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Purification, crystallization and preliminary crystallographic studies on 2-dehydro-3-deoxygalactarate aldolase from *Leptospira interrogans*

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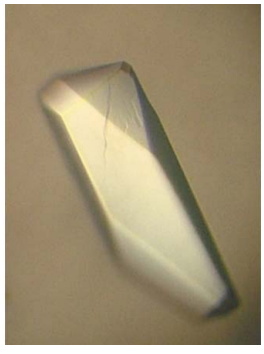

2-Dehydro-3-deoxygalactarate (DDG) aldolase is a member of the class II aldolase family and plays an important role in the pyruvate-metabolism pathway, catalyzing the reversible aldol cleavage of DDG to pyruvate and tartronic semialdehyde. As it is a potential novel antibiotic target, it is necessary to elucidate the catalytic mechanism of DDG aldolase. To determine the crystal structure, crystals of DDG aldolase from *Leptospira interrogans* were obtained by the hanging-drop vapour-diffusion method. The crystals diffracted to 2.2 Å resolution using a Cu $K\alpha$ rotating-anode X-ray source. The crystal belonged to space group $C2$, with unit-cell parameters a = 293.5, b = 125.6, c = 87.6 Å, β = 100.9°. The V_M is calculated to be 2.4 Å^3 Da^{-1}, assuming there to be 12 protein molecules in the asymmetric unit.

1. Introduction

Leptospirosis occurs as severe worldwide epidemics, with outbreaks most common in agricultural populations. This disease is caused by the nosogenetic bacterium *Leptospira interrogans*, which infects people through broken skin, conjunctiva and the nasopharyngeal mucosa (Sambsiava *et al.*, 2003; Vijayachari *et al.*, 2003). Rice-producing regions such as China, India and southeast Asia are the primarily affected countries. In recent years, leptospirosis outbreaks have also occurred in France and Canada, both owing to infection by pets (World Health Organization, 2006).

Amino-acid sequence alignment suggests that 2-dehydro-3-deoxygalactarate (DDG) aldolase (EC 4.1.2.20) from *L. interrogans* belongs to the class II aldolase family, members of which are often found in bacteria and other lower organisms and require a metal cofactor for activity (Izard & Sygusch, 2004). This enzyme catalyzes the reversible aldol cleavage of DDG to pyruvate and tartronic semialdehyde (Blackwell *et al.*, 1999). Because DDG aldolase plays an essential role in the pyruvate-metabolism pathway of *L. interrogans* and has never been found in higher organisms, it is considered to be a potential target for novel antibiotics (Izard & Blackwell, 2000).

To date, only the crystal structure of DDG aldolase from *Escherichia coli* (33% identity to DDG aldolase from *L. interrogans*) has been reported, on the basis of which a possible catalytic mechanism was proposed (Izard & Blackwell, 2000). However, no structure of DDG aldolase from any other organism is available. Thus, whether the conclusion from the *E. coli* DDG aldolase structure is generally applicable is unknown.

In this paper, we report the expression, purification, crystallization and preliminary X-ray diffraction analysis of DDG aldolase from *L. interrogans*. The diffraction data were collected to 2.2 Å resolution.

2. Materials and methods

2.1. Cloning and purification

The full-length gene fragment encoding DDG aldolase was amplified from *L. interrogans* genomic DNA and subcloned into a modified pET22b(+) vector (Novagen) in which a stop codon (TAG) was introduced before the His tag. The recombinant protein was

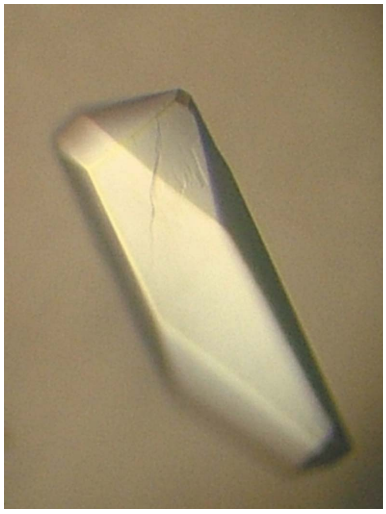

Figure 1
Crystal of *L. interrogans* DDG aldolase grown by the hanging-drop method.

highly expressed in *E. coli* strain BL21 (DE3) after induction by IPTG.

Harvested *E. coli* cells were disrupted by sonication for 600 s at 273 K with 20 m*M* Tris–HCl pH 7.15 and 10 µ*M* PMSF. After centrifugation at 15 000*g* (at 277 K) for 20 min, the pellet was discarded and the supernatant was further centrifuged at 100 000*g* (at 277 K) for 2 h to remove cell membranes containing reduced nicotinamide adenine dinucleotide (NADH) oxidase (Blackwell *et al.*, 1999).

