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Cloning, expression, purification, crystallization and preliminary X-ray diffraction analysis of 2-keto-3-deoxy-6-phosphogluconate aldolase from *Zymomonas mobilis* ZM4

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Zymomonas mobilis ZM4 is an organism optimized for ethanol production which uses the Entner–Doudoroff (ED) pathway for the breakdown of glucose. The key enzyme in this process is 2-keto-3-deoxy-6-phosphogluconate (KDPG) aldolase, which produces glyceraldehyde 3-phosphate and pyruvate. In order to provide a molecular background for the KDPG aldolase from this ethanologenic organism (zmKDPG aldolase), the ZMO0997 gene of *Z. mobilis* ZM4 coding for zmKDPG aldolase was cloned and expressed and the purified protein was crystallized from 25%(*w*/*v*) polyethylene glycol 3350 and 0.1 *M* bis-tris pH 5.5. Diffraction data were collected to 1.8 Å resolution using synchrotron radiation. The crystal belonged to the orthorhombic space group $P2_12_12_1$, with unit-cell parameters a = 63.7, b = 83.0, c = 117.2 Å. A trimeric zmKDPG aldolase molecule was present in the asymmetric unit, resulting in a crystal volume per unit protein weight of 2.40 Å^3 Da^{-1} and a solvent content of 48%.

1. Introduction

Fossil fuels can give rise to many environmental problems. In addition, their mass supply is limited. Therefore, interest in the production of biofuels from natural resources has gradually increased. However, biofuel production using living organisms is restricted by the high concentration of substrates, limitation of nutrients, sensitivity to acetic acid and rapid shifts in pH and temperature during the fermentation process. Moreover, the accumulation of alcohols often leads to reduced growth and eventually to death of the fermenting microbes. In this respect, *Zymomonas mobilis*, which is one of the most effective ethanol-producing organisms, is a powerful candidate for bioethanol production because of its higher ethanol tolerance to up to 16%(*v*/*v*) (Rogers *et al.*, 2007) and its ethanol yields, which are close to the theoretical maximum (Lee *et al.*, 1980).

In many organisms, glucose is metabolized *via* the Embden–Meyerhof–Parnas (EMP) pathway, in which the phosphorylated glucose is isomerized and a successive phosphorylation reaction occurs before the next aldol-cleavage reaction. However, according to the recently reported genomic sequence of the ethanologenic bacterium *Z. mobilis* ZM4 (Seo *et al.*, 2005) this microorganism lacks recognizable genes for 6-phosphofructokinase, which is an essential enzyme in the EMP pathway. The lack of 6-phosphofructokinase in *Z. mobilis* requires a different pathway for glucose metabolism, in which sugar-ring opening occurs without isomerization of a glucose to a fructose derivative and phosphorylation before cleavage of the six-carbon sugar into two three-carbon sugar molecules: the so-called Entner–Doudoroff (ED) pathway. This cleavage reaction is possible in *Z. mobilis* because of the presence of a specific KDPG aldolase, zmKDPG aldolase (for further details, see Romano & Conway, 1996).

zmKDPG aldolase shows 54% amino-acid sequence identity and 70% sequence similarity to *Escherichia coli* KDPG (ecKDPG) aldolase. Although several structures of trimeric ecKDPG aldolase have been reported (Wymer *et al.*, 2001; Bell *et al.*, 2003; Fullerton *et al.*, 2006), structural information on this key aldolase in the ED pathway of the ethanologenic bacterium *Z. mobilis* is not available. In order to understand its molecular background, we cloned, expressed,

purified and crystallized zmKDPG aldolase. Here, we report the analysis of its X-ray diffraction data.

