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Crystallization and preliminary X-ray analysis of 5-keto-D-gluconate reductase from *Gluconobacter suboxydans* IFO12528 complexed with 5-keto-D-gluconate and NADPH

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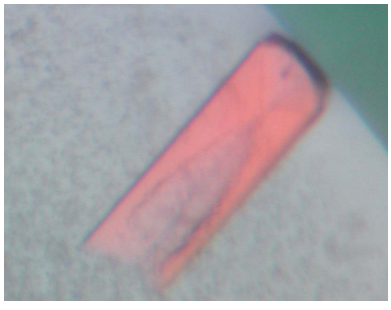

NADPH-dependent 5-keto-D-gluconate reductase from *Gluconobacter suboxydans* IFO12528 (5KGR) catalyzes oxidoreduction between 5-keto-D-gluconate and D-gluconate with high specificity. 5KGR was expressed in *Escherichia coli*, purified and crystallized with 5-keto-D-gluconate and NADPH using the sitting-drop vapour-diffusion method at 288 K. A crystal of the 5KGR–NADPH complex was obtained using reservoir solution containing PEG 4000 as a precipitant and diffracted X-rays to 1.75 Å resolution. The crystal of the complex belonged to space group $P4_22_12$, with unit-cell parameters $a = b = 128.6$, $c = 62.9$ Å. A crystal of the 5KGR–NADPH–5-keto-D-gluconate complex was prepared by soaking the 5KGR–NADPH complex crystal in reservoir solution supplemented with 100 m*M* 5-keto-D-gluconate and 10 m*M* NADPH for 20 min and diffracted X-rays to 2.26 Å resolution. The crystal of the ternary complex belonged to space group $P4_22_12$, with unit-cell parameters $a = b = 128.7$, $c = 62.5$ Å. Both crystals contained two molecules in the asymmetric unit.

1. Introduction

The genus *Gluconobacter* belongs to a group of acetic acid bacteria and is known to oxidize various sugars and sugar alcohols, leading to the accumulation of oxidized products in the culture medium. The oxidized sugars and sugar alcohols are usually very difficult for other microorganisms to utilize and are harmful to the growth of other microorganisms, whereas *Gluconobacter* can utilize the oxidized products as carbon sources. Oxidized sugars and sugar alcohols are industrially useful as starting materials in the synthesis of various pharmaceuticals and chemicals (Hölscher *et al.*, 2009). 5-Keto-D-gluconate produced by *Gluconobacter* is an important compound in the industrial production of L-(+)-tartaric acid (Matzerath *et al.*, 1995), xylaric acid (Fleche, 1998) and valuable flavouring compounds such as 4-hydroxy-5-methyl-2,3-dihydrofuranone-3 (De Rooij, 1984; Salusjärvi *et al.*, 2004). Moreover, 5-keto-D-gluconate is a probable precursor for the industrial production of vitamin C by Gray's method (Gray, 1947*a*,*b*).

5-Keto-D-gluconate reductase (EC 1.1.1.69) from *G. suboxydans* IFO12528 (5KGR) is an NADPH-dependent oxidoreductase that belongs to the short-chain dehydrogenase/reductase (SDR) family (Klasen *et al.*, 1995). 5KGR catalyzes oxidoreduction between 5-keto-D-gluconate and D-gluconate with high substrate specificity (Ameyama & Adachi, 1982). In *Gluconobacter*, 5KGR plays a critical role in the assimilation of 5-keto-D-gluconate in the cytoplasm and regulates cell growth and energy generation (Matsushita *et al.*, 1994). In addition, 5KGR is used as a quantitation tool for 5-keto-D-gluconate. To elucidate the structural basis of the catalytic mechanism and substrate specificity of 5KGR, we report the expression, purification, crystallization and preliminary X-ray analysis of 5KGR.

2. Materials and results

2.1. Overexpression and purification

The 5KGR-coding gene from *G. suboxydans* IFO12528 (GenBank code GOX2187; Prust *et al.*, 2005) was cloned into the *Nco*I/*Bam*HI

