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Rhombohedral crystals of 2-dehydro-3-deoxygalactarate aldolase from *Escherichia coli*

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2-Dehydro-3-deoxygalactarate (DDG) aldolase (E.C. 4.1.2.20) catalyzes the reversible aldol cleavage of DDG and 2-dehydro-3-deoxyglucarate to pyruvate and tartronic semialdehyde. Rhombohedral crystals of recombinant DDG aldolase from *Escherichia coli* K-12 were obtained. The crystals belong to space group *R*32 with unit-cell parameters $a = 93$ Å, $\alpha = 85^\circ$. The crystals diffract to beyond 1.8 Å resolution on a Cu $K\alpha$ rotating-anode generator. The asymmetric unit is likely to contain two molecules, corresponding to a packing density of 1.34 Å^3 Da^{-1}.

1. Introduction

The aldol condensation is an important reaction in synthetic organic chemistry, and the use of aldolase enzymes for the stereochemical control of such reactions is an attractive alternative to conventional chemical methods. The potential of aldolases, particularly the more stable class II enzymes, is widely recognized in synthetic chemistry (Wong & Whitesides, 1994; Fessner, 1998) and there are numerous examples where aldolases have proven effective in biotransformations and synthetic organic chemistry, such as the synthesis of novel antibiotics (Wagner *et al.*, 1995; Barbas *et al.*, 1997).

Aldolases display an $(\alpha/\beta)_8$ barrel fold, as first observed in triosephosphate isomerase (Banner *et al.*, 1975), which is now the most frequently occurring protein fold (Brändén, 1991). Aldolases are mechanistically divided into two classes. The class I aldolases use an active-site lysine residue in Schiff-base formation with substrate, whilst the class II enzymes require a metal cofactor (Rutter, 1964). Class I enzymes are generally found in higher organisms, whereas class II enzymes are found in bacteria and other lower organisms. One such class II enzyme is 2-dehydro-3-deoxygalactarate (DDG) aldolase, which catalyzes the reversible aldol cleavage of DDG and 2-dehydro-3-deoxyglucarate to pyruvate and tartronic semialdehyde (Fish & Blumenthal, 1966). DDG aldolase requires a divalent metal ion (such as Mg^{2+}, Co^{2+} or Mn^{2+}) for catalysis. Type IIA aldolases use dihydroxyacetone phosphate (DHAP) as a nucleophilic substrate, which upon reaction with an aldehyde forms a ketose-1-phosphate sugar (Drueckhammer *et al.*, 1991). Type IIB aldolases, such as DDG aldolase, use pyruvic acid or phosphoenolpyruvate as the nucleophile to form a 3-deoxy-2-keto acid product (Drueckhammer *et al.*, 1991). A third type of aldolase (type IIC), deoxyribose-5-phosphate aldolase, uses acetaldehyde as a nucleophilic substrate to form a 2-deoxyaldose sugar (Drueckhammer *et al.*, 1991).

DDG aldolase has a considerable advantage with respect to other aldolases owing to its low substrate specificity and its ability to condense a wide range of aldehydes with pyruvic acid. Elucidation of the reaction mechanism of DDG aldolase may pave the way for development and design of selective inhibitors for therapeutic uses. Moreover, this knowledge should add to the progress in rational protein engineering towards altered substrate specificity and the generation of new catalysts for synthetic chemistry.

The recombinant DDG aldolase gene has been overexpressed, the protein purified, its DDG aldolase activity confirmed and crystallized. The polypeptide chain has a molecular weight of 27.4 kDa. A search for heavy-atom derivatives for a structure solution *via* isomorphous replacement has been initiated.

2. Methods and results

A single colony of the *Escherichia coli* strain BL21 (DE3) carrying the expression vector pT7-7 containing the DDG aldolase gene was added to 5 ml of LB medium, consisting of 10 g tryptone, 5 g yeast extract and 10 g NaCl per litre, with 0.1 mg ml^{-1} ampicillin and was incubated overnight at 310 K. This 5 ml aliquot was used to inoculate 100 ml LB medium containing 0.1 mg ml^{-1} ampicillin, which was then incubated at 303 K until an optical density at 680 nm of 0.3 was reached. Protein production was induced by adding isopropyl-1-thio-β-D-galactopyranoside to a final concentration of 0.01 m*M*. After 18 h growth at 293 K, cells were harvested in the stationary phase by

Table 1
Data-reduction statistics of DDG aldolase.

