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Crystallization and preliminary crystallographic analysis of 2-keto-3-deoxygluconate kinase from *Thermus thermophilus*

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2-Keto-3-deoxygluconate kinase (KDGK) catalyzes the phosphorylation of 2-keto-3-deoxygluconate (KDG) to 2-keto-3-deoxy-6-phosphogluconate. Two crystal forms of KDGK from *Thermus thermophilus* were obtained by vapour-diffusion and microbatch methods. Crystals in the form of triangular plates (TtKDGK-1) were obtained that belong to space group $P3$, with unit-cell parameters $a = b = 145.83$, $c = 74.63$ Å, and diffract to 3.2 Å. These crystals exhibited nearly perfect hemihedral twinning. Assigning six subunits of TtKDGK to the asymmetric unit of the crystal corresponds to a 46.2% solvent content. A single plate-like crystal (TtKDGK-2) belonged to space group $P6_3$, with unit-cell parameters $a = b = 84.83$, $c = 168.49$ Å, and diffracts to 2.25 Å. This crystal exhibits only partial hemihedral twinning, with a twin fraction of 24.4%. Diffraction-quality crystals of TtKDGK with bound ATP (TtKDGK–ATP), $a = b = 84.72$, $c = 321.61$ Å and with bound KDG plus the ATP analogue AMP-PNP (TtKDGK–ATP–KDG), with unit-cell parameters $a = b = 84.32$, $c = 168.7$ Å, were also prepared and characterized.

1. Introduction

Many kinds of bacteria and archaea possess the metabolic pathway for survival in the presence of gluconate as a nutrient. In *Clostridia*, gluconate is dehydrated to form 2-keto-3-deoxyglucoate (KDG), an intermediate in the modified Entner–Doudoroff pathway (Conway, 1992), by gluconate dehydratase. KDG is then phosphorylated to form 2-keto-3-deoxy-6-phosphogluconate (KDGP) by 2-keto-3-deoxygluconate kinase (KDGK). Pyruvate and glyceraldehyde 3-phosphate are produced from KDGP by KDGP aldolase (Bender *et al.*, 1971). KDG is also an important intermediate for the degradation of extracellular pectin, which is processed and incorporated into cells and then converted to KDG intracellularly in *Erwinia chrysanthemi* (Hugouvieux-Cotte-Pattat *et al.*, 1996). Extracellular KDG is also utilized by incorporation *via* the KDG transporter in *Escherichia coli* (Mandrand-Berthelot *et al.*, 1984) and *Er. chrysanthemi* (Hugouvieux-Cotte-Pattat *et al.*, 1996). KDG is also reported to be produced from D-glucosaminate by D-glucosaminate dehydratase in *Pseudomonas fluorescens* (Iwamoto & Imanaga, 1991).

From analysis of its amino-acid sequence, KDGK has been reported to belong to the PfkB family of carbohydrate kinases (Bork *et al.*, 1993). The crystal structures of several PfkB family members, including the crystal structure of *E. coli* ribokinase (EcRK; Sigrell *et al.*, 1998), have been reported recently. In this report, we describe the crystallization and preliminary crystallographic studies of *Thermus thermophilus* KDGK (TtKDGK) in two ligand-free forms (TtKDGK-1 and TtKDGK-2), an ATP-bound form (TtKDGK–ATP) and in a form with KDG and an ATP analogue (AMP-PNP) bound (TtKDGK–ATP–KDG).

2. Materials and methods

2.1. Expression and purification

The protocols used for the expression and purification of TtKDGK were similar to those described for phosphopantetheine adenylyltransferase (Takahashi *et al.*, 2004).

2.2. Dynamic light-scattering measurements

Dynamic light-scattering measurements of the purified protein were performed using DynaPro MS/X (Protein Solutions). Protein samples (0.72 mg ml^{-1}) were prepared in solutions of 20 m*M* Tris–HCl buffer pH 8.0 containing 200 m*M* NaCl. Protein samples were filtered through 0.1 µm glass filters (Anodisc13, Whatman) before measurement. Measurements were performed at 291 K. All data were analyzed using *DYNAMICS* software (Protein Solutions).

2.3. Preparation of KDG

KDG was prepared from oxaloacetic acid and D-glyceraldehyde according to a pre-

viously described procedure (Portsmouth, 1968).

2.4. Crystallization

Crystals of TtKDGK were obtained by the microbatch method (Chayen *et al.*, 1990) using a TERA crystallization robot and a screening kit designed for high-throughput protein crystallization (Sugahara & Miyano, 2002). A 0.5 µl aliquot of the screen solution was mixed with 0.5 µl of 23 mg ml^{-1} protein solution, covered with 15 µl of a silicone and paraffin oil mixture and kept at a temperature of 291 K. One diffraction-quality plate-like crystal appeared after one month from a screen solution containing 44%(*v*/*v*) MPD, 10%(*v*/*v*) dioxane and 0.1 *M* Na HEPES buffer pH 7.7 and grew to final dimensions of 0.5 × 0.25 × 0.05 mm within a month of appearance. Numerous attempts to reproduce similar crystals by microbatch or vapour-diffusion methods were unsuccessful.