site of pET-28a(+) (Saichana *et al.*, 2007). The recombinant plasmid was transformed into *Escherichia coli* Rosetta (DE3) cells (Novagen). 5KGR expression was induced by the addition of 1 m*M* (final concentration) isopropyl *β*-D-1-thiogalactopyranoside (IPTG) when the optical density of the medium at 600 nm reached 1.0. The cells were cultivated for a further 5 h at 303 K to accumulate the target protein. The harvested cells were resuspended in 10 m*M* MES buffer pH 6.0 containing 1 m*M* EDTA and 1 m*M* dithiothreitol (DTT; Wako) and then disrupted by sonication. After centrifugation at 40 000*g* for 30 min, the supernatant was applied onto a DEAE-cellulose (Sigma) column pre-equilibrated with the same buffer. 5KGR was eluted with a linear gradient of 0–0.3 *M* NaCl. The fractions containing 5KGR were pooled and dialyzed against 10 m*M* MES buffer pH 6.0 containing 1 m*M* tris(2-carboxyethyl)phosphine hydrochloride (TCEP; Pierce Chemical). All purification procedures were performed at 277 K. The purified 5KGR was concentrated to 9 mg ml^{-1} for crystallization.

2.2. Crystallization

For cocrystallization of 5KGR with its cofactor NADPH, NADPH was added to the protein solution to a final concentration of 5 m*M*. Initial crystallization trials of the 5KGR–NADPH complex were performed by the sitting-drop vapour-diffusion method using the sparse-matrix screening kits Crystal Screen HT (Hampton Research) and Wizard I and II (Emerald BioSystems) at 293 K. After the crystallization conditions had been refined, the best crystal of the 5KGR–NADPH complex (Fig. 1) was obtained by mixing 1.2 µl 5KGR–NADPH solution with 1.0 µl reservoir solution consisting of 27%(*w*/*v*) PEG 4000, 180 m*M* ammonium sulfate and 100 m*M* sodium acetate trihydrate pH 4.6 at 288 K. To obtain a crystal of the 5KGR–NADPH–5-keto-D-gluconate complex, crystals of 5KGR–NADPH were soaked in reservoir solution supplemented with 100 m*M* 5-keto-D-gluconate and 10 m*M* NADPH for 20 min.

2.3. Data collection and processing

The 5KGR crystals were flash-cooled to 95 K in a nitrogen stream for data collection. For cryoprotection, each crystal was transferred to reservoir solution containing 20%(*v*/*v*) ethylene glycol for a few seconds. X-ray diffraction data sets for the 5KGR–NADPH complex and the 5KGR–NADPH–5-keto-D-gluconate complex were collected on beamline BL5A at Photon Factory (Tsukuba, Japan) using an ADSC Quantum 315 CCD detector. The best crystals of the 5KGR–NADPH and the 5KGR–NADPH–5-keto-D-gluconate complexes diffracted X-rays to 1.75 and 2.26 Å resolution, respectively (Fig. 2). The diffraction data were indexed, integrated and scaled with *XDS* (Kabsch, 2010). The crystal of the 5KGR–NADPH complex was found to belong to the primitive tetragonal space group $P4_22_12$, with unit-cell parameters $a = b = 128.6$, $c = 62.9$ Å. The crystal of the 5KGR–NADPH–5-keto-D-gluconate complex was found to belong to the primitive tetragonal space group $P4_22_12$, with unit-cell parameters $a = b = 128.7$, $c = 62.5$ Å. According to Matthews coefficient calcu-

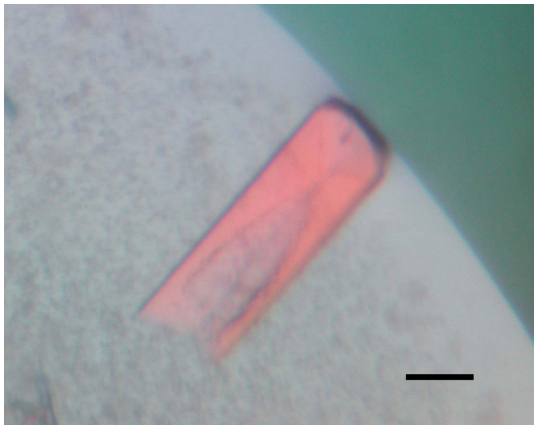

Figure 1
A crystal of 5KGR complexed with NADPH. The scale bar is 100 µm in length.