2. Methods

2.1. Cloning, expression and purification of zmKDPG

The *Z. mobilis* ZM4 gene coding for zmKDPG (ZMO0997; Met1–Ala208) was amplified from *Z. mobilis* ZM4 chromosomal DNA by the polymerase chain reaction (PCR). The PCR product was then cloned into pProEx HTc (Invitrogen), which expresses 25 extra amino acids with six consecutive His residues at the N-terminus. The expression construct was transformed into *E. coli* BL21 (DE3) Star and grown in LB medium containing 100 µg ml^{-1} ampicillin at 310 K. After induction with 1.0 m*M* IPTG for a further 8 h at 310 K, the culture was harvested by centrifugation at 5000*g* and 277 K. The cell pellet was resuspended in ice-cold buffer *A* (20 m*M* Tris–HCl pH 7.5 and 500 m*M* NaCl) and disrupted by ultrasonication. The cell debris was removed by centrifugation at 11 000*g* for 1 h. The zmKDPG fusion protein was purified using a 5 ml HisTrap chelating column (GE Healthcare, Uppsala, Sweden). After treatment with rTEV to cleave the six-His tag and the removal of salts by dialysis, the protein containing five additional amino acids (YFQGA) at the N-terminus was purified using a 5 ml HiTrap Q anion-exchange column (GE Healthcare, Uppsala, Sweden). The purified protein was >95% pure as judged by Coomassie Blue-stained SDS–PAGE (data not shown).

2.2. Crystallization

For crystallization, the purified zmKDPG protein was concentrated to 8.2 mg ml^{-1} in a buffer consisting of 20 m*M* Tris–HCl pH 7.5 and 300 m*M* NaCl. The protein concentration was determined using an extinction coefficient of 0.331 mg ml^{-1} cm^{-1} at 280 nm, which was calculated from its amino-acid sequence. Crystallization of zmKDPG was attempted at 295 K by the sitting-drop vapour-diffusion method. The initial trials used Crystal Screens 1 and 2 and Index 1 and 2 (Hampton Research, Riverside, California, USA). For each crystallization trial, a 2 µl drop was prepared by mixing 1 µl purified protein solution (8.2 mg ml^{-1}) with an equal volume of reservoir solution. The reservoir contained 80 µl of the precipitating solution. Small crystals were obtained in 2 d from five of the drops [solution Nos. 28, 30, 32 and 47 of Crystal Screen (CS) 1 and solution No. 6 of CS 2]. The most promising results were obtained using solution No. 28 of CS 1 [30%(*w*/*v*) polyethylene glycol, 0.1 *M* sodium cacodylate pH 6.5 and 0.2 *M* sodium acetate] and this condition was optimized by changing the precipitant and protein concentration, the buffer pH, the temperature and the vapour-diffusion method to obtain crystals that were suitable for X-ray diffraction.

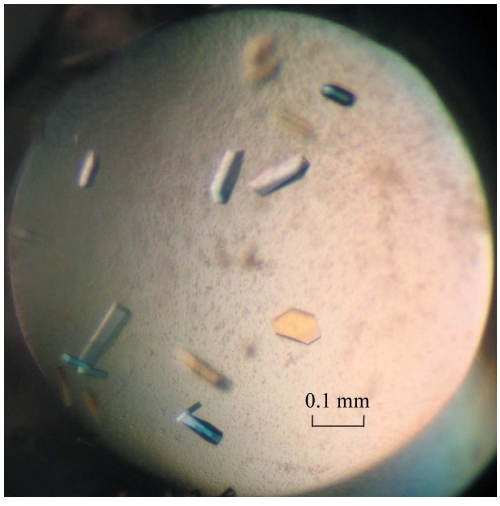

Figure 1
Crystals of zmKDPG. The crystals were grown at 291 K within 1 d and had maximum dimensions of approximately 0.1 × 0.05 × 0.05 mm.

Table 1
Data-collection statistics for zmKDPG.

Values in parentheses are for the highest resolution shell.

Wavelength (Å)	1.00
Space group	$P2_12_12_1$
Unit-cell parameters (Å)	a = 63.7, b = 83.0, c = 117.2
Resolution range (Å)	50.0–1.8 (1.86–1.8)
Measured reflections	470412
Unique reflections	57603 (5519)
Multiplicity	8.2
Temperature (K)	100
Matthews coefficient (Å^3 Da^{-1})	2.40
Solvent content (%)	48
No. of molecules in the asymmetric unit	3
Completeness (%)	98.5 (95.8)
Mean $I/\sigma(I)$	17.0 (2.2)
$R_{\rm merge}$† (%)	7.7 (43.0)

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ is the observed intensity of an individual reflection and $\langle I(hkl)\rangle$ is the mean intensity of that reflection.

