* strain (Sun *et al.*, 1999) have been reported and indicate that the bacterial and eukaryotic PGIs consist of a homodimer of 60–70 kDa subunits that carries out the isomerase activity. Interestingly, the structure of an archaeal PGI from the hyperthermophile *Pyroccus furiosus* (Pfu) consisted of a cupin-based fold which was different from the $\alpha\beta\alpha$ sandwich fold of the previously solved structures of bacterial and eukaryotic PGIs (Berrisford *et al.*, 2003). The structure of PGI from *B. stearothermophilus* (PDB code 2pgi) was reported by Sun *et al.* (1999) and the thermostable enzyme was purified by incubating the cell lysate at 343 K. The PGI from mesophilic *B. subtilis* studied here is thermolabile and is stable for less than 4.2 min at 336 K (data not shown). These enzymes share 67% amino-acid sequence identity but have rather different thermostabilities. A comparison of the two enzymes is important in order to understand the structural features that are essential for protein thermostability and to investigate whether these differences can provide further information on the catalytic mechanism of PGI in mesophilic organisms. In this report, we describe the crystallization and preliminary X-ray diffraction studies of the *B. subtilis* PGI protein.

2. Materials and methods

2.1. Expression and purification

Escherichia coli DF214 (pgi-*Mu*, *zwf*Δ) cells containing a pUC18-based expression vector encoding full-length *B. subtilis* phosphoglucose isomerase were a kind gift from Dr Meng (National ChungHsing University). The enzyme was expressed under the control of *Bacillus*'s own promoter in a double mutant *E. coli* DF214 (pgi-*Mu*, *zwf*Δ) with a Mu insertion in *pgi* and a deletion in *zwf* (glucose 6-phosphate dehydrogenase; Vinopal *et al.*, 1975). An aliquot of a 4 ml overnight culture was used to inoculate 2.0 l of 2×TY medium (16 g l^{-1} tryptone, 10 g l^{-1} yeast extract, 5 g l^{-1} NaCl) containing ampicillin (150 µg ml^{-1}), which was followed by incubation at 310 K and 250 rev min^{-1} for 30 h without IPTG induction. Cells were harvested by centrifugation, resuspended in lysis buffer (100 m*M* Tris–HCl pH 8.0, 100 m*M* NaCl, 10 m*M* β-mercaptoethanol, 1 m*M* EDTA, 0.01% NaN_3 and 0.4 m*M* phenylmethanesulfonyl fluoride) and lysed with a Microfluidics Model M-110EH-30 microfluidizer at 76 MPa in three cycles. The lysate was clarified by centrifugation at 12 000*g* for 1 h at 277 K and the soluble protein was precipitated by adding solid ammonium sulfate to a final saturation of 80%. The crude protein pellet was collected by centrifugation at 10 000*g* for 1 h, redissolved in 200 ml lysis buffer and dialyzed into buffer *A* (50 m*M* Tris–HCl pH 8.0, 50 m*M* NaCl, 10 m*M* β-mercaptoethanol, 10 m*M* EDTA, 0.01% NaN_3). Three sequential chromatographic steps were used to purify the protein to homogeneity. The resuspended pellet was applied onto a Q Sepharose Fast Flow (Pharmacia) column, which was washed with four column volumes of buffer *A* and eluted with a five-column-volume gradient from 0.1 to 1.0 *M* NaCl in buffer *A*. The eluted protein fractions were pooled and dialyzed into buffer *A* and applied onto a Source 15Q column (Pharmacia), which was washed with five column volumes of buffer *A* and eluted with an eight-column-volume gradient from 150 to 350 m*M* NaCl in buffer *A*. The 95% pure protein solution with solid ammonium sulfate added to a final concentration of 1.5 *M* was passed through a phenyl Sepharose column (Pharmacia) and eluted with a four-column-volume gradient from 1.5 to 0 *M* ammoinum sulfate in buffer *A*. The peak fractions were pooled and dialyzed into buffer *B* (75 m*M* Tris–HCl pH 7.5, 25 m*M* NaCl, 1 m*M* DTT, 0.2 m*M* EDTA, 0.01% NaN_3), concentrated to 22 mg ml^{-1} and stored at 193 K. At each purification stage, the homogeneity of the protein solution was determined by SDS–PAGE analysis of serially diluted protein samples to check for contaminants and degradation. The final protein concentration was determined using the A_{280} with an extinction coefficient of 0.920 ml mg^{-1} cm^{-1}.