Reproducible crystallization conditions were obtained by further screening at a temperature of 298 K in 96-well plates using the sitting-drop vapour-diffusion method. The Crystal Screen I and Crystal Screen II (Hampton Research) sets of screening conditions (Jancarik & Kim, 1991; Cudney *et al.*, 1994) were used for the screening. Drops consisting of 1 µl of 10 mg ml^{-1} protein solution mixed with 1 µl reservoir solution were equilibrated against 100 µl reservoir solution. Some of the conditions produced small aggregated crystals. Based on the conditions producing these crystals, grid screens with varying precipitant concentration, salt type and concentration and pH were prepared and tested. Finally, we obtained two types of crystals. Triangular plate-shaped crystals (Fig. 1*a*) grew to dimensions of 0.1 × 0.1 × 0.01 mm in 2–4 d using reservoir solution containing 28%(*v*/*v*) MPD, 10 m*M* calcium chloride and 100 m*M* trisodium citrate buffer pH 5.6. Crystals in the form of hexagonal plates (Fig. 1*b*) grew to dimensions of 0.2 × 0.2 × 0.02 mm within a month using a reservoir solution containing 0.35–0.45 *M* ammonium sulfate and 0.1 *M* Tri–HCl buffer pH 8.5. The latter conditions were used for the protein co-crystallization with ligands, as described below. The triangular-shaped crystal and the crystal grown using the TERA crystallization robot were used for the collection of native X-ray diffraction data sets TtKDGK-1 and TtKDGK-2, respectively.

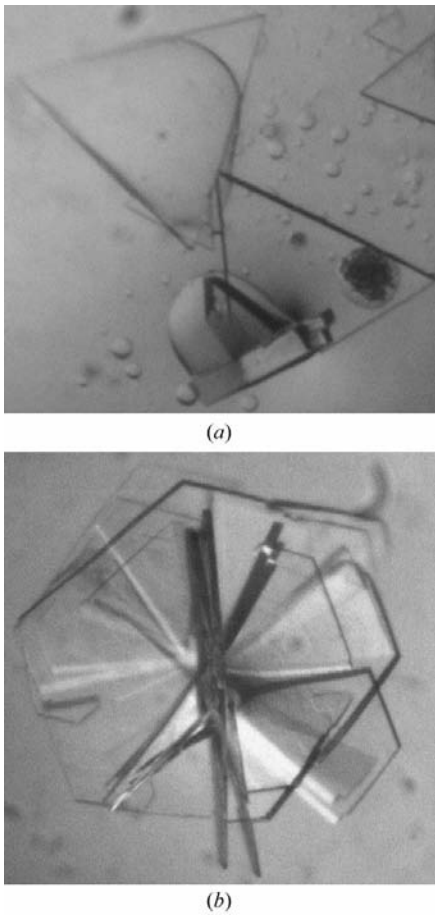

Figure 1
Photomicrographs of (*a*) triangular and (*b*) hexagonal plate-shaped crystals.

Table 1
Crystal parameters and data collection statistics.

Crystal	TtKDGK-1	TtKDGK-2	TtKDGK–ATP	TtKDGK–ATP–KDG
Unit-cell parameters				
a (Å)	145.83	84.83	84.72	84.32
c (Å)	74.63	168.49	321.61	168.7
Space group	*P*3	$P6_3$	$P6_3$	$P6_3$
Z	18	12	24	12
Solvent content (%)	46.2	53.0	50.6	52.5
Temperature (K)	100	100	100	100
Beamline (SPring-8)	BL44B2	BL44B2	BL26B1	BL26B1
Detector	MAR CCD	MAR CCD	R-AXIS V	R-AXIS V
Wavelength (Å)	1.000	1.040	1.000	1.000
Resolution (Å)	50–3.2 (3.31–3.2)	50–2.25 (2.33–2.25)	50–2.6 (2.69–2.6)	30–2.1 (2.18–2.1)
Observations	92978	296182	89307	177500
Unique reflections	27126	32439	36923	38730
Completeness (%)	92.9 (82.0)	99.5 (97.1)	91.7 (87.8)	97.9 (96.2)
R_{sym}	0.095 (0.261)	0.061 (0.400)	0.081 (0.284)	0.056 (0.355)
$I/\sigma(I)$	9.5 (2.0)	29.5 (2.9)	9.9 (1.8)	19.6 (2.7)
Twinning operator	*h*, −*h* − *k*, −*l*	*h*, −*h* − *k*, −*l*	*h*, −*h* − *k*, −*l*	*h*, −*h* − *k*, −*l*
Twinning fraction	0.5	0.244	0.5	0.5

2.5. Preparation of ligand-bound crystals

Crystals with hexagonal plate shapes also grew in the presence of ATP and magnesium chloride or KDG plus AMP-PNP and magnesium chloride, with a 5 m*M* concentration of each. In spite of co-crystallization with ligands, tight ligand binding to protein was not observed (Tahirov *et al.*, unpublished results). This could be explained by the presence of ammonium sulfate at higher concentration. In order to avoid the interference of ammonium sulfate, it was gradually replaced by magnesium chloride and crystals were kept in this solution for 1 h prior to cryoprotection. The cryoprotectant contained 33%(*v*/*v*) ethylene glycol in addition to magnesium chloride, ligands and buffer. Although it was easy to obtain hexagonal plate-shaped crystals, most of them appear to be aggregates or twins and diffracted poorly. Eventually, we succeeded in finding two single crystals, one of TtKDGK–ATP and another of TtKDGK–ATP–KDG, which could be used for collecting diffraction data sets.

