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Cloning, expression, purification, crystallization and preliminary X-ray diffraction analysis of 2-keto-3-deoxy-6-phosphogluconate (KDPG) aldolase from *Streptococcus suis* serotype 2

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2-Keto-3-deoxy-6-phosphogluconate (KDPG) adolase from pathogenic *Streptococcus suis* serotype 2 was crystallized using the hanging-drop vapour-diffusion method at 291 K. X-ray diffraction data were collected to 2.8 Å resolution. The crystal belonged to space group *R*32, with unit-cell parameters $a = b = 126.4$, $c = 415.9$ Å, $\alpha = \beta = 90$, $\gamma = 120^\circ$. Assuming the presence of six molecules in the asymmetric unit gave a V_M value of 2.32 Å^3 Da^{-1} and a solvent content of 47.12%.

1. Introduction

Streptococcus suis is an important pathogen that is associated with a wide range of diseases in pigs and humans, including meningitis, septicaemia, endocarditis, arthritis and septic shock (Tang *et al.*, 2006; Lun *et al.*, 2007). Sugar acids, typically gluconate, are very important carbon and energy sources for several species of bacteria (including *S. suis*), especially when these organisms enter the stationary phase of growth (Adachi *et al.*, 1979). Sugar acids can be converted to 2-keto-3-deoxy-6-phosphogluconate (KDPG) *via* the Entner–Doudoroff pathway, which exists extensively in bacterial species (Peekhaus & Conway, 1998). The accumulation of KDPG in bacteria is often correlated with an immediate and significant decrease in growth (Fuhrman *et al.*, 1998). The enzyme KDPG aldolase, which is responsible for further degradation of KDPG, is therefore an attractive target for drug therapy in the case of human *S. suis* infections.

KDPG aldolase (EC 4.1.2.14) catalyzes the reversible cleavage of KDPG to pyruvate and glyceraldehyde-3-phosphate. The enzyme is a class I aldolase and its reaction mechanism involves the formation of a Schiff-base intermediate between a catalytic lysine and a keto substrate (Allard *et al.*, 2001). The structures of several KDPG aldolases from *Escherichia coli* (Allard *et al.*, 2001), *Pseudomonas putida* (Mavridis *et al.*, 1982), *Thermotoga maritima* (Fullerton *et al.*, 2006) and *Haemophilus influenzae* have been solved to date (PDB codes 1wau, 1mxs, 1kga, 1eua, 1fq0, 2c0a, 1wbh, 1eun, 1fwr and 1vhc). On the basis of the aldolase mechanistic pathway, the effects of several pyruvate analogues as inhibitors of KDPG aldolase have been examined (Braga *et al.*, 2004). For the purpose of the treatment of human *S. suis* infections, it is necessary to design novel inhibitors against targets such as KDPG aldolase, considering the presence of pyruvate in humans.

Here, we report the expression, purification, crystallization and preliminary X-ray diffraction analysis of *S. suis* KDPG aldolase, the crystal structure of which will provide a structural basis for the design of novel inhibitors against human *S. suis* infections.

2. Experimental methods

2.1. Expression and purification of recombinant KDPG aldolase

The gene encoding full-length KDPG aldolase (NCBI gene ID ABP90126) was amplified by PCR using *S. suis* serotype 2 (strain 05ZYH33) genomic DNA as a template (forward primer, 5′-GGAATTC**CATATG**TTGGAGGTCAAGATGTTA-3′; reverse primer, 5′-CCG**CTCGAG**TCCCTCCAAAGCTGC-3′). The forward and reverse

primers contained an *Nde*I and an *Xho*I restriction-enzyme site (bold), respectively. The PCR product was digested with these two restriction endonucleases, inserted into the vector pET21a (Novagen) containing a C-terminal 6×His tag and transformed into *Escherichia coli* strain BL21 (DE3). Thus, the amino-acid sequence at the C-terminus of the expressed KDPG aldolase consists of its last residue plus LEHHHHHH. The cells were grown in LB medium supplemented with 10 µg ml^{-1} ampicillin at 310 K until the OD_{600} reached 0.8. Protein production was induced by the addition of 0.1 m*M* isopropyl *β*-D-1-thiogalactopyranoside (IPTG) followed by incubation at 310 K for 5 h. Cells were harvested by centrifugation, resuspended in lysis buffer containing 20 m*M* Tris–HCl pH 8.0 and 50 m*M* NaCl and homogenized by sonication. Cellular debris was removed by centrifugation. The resulting supernatant was applied onto a nickel-affinity column (Qiagen) pre-equilibrated with lysis buffer. The column was washed with lysis buffer until the elute did not turn blue when detected using Coomassie Brilliant Blue G250. The protein was eluted with lysis buffer containing an increasing linear concentration gradient of imidazole and then dialyzed against lysis buffer. After concentration, the preparation was loaded onto a Superdex 200 FPLC column (Amersham Biosciences, New Jersey, USA) equilibrated with the same dialysis buffer. Fractions containing KDPG aldolase were pooled and concentrated using Amicon Ultra-15 filters. The protein purity was detected by SDS–PAGE and the native molecular weight was measured by gel filtration on a Superdex 75 column (Amersham Biosciences, New Jersey, USA) and dynamic light-scattering (DLS) experiments. Briefly, purified KDPG aldolase (about 3 mg ml^{-1}) after size-exclusion chromatography was used for DLS analysis. DLS measurements were carried out at 298 K using a DynaPro Titan instrument (Wyatt Technology, Santa Barbara, California, USA). Protein concentrations were determined using the BCA protein assay according to the manufacturer's instructions (Pierce).