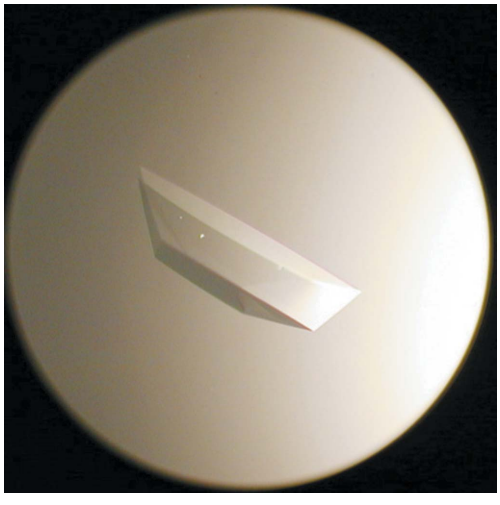

Figure 1
A single crystal of phosphoglucose isomerase from *B. subtilis* with dimensions of 0.25 × 0.25 × 0.7 mm.

2.2. Crystallization and data collection

Crystallization trials were carried out using the hanging-drop vapour-diffusion method at 293 K. In each drop, 2 µl protein solution (22 mg ml^{-1} in buffer *B*) and 2 µl reservoir solution were mixed and equilibrated against 1.0 ml reservoir solution. Prismatic rod-shaped crystals appeared in one week with reservoir conditions consisting of 100 m*M* Tris–HCl pH 7.2, 20–23% PEG 400, 80–100 m*M* $MgCl_2$, 1 m*M* β-mercaptoethanol and 0.01% NaN_3. These crystals were fragile and cracked easily on attempting to stabilize them in various compositions of cryoprotectants and oil. Finally, crystals of good size that were suitable for cooling to 100 K for data collection were obtained using a reservoir solution consisting of 100 m*M* Tris–HCl pH 6.5, 15% PEG 400, 30% ethylene glycol, 80 m*M* $MgCl_2$, 1 m*M* β-mercaptoethanol and 0.01% NaN_3 (Fig. 1).

Data collection was carried out in-house on a Rigaku RUH3R generator equipped with a copper rotating-anode generator operating at a 5.0 kW power rating (50 kV, 100 mA). The X-ray beam was focused and monochromated using confocal blue optics and tuned to Cu *Kα* radiation of 1.5418 Å wavelength. A crystal harvested directly from the glass cover slip using a mohair cryoloop was flash-cooled in a cold nitrogen-gas stream at 100 K. An *in situ* flash-annealing technique was applied to the cooled crystal in which the cold nitrogen-gas stream was blocked three times for 3 s, with intervals of 10 s between the thawing processes. The resolution limit of the crystal was then checked using an R-AXIS IV^{++} image-plate system. Data were collected by the standard oscillation method using a crystal-to-detector distance of 120 mm. A total of 289 images were collected in 0.5° increments with an exposure time of 13 min per image. The data