2.6. Data collection

For data collection, the crystals were mounted in nylon-fibre loops and flash-cooled in a dry nitrogen stream at 100 K. Complete data sets were collected at 100 K using synchrotron radiation at SPring-8 beamlines BL44B2 and BL26B1 (Table 1). All intensity data were indexed, integrated and scaled with *DENZO* and *SCALEPACK* as implemented in the *HKL*2000 program package (Otwinowski, 1993; Otwinowski & Minor, 1997). The crystal parameters and data-processing statistics are summarized in Table 1. Examples of diffraction images are shown in Fig. 2.

3. Results and discussion

The triangular plate-shaped TtKDGK crystals (TtKDGK-1) have trigonal symmetry and diffracted to 3.2 Å, while the hexagonal plate-shaped crystals (TtKDGK-2) had hexagonal symmetry and diffracted to

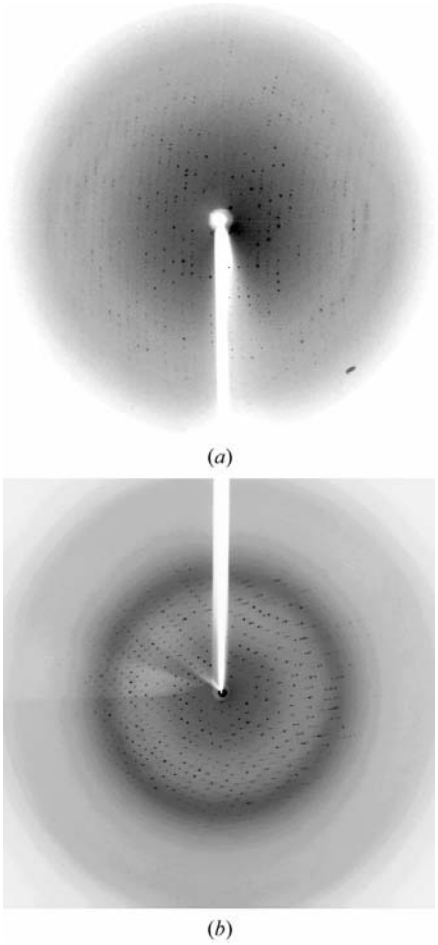

Figure 2
Examples of X-ray diffraction images obtained from the crystals of (*a*) TtKDGK-1 on a MAR CCD and (*b*) TtKDGK-ATP-KDG on an R-AXIS V detector. The resolutions at the edges of the images are 3 Å for (*a*) and 2.05 Å for (*b*).

2.25 Å (Table 1 and Fig. 2). Diffraction data processing and an inspection of the reflection conditions showed that the space group of the TtKDGK-1 crystals was most likely to be *P*321. However, calculation of the twin-fraction content (Yeates, 1997) with *CNS* (Brünger *et al.*, 1998) revealed the presence of perfect hemihedral twinning, indicating the true space group of TtKDGK-1 crystals to be *P*3. The space group of the TtKDGK-2 crystal was $P6_3$ and the crystal exhibited only partial hemihedral twinning. Solvent-content calculations (Matthews, 1968) show that six subunits of TtKDGK can be placed in the TtKDGK-1 crystal asymmetric unit, with a corresponding crystal solvent content of 46.2%. In the case of TtKDGK-2 crystals, the solvent content was 53% assuming the presence of two subunits of TtKDGK in the asymmetric unit. Dynamic light-scattering measurements show TtKDGK to be monodisperse with a molecular weight of 158 kDa. This suggests that TtKDGK subunits may form hexamers both in solution and in crystals. The structure has been solved by the molecular-replacement method using the coordinates of *E. coli* ribokinase (Sigrell *et al.*, 1998). The sequence identity between *E. coli* ribokinase and TtKDGK is 26%. The partially hemihedrally twinned reflection data was detwinned using the 'detwin_partial.inp' protocol of *CNS* and was used for density modification with starting phases calculated from the molecular-replacement model. After the rebuilding of the initial model, the structure was refined with standard protocols in *CNS* for hemihedrally twinned crystals (Brünger *et al.*, 1998). Further details of structure determination and overcoming the twinning problem during the model building and refinement will be reported elsewhere. Additionally, the prepared diffraction-quality crystals of TtKDGK–ATP and TtKDGK–ATP–KDG may reveal the mechanisms of binding and phosphorylation of KDG.

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