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Purification, crystallization and preliminary X-ray analysis of glucokinase from *Streptomyces griseus* in complex with glucose

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Glucokinase catalyzes the phosphorylation of glucose using ATP to yield glucose 6-phosphate. SgGlkA is a bacterial group III glucokinase from *Streptomyces griseus* that seems to play a regulatory role in carbon catabolite repression in this organism. SgGlkA was expressed in *Escherichia coli*, purified and crystallized using the sitting-drop vapour-diffusion method at 293 K. A crystal of SgGlkA in complex with glucose was obtained using a reservoir solution consisting of 0.9 *M* sodium/potassium tartrate, 0.2 *M* NaCl and 0.1 *M* imidazole pH 8.1 and diffracted X-rays to 1.84 Å resolution. The crystal of SgGlkA in complex with glucose belonged to space group $P6_222$ or $P6_422$, with unit-cell parameters $a = b = 109.19$, $c = 141.18$ Å. The crystal contained one molecule in the asymmetric unit.

1. Introduction

The genus *Streptomyces* is characterized by its complex morphological differentiation and by its ability to produce a wide variety of secondary metabolites such as antibiotics, immunosuppressants, insecticides and antitumour agents (Demain, 2000; Claessen *et al.*, 2006). Because of the commercial importance of these compounds, there is great interest in *Streptomyces* as an industrial microorganism. The production of secondary metabolites in *Streptomyces* is frequently affected by carbon catabolite repression (CCR; Görke & Stülke, 2008; Sánchez *et al.*, 2010). CCR is a widespread phenomenon in many bacteria; it can be defined primarily as the repression of enzyme activities for the catabolism of unfavourable carbon sources by the presence of a preferable catabolite in the growth medium. Although CCR mechanisms in Gram-negative bacteria and low-G+C-content Gram-positive bacteria have been well characterized, the CCR mechanism in high-G+C-content Gram-positive bacteria, including *Streptomyces*, is little understood. To date, it is known that CCR in *Streptomyces* is independent of the phosphotransferase (PTS) system and that glucokinase may be a central player in CCR (Angell *et al.*, 1992, 1994; Kwakman & Postma, 1994).

Glucokinase (EC 2.7.1.2) catalyzes the phosphorylation of glucose to yield the metabolic intermediate glucose 6-phosphate using ATP. Microbial glucokinases have been classified into three groups (groups I–III; Lunin *et al.*, 2004). Group I consists of ADP-dependent glucokinases from archaea (PFAM accession No. PF04587; Ronimus & Morgan, 2004; Sakuraba *et al.*, 2002). Group II glucokinases are ATP-dependent glucokinases that do not contain the ROK (repressors, open reading frames and kinases) sequence motif (PFAM accession No. PF02685), including the well characterized *Escherichia coli* glucokinase (Meyer *et al.*, 1997; Lunin *et al.*, 2004). Group III consists of glucokinases belonging primarily to Gram-positive bacteria and archaea, and contains the ROK sequence motif with a widely conserved cysteine-rich motif: **C***X***C**G*XX*G**C***X*E (conserved cysteine residues are shown in bold; PFAM accession No. PF00480; Mesak *et al.*, 2004; Titgemeyer *et al.*, 1994). No structures have been determined for this group of glucokinases. Although the cysteine residues in the conserved motif are indispensable for the enzymatic activity of

group III glucokinases (Mesak *et al.*, 2004), it remains unclear why these cysteine residues are important in the catalytic mechanism of group III glucokinases.

Glucokinase from *S. griseus* (SgGlkA) belongs to group III. To elucidate the structural basis of the catalytic mechanism underlying group III glucokinases in bacteria, especially the importance of the cysteine-rich motif in their catalysis, and to shed light on the molecular function of SgGlkA as a putative regulator in CCR, we purified and crystallized SgGlkA in order to determine its structure by X-ray crystallography.

2. Materials and methods

2.1. Overexpression, purification and crystallization

Recombinant SgGlkA with a C-terminal 6×His tag (SgGlkA-EHHHHHH) was overexpressed in *E. coli* using a T7 RNA polymerase-based system. The SgGlkA gene was obtained by PCR from genomic DNA of *S. griseus* and was cloned into the *Nde*I/*Xho*I site of the pET-26b plasmid (Novagen). SgGlkA was overexpressed in *E. coli* Rosetta (DE3) (Novagen) harbouring the constructed plasmid. SgGlkA 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 0.6. The cells were harvested after overnight culture at 298 K. The harvested cells were resuspended in 50 m*M* Tris–HCl pH 8.0, 300 m*M* NaCl, 10 m*M* imidazole and were disrupted by sonication. After centrifugation at 40 000*g* for 30 min, the supernatant was applied onto an Ni–NTA agarose (Qiagen) column. The bound protein was eluted with a buffer solution consisting of 50 m*M* Tris–HCl pH 8.0, 300 m*M* NaCl and 250 m*M* imidazole. SgGlkA was further purified using a Resource Q (GE Healthcare) 6 ml column pre-equilibrated with 20 m*M* Tris–HCl pH 8.0 and was eluted with a linear gradient of 0–1 *M* NaCl. The purified protein was dialyzed against 10 m*M* Tris–HCl pH 8.0, 1 m*M* $MgCl_2$ and 10 m*M* D-glucose and concentrated to 13.3 mg ml^{-1} for crystallization. All purification experiments were performed at 277 K.

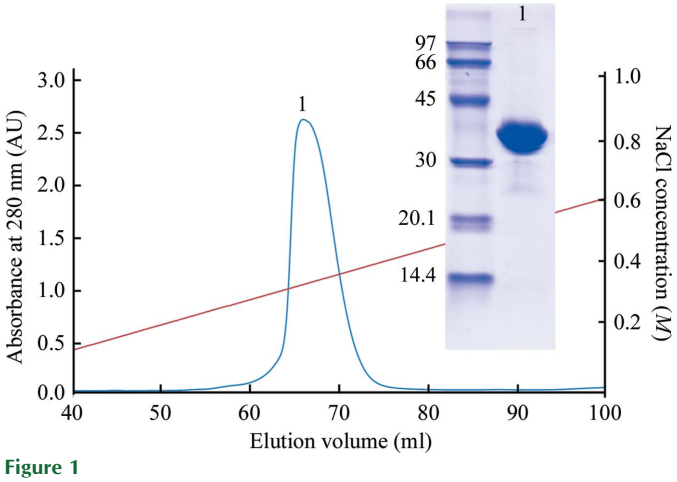

Figure 1
Purification of SgGlkA by anion-exchange chromatography. Peak 1 represents SgGlkA, which eluted at an NaCl concentration of 0.32 *M*. Inset: SDS–PAGE gel (15%) for peak 1; molecular-weight markers are labelled in kDa. The approximate molecular weight of SgGlkA is 33 kDa.

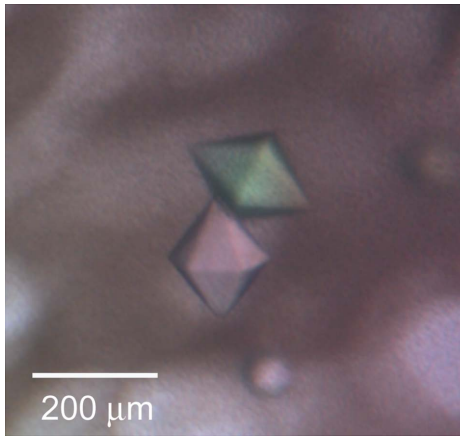

Figure