2.2. Crystallization

Crystallization screening was carried out with Crystal Screens I and II, PEG/Ion Screen and Index Screen (Hampton Research). Initial attempts to crystallize KDPG aldolase were performed at 291 K using the hanging-drop vapour-diffusion method by mixing 1 µl 5–15 mg ml^{-1} protein (in a buffer containing 20 m*M* Tris–HCl pH 8.0 and 50 m*M* NaCl) and 1 µl reservoir solution. The reservoir volume was 200 µl. Needle-like crystals appeared within 3 d in six different conditions. Diffraction-quality crystals were obtained in the following three conditions: (i) 0.2 *M* ammonium acetate, 0.1 *M* sodium citrate pH 5.6, 30% PEG 4000, (ii) 0.2 *M* potassium sodium tartrate, 0.1 *M* trisodium citrate pH 5.6, 2.0 *M* ammonium sulfate, (iii) 0.2 *M* magnesium formate, 20% PEG 3350. Using a protein concentration of 5 mg ml^{-1}, further optimization of condition (ii) gave well diffracting crystals (Fig. 1).

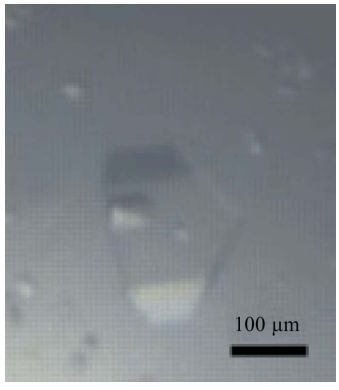

Figure 1
Representative crystal of *S. suis* KDPG aldolase.

2.3. Diffraction data collection and processing

Prior to data collection, the crystal was immersed for more than 30 s in mother liquor with 1.6 *M* trisodium citrate added as a cryoprotectant. X-ray diffraction data were collected at 100 K using in-house Cu *Kα* X-rays with an R-AXIS IV^{++} imaging plate (Rigaku, Japan). The data were indexed, integrated and scaled with *HKL*-2000 (Otwinowski & Minor, 1997).

3. Results and discussion

The gene encoding *S. suis* KDPG aldolase was cloned and the protein was overexpressed and purified. The molecular weight of KDPG aldolase was about 23 kDa as judged by SDS–PAGE, which is in agreement with the calculated molecular weight of 22 920 Da including the His tag. During gel filtration on a Superdex 75 column, KDPG aldolase eluted at the volume expected for a molecular weight of about 60 kDa (Fig. 2). This result coincided with DLS experiments (Fig. 3) suggesting that the protein exists as a trimer in solution. In the

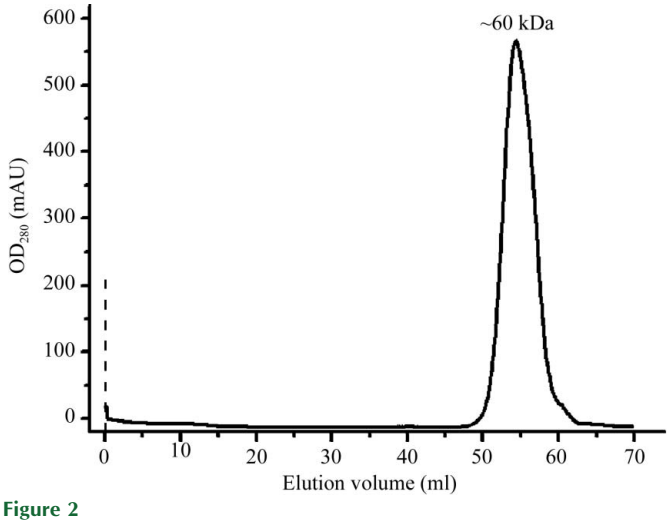

Figure 2
Gel-filtration chromatogram of *S. suis* KDPG aldolase loaded onto Superdex 75.