Total data	869856
Unique data	46240
Redundancy	18.8
$F^2 > 3\sigma(F^2)$ (100–1.8 Å) (%)	87.2
$F^2 > 3\sigma(F^2)$ (1.86–1.8 Å) (%)	45.9
Average $F^2/\sigma(F^2)$	23.5

Resolution range (Å)	$R_{\rm merge}$†	Completeness
100.00–3.88	0.072	0.996
3.88–3.08	0.086	1.000
3.08–2.69	0.107	1.000
2.69–2.44	0.127	1.000
2.44–2.27	0.140	1.000
2.27–2.13	0.151	1.000
2.13–2.03	0.164	0.999
2.03–1.94	0.177	0.911
1.94–1.86	0.199	0.757
1.86–1.80	0.225	0.599
100.00–1.80	0.099	0.927

† $R_{\rm merge} = \sum_{\rm unique\ reflections}(\sum_{i=1}^{N} |I_i - \overline{I}|)/\sum_{\rm unique\ reflections} \times (\sum_{i=1}^{N} I_i)$.

centrifugation at 12000*g* for 20 min. The pellet was resuspended in 4 ml 0.05 *M* potassium phosphate buffer (pH 7.15) and the cells were disrupted by sonication for 30 s at 273 K in an MSE ultrasonic oscillator operating at an amplitude of 10 µm. The sonicate was centrifuged at 15000*g* (at 273 K) for 20 min. The pellet was discarded and the supernatant centrifuged further at 120000*g* (at 277 K) for 2 h to remove cell membranes containing reduced nicotinamide adenine dinucleotide (NADH) oxidase.

DDG aldolase was assayed by monitoring the rate of pyruvate formation from DDG at 303 K in a coupled assay with NADH and lactate dehydrogenase. 1 ml of the reaction mixture contained potassium phosphate buffer pH 8 (100 µmol), $MgSO_4$ (5 µmol), NADH (0.15 µmol), crystalline lactate dehydrogenase (1 unit) and aldolase enzyme. After monitoring the A_{340} signal over a 7 min period DDG (1.12 µmol) was added and the absorbance at 340 nm measured. A control assay lacked the lactate dehydrogenase. One unit is the amount of enzyme which catalyzes the formation of 1 µmol pyruvate per minute at 303 K. The specific activity of the purified recombinant DDG aldolase was over 3×10^{-6} mol s^{-1} mg^{-1}.

Crystallization experiments were performed at room temperature using the hanging-drop vapour-diffusion technique. The purified enzyme was dialyzed into 20 m*M* potassium phosphate buffer at pH 6.7 containing 5 m*M* magnesium sulfate and then concentrated to 7 mg ml^{-1}. Crystals of native DDG aldolase were obtained from 4% polyethylene glycol (PEG) with a molecular weight of 3000 as a precipitant and 0.2 *M* potassium phosphate buffer (pH 4.5). Initial crystallization droplets of 3 µl resulted in DDG aldolase crystals of dimensions up to 0.1 mm. Increasing the drop size to 12 µl resulted in DDG aldolase crystals up to 0.2 mm in each dimension within a few days. Crystals were cryoprotected by including 35% glycerol in the mother liquor.

X-ray data were collected from a DDG aldolase crystal at 100 K using a Cu $K\alpha$ rotating-anode source with an R-AXIS IV imaging plate. The crystal-to-detector distance was set to 150 mm, with a 1° oscillation per image and an exposure time of 30 min per frame. All data were processed using the programs *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997). Data statistics are given in Table 1.

The results of autoindexing with *DENZO* (Otwinowski & Minor, 1997) are consistent with the crystals having a primitive rhombohedral lattice, with unit-cell parameters a = 93 Å, α = 85° in the rhombohedral setting (equivalent to $a = b$ = 125.6, c = 174.3 Å in the hexagonal setting). Analysis of simulated $hk0$ precession photographs showed 6*mm* symmetry, with mirror planes every 60° about c^*. This indicates the presence of twofold axes perpendicular to and related by a threefold axis. The twofold axes lie normal to the mirror plane. Thus, the crystals belong to space group $R32$. Furthermore, the $R_{\rm merge}$ ($\sum |I - \overline{I}|/\sum I$) for data reduced in space group $R32$ was not of lesser quality than for data reduced in space group $R3$.

An assumption of two protomers per asymmetric unit leads to an acceptable packing density V_m (Matthews, 1968) of 1.34 $Å^3$ Da^{-1}, corresponding to a solvent content of about 0.48. The self-rotation function, calculated with either the program *GLRF* (Tong & Rossmann, 1990) or *POLARRFN* (Collaborative Computational Project, Number 4, 1994), only showed significant peaks above the noise level in the $\kappa = 180°$ section at $\omega = 88°$ and $\varphi = 150°$, with a peak height of 30% relative to the origin. This is indication of a non-crystallographic twofold axis perpendicular to c^* lying in between the crystallographic dyads. Together with the absence of non-origin peaks in the $\kappa = 120°$ or $\kappa = 90°$ sections, this suggests that the DDG aldolase crystal structure is a hexamer with point group 32. In this case, its triad axis must be parallel to a crystallographic axis.

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