2.3. X-ray data collection

For diffraction experiments, crystals were briefly immersed into precipitant solution containing 20%(*v*/*v*) glycerol as a cryoprotectant and immediately placed in a 100 K nitrogen-gas stream. Native X-ray diffraction data were collected at MAX4A, Pohang Accelerator Laboratory (PAL, Republic of Korea) using 1° oscillation per image and a crystal-to-detector distance of 200 mm. The crystal was exposed for 10 s per image at a wavelength of 1.00 Å. A data set of 180 images was collected to 1.80 Å resolution from a single crystal. The data were

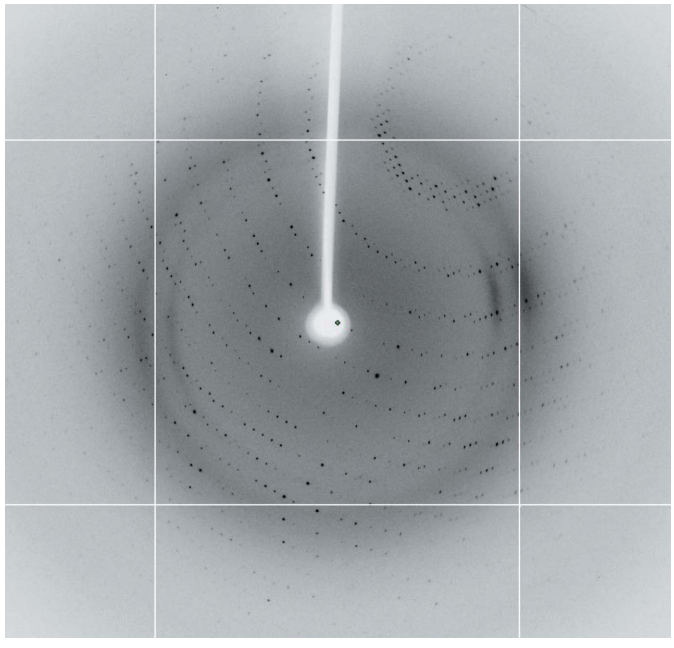

Figure 2
Representative X-ray diffraction image from zmKDPG. The crystal was exposed for 10 s over a 1° oscillation range. The edge of the detector corresponds to a resolution of 1.60 Å.

indexed and scaled with *HKL*-2000 (Otwinowski & Minor, 1997). The data-collection statistics are summarized in Table 1.

3. Results and discussion

Recombinant zmKDPG protein was successfully expressed in *E. coli* and purified. The purified protein was concentrated to 8.2 mg ml^{-1} in 20 m*M* Tris–HCl pH 7.5 and 300 m*M* NaCl. Crystals that were suitable for diffraction experiments were obtained within 1 d using the hanging-drop vapour-diffusion method at 291 K by mixing 1 µl protein solution and 1 µl reservoir solution and equilibrating against 200 µl reservoir solution, which consisted of 25%(*w*/*v*) polyethylene glycol 8000 and 0.1 *M* bis-tris pH 5.5. The dimensions of the crystal used for data collection were approximately 0.1 × 0.05 × 0.05 mm (Fig. 1) and the crystal diffracted to 1.8 Å resolution (Fig. 2). The crystal belonged to the orthorhombic space group $P2_12_12_1$, with unit-cell parameters a = 63.7, b = 83.0, c = 117.2 Å.

Phasing information for the zmKDPG aldolase crystal was obtained by the molecular-replacement method with the *CCP*4 version of *Phaser* (McCoy *et al.*, 2007), using the structure of ecKDPG aldolase (PDB code 1fq0; Wymer *et al.*, 2001), which has 50% sequence identity, as the search probe. After a fast rotational and translational search, the *Phaser* scores were 7.8 and 29.4, respectively, with a log-likelihood gain of 1352. Like ecKDPG, the asymmetric unit contains a trimer of zmKDPG, resulting in a corresponding crystal volume per protein weight of 2.40 Å^3 Da^{-1} and a solvent content of 48% (Matthews, 1968). Crystallographic model building and refinement of the structure to 1.8 Å resolution are now in progress.

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