Purification of the soluble DDG aldolase protein was carried out by ion-exchange chromatography using a DEAE-Sepharose column (Amersham Biosciences). DDG aldolase was eluted in 20 m*M* Tris–HCl buffer pH 8.0 with a 0–500 m*M* sodium chloride gradient. The target protein was collected and applied onto a Superdex 75 size-exclusion chromatography column (Amersham Biosciences) that had been pre-equilibrated with 20 m*M* Tris–HCl pH 8.0 and 100 m*M* sodium chloride. SDS–PAGE showed the protein sample to be more than 95% pure. The protein concentration was measured by the Bradford method (Bio-Rad Protein Assay) using bovine serum albumin as the standard (Bradford, 1976). The purified and concentrated DDG aldolase (7.5 mg ml^{-1}) was stored in 20 m*M* Tris–HCl pH 7.15.

2.2. Protein crystallization

Protein crystals were grown at constant temperature (295 K) by the hanging-drop vapour-diffusion method using 0.4 ml mother liquor in the reservoirs and crystallization drops made by mixing 2 µl protein solution and 2 µl reservoir solution. Hampton Research Crystal Screen and Crystal Screen 2 kits yielded small crystals in condition Nos. 42 (0.05 *M* monopotassium dihydrogen phosphate, 20% PEG 8000), 46 (0.2 *M* calcium acetate hydrate, 0.1 *M* sodium cacodylate pH 6.5, 18% PEG 8000) and 87 (0.1 *M* HEPES pH 7.5, 10% PEG 8000, 8% ethylene glycol), all of which use polyethylene glycol 8000 as the precipitant. By adjusting the concentration of PEG 8000 and the pH value larger crystals could be obtained, but they diffracted with very high mosaicity. Because Mg^{2+} or Mn^{2+} may be essential for DDG aldolase activity, various concentrations of Mg^{2+} or Mn^{2+} were added to the crystallization solutions. Finally, crystals of DDG aldolase of good quality were obtained from mother liquor containing 10% PEG 8000, 100 m*M* Tris–HCl buffer pH 6.8 and 20 m*M* manganese chloride. Typical crystals are 0.3 × 0.2 × 1 mm in size (Fig. 1).

Table 1
Diffraction data-collection and processing statistics of *L. interrogans* DDG aldolase.

Wavelength (Å)	1.5418
Temperature (K)	100
Space group	*C*2
Unit-cell parameters (Å, °)	a = 293.5, b = 125.6, c = 87.6, $\alpha = \gamma$ = 90, β = 100.9
Total no. of reflections	560840
Unique reflections	151329
Resolution range	35.6–2.2 (2.3–2.2)
$R_{\rm merge}$†	0.073 (0.382)
Completeness	98.3 (93.2)
Mean $I/\sigma(I)$	14.4 (3.5)
$V_{\rm M}$ (Å^3 Da^{-1})	2.4
Z	12
Solvent content (%)	48.1

† $R_{\rm merge} = \sum_h \sum_j |\langle I(h)\rangle - I(h)_j| / \sum_h \sum_j I(h)_j$, where $I(h)_j$ is the observed reflection intensity and $\langle I(h)\rangle$ is the mean intensity of the reflection.

2.3. Diffraction data collection

Prior to data collection, crystals were cryoprotected in a buffer consisting of 20% glycerol added to the mother liquor and were then flash-cooled in liquid nitrogen. Diffraction data from DDG aldolase were collected on a Cu $K\alpha$ rotating-anode source (Rigaku Micro007) with a MAR Research dtb345 imaging plate. The crystal-to-detector distance was set to 200 mm, with a 1° oscillation per image and an exposure time of 10 min per frame. All data were processed using *MOSFLM* v.6.2.3 (Leslie, 1992). Data statistics are given in Table 1.

3. Results and discussion

Based on size-exclusion chromatography (results not shown), *L. interrogans* DDG aldolase appears to be a hexamer as is the case for *E. coli* DDG aldolase (Izard & Blackwell, 2000). Calculation of the $V_{\rm M}$ value indicates that there could be two hexamers in the asymmetric unit, with a $V_{\rm M}$ value of 2.4 Å^3 Da^{-1}.

We are currently determining the structure of DDG aldolase using molecular replacement with *E. coli* DDG aldolase (PDB code 1dxe) as the search model. Efforts to obtain crystals of *L. interrogans* DDG aldolase in complex with various substrates (pyruvate, tartronic semialdehyde) are under way.

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