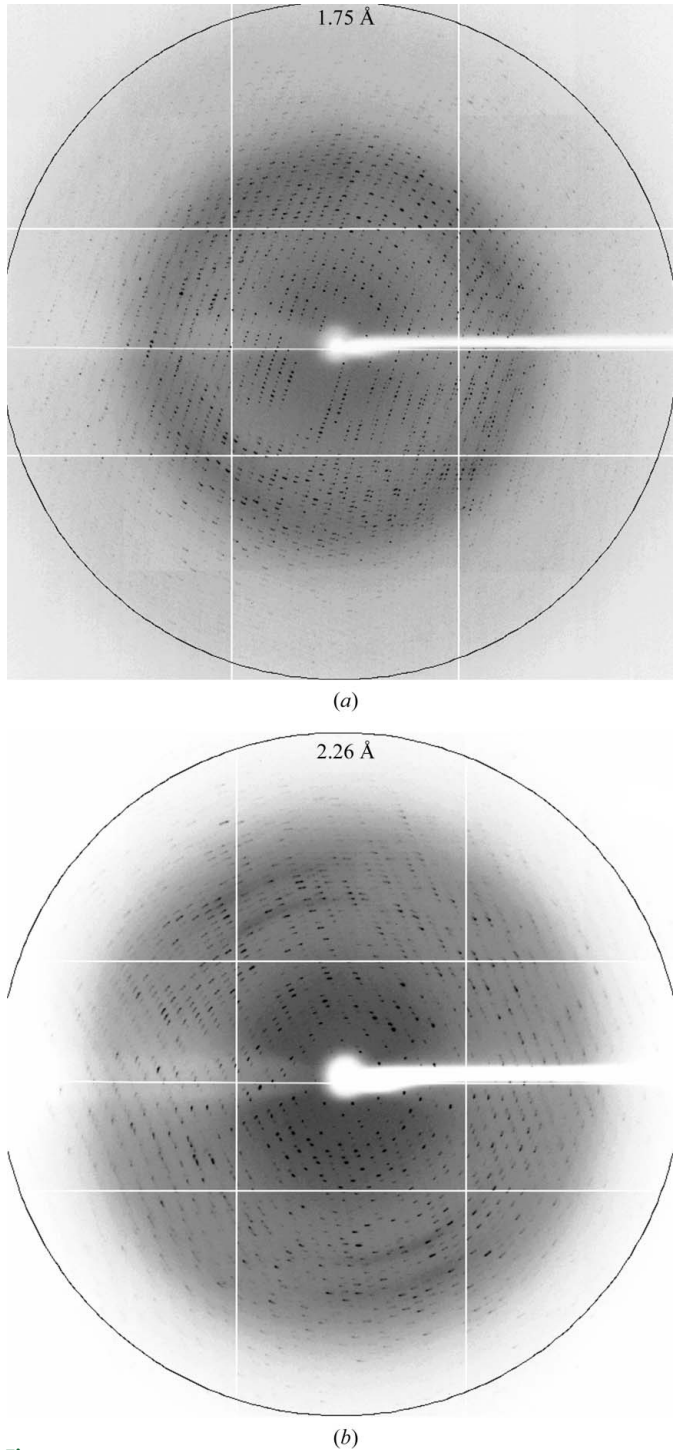

Figure 2
X-ray diffraction images of (*a*) the 5KGR–NADPH complex crystal and (*b*) the 5KGR–NADPH–5-keto-D-gluconate complex crystal. The circles display resolutions of (*a*) 1.75 Å and (*b*) 2.26 Å.

Table 1
Summary of data-collection statistics.

Values in parentheses are for the highest resolution shell.

	5KGR–NADPH	5KGR–NADPH–5-keto-D-gluconate
Beamline	PF BL5A	
Wavelength (Å)	1.00000	
Space group	$P4_22_12$	$P4_22_12$
Unit-cell parameters (Å)	$a = b = 128.6$, $c = 62.9$	$a = b = 128.7$, $c = 62.5$
Resolution (Å)	50.0–1.75 (1.80–1.75)	20.0–2.26 (2.32–2.26)
No. of measurements	733651	319034
No. of unique reflections	53545	24944
Completeness (%)	99.9 (100.0)	99.0 (90.0)
$R_{\rm merge}$†	0.070 (0.338)	0.080 (0.342)
$\langle I/\sigma(I)\rangle$	26.0 (6.5)	25.0 (2.9)

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ is the ith intensity measurement of reflection hkl, including symmetry-related reflections, and $\langle I(hkl)\rangle$ is its average.

lations (Matthews, 1968), both of the crystals contained two 5KGR molecules in the asymmetric unit, with solvent contents of 48.5 and 48.3%, respectively. Data-collection statistics for the crystals are provided in Table 1. Structure determination by the molecular-replacement method is under way using *MOLREP* (Vagin & Teplyakov, 2010), with the coordinates of gluconate 5-dehydrogenase (TM0441) from *Thermotoga maritima* (PDB code 1vl8; 39% sequence identity; Joint Center for Structural Genomics, unpublished work) as a search model.

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