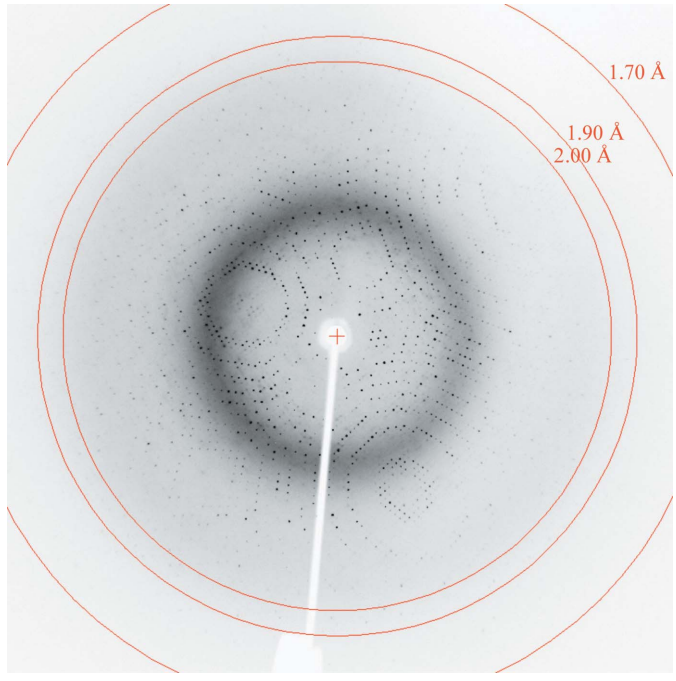

Figure 2
A typical X-ray diffraction pattern. The diffraction image was collected on an R-AXIS IV^{++} image-plate detector. The oscillation range was 0.5° and the crystal-to-detector distance was 120 mm.

Table 1
Data-collection and processing statistics.

Values in parentheses are for the highest resolution shell.

Space group	*C*2
Unit-cell parameters (Å, °)	*a* = 145.7, *b* = 136.0, *c* = 109.1, β = 119.4
Resolution range (Å)	28.5–1.9 (1.97–1.90)
Total reflections	534935
Unique reflections	142535
Data redundancy	3.8 (3.7)
Completeness	98.1 (96.4)
$I/\sigma(I)$	17.4 (5.8)
$R_{\rm merge}$† (%)	4.6 (17.2)

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ is the observed intensity of reflection *hkl* and $\langle I(hkl)\rangle$ is the average intensity of multiple observations of symmetry-related reflections.

were processed using *CrystalClear* (Rigaku MSC, The Woodlands, Texas, USA).

3. Results and discussions

The phosphoglucose isomerase from *B. subtilis* was purified to near-homogeneity, with a final yield of 48 mg per litre of cell culture. Addition of ethylene glycol (but not glycerol, which inhibited crystal formation) to the crystallization solution was necessary to prevent crystal cracking and ice formation during the flash-cooling process. Several attempts, including cross-linking the crystals with glutaraldehyde (Li *et al.*, 1995), adding sucrose or glycerol to the cryoprotectant and quick dippping or slow equilibration of the crystals in cryoprotectant containing a higher concentration of PEG 400 or glycerol, failed to stabilize the crystals during the flash-cooling process. At room temperature, diffraction from these crystals extended to 2.1 Å resolution using a laboratory X-ray source. After flash-cooling of the protein crystals, the diffraction spots became mosaic and the resolution was reduced to 2.4 Å (Bunick *et al.*, 1998; Harp *et al.*, 1998). *In situ* flash-annealing of the cooled crystal at 100 K, which utilized brief thawing and rapid cooling cycles of the crystal in the cold nitrogen stream, greatly improved the resolution limit to 1.9 Å and reduced the mosaicity (Fig. 2), which was previously manifested by the long tails in the diffraction patterns. Data were collected from the best crystal and after processing by *CrystaClear* (Rigaku MSC, The Woodlands, Texas, USA) gave an overall completeness of 98.1%, an overall $R_{\rm merge}$ of 4.6% and a crystal mosaicity of 0.69°. The statistics of the collected data are summarized in Table 1. The unit-cell parameters of the crystal are *a* = 145.7, *b* = 136.0, *c* = 109.1 Å, β = 119.4° and the space group is *C*2. Assuming the presence of four molecules in the asymmetric unit and a molecular weight of 50 kDa, the calculated solvent content is 46.8% and the Matthews coefficient ($V_{\rm M}$) is 2.3 Å^3 Da^{-1}. Solution of the phase problem by molecular replacement using the structure of phosphoglucose isomerase from *B. thermophilus* (67% identity over 453 amino acids; PDB code 2pgi; Sun *et al.*, 1999) as a search model found a solution consisting of four molecules with a correlation coefficient ranging from 22% to 54%. Self-rotation function calculations also showed two sets of three well defined noncrystallographic twofold axes, which represent the twofold rotation symmetry of the packing of the four molecules in the asymmetric unit. Crystallographic refinement of the search model is currently under way.

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