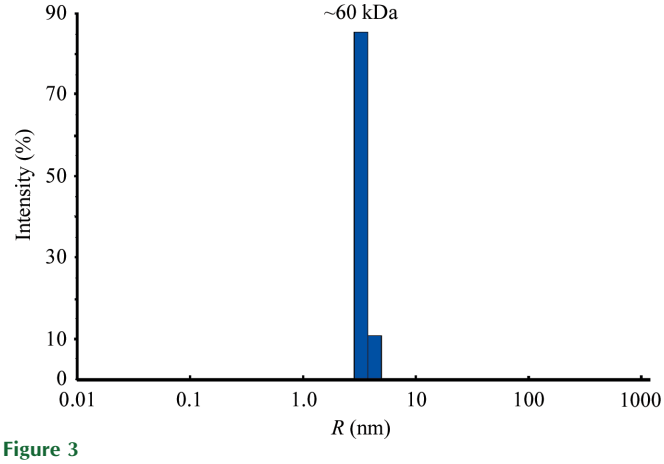

Figure 3
DLS experiment result, showing a single peak of about 60 kDa.

Table 1
Data-collection and processing statistics.

Values in parentheses are for the highest resolution shell.

Space group	*R*32
Unit-cell parameters (Å, °)	$a = b = 126.4$, $c = 415.9$, $\alpha = \beta = 90$, $\gamma = 120$
Resolution range (Å)	50–2.8 (2.9–2.8)
No. of unique reflections	32015
No. of observed reflections	259264
Completeness (%)	98.9 (99.9)
Redundancy	8.2 (8.2)
$I/\sigma(I)$	11.7 (4.7)
$R_{\rm merge}$† (%)	16.4 (55.0)

† $R_{\rm merge} = \sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl} \sum_i I_i(hkl)$.

initial crystallization screen, crystals of KDPG aldolase were obtained in the presence of ammonium sulfate, PEG 3350, PEG 4000 or PEG 6000. The pH value (5.6–8.5) did not seem to affect the growth of the crystals. Large well diffracting crystals were obtained using a reservoir solution consisting of 0.2 *M* potassium sodium tartrate, 0.1 *M* trisodium citrate pH 5.6 and 1.8 *M* ammonium sulfate. The crystals grew to final dimensions of 0.2 × 0.3 × 0.5 mm within 3 d. The KDPG aldolase crystals belonged to space group *R*32, with unit-cell parameters $a = b = 126.4$, $c = 415.9$ Å, $\alpha = \beta = 90$, $\gamma = 120^\circ$. Using the molecular weight of the monomer (22 920 Da), a Matthews coefficient (Matthews, 1968) of 2.32 Å^3 Da^{-1}, corresponding to a solvent content of 47.12%, was obtained assuming the presence of six monomers (two trimers) in the asymmetric unit. Data-collection and processing statistics are given in Table 1. Attempts to solve the structure of *S. suis* KDPG aldolase by molecular-replacement procedures or by anomalous diffraction using heavy-atom derivatives are in progress.

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References

Adachi, O., Shinagawa, E., Matsushita, K. & Ameyama, M. (1979). *Agric. Biol. Chem.* **43**, 75–83.
Allard, J., Grochulski, P. & Sygusch, J. (2001). *Proc. Natl Acad. Sci. USA*, **98**, 3679–3684.
Braga, R., Hecquet, L. & Blonski, C. (2004). *Bioorg. Med. Chem.* **12**, 2965–2972.
Fuhrman, L. K., Wanken, A., Nickerson, K. W. & Conway, T. (1998). *FEMS Microbiol. Lett.* **159**, 261–266.
Fullerton, S. W., Griffiths, J. S., Merkel, A. B., Cheriyan, M., Wymer, N. J., Hutchins, M. J., Fierke, C. A., Toone, E. J. & Naismith, J. H. (2006). *Bioorg. Med. Chem.* **14**, 3002–3010.
Lun, Z. R., Wang, Q. P., Chen, X. G., Li, A. X. & Zhu, X. Q. (2007). *Lancet Infect. Dis.* **7**, 201–209.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Mavridis, I. M., Hatada, M. H., Tulinsky, A. & Lebioda, L. (1982). *J. Mol. Biol.* **162**, 419–444.
Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
Peekhaus, N. & Conway, T. (1998). *J. Bacteriol.* **180**, 3495–3502.
Tang, J. *et al.* (2006). *PloS Med.* **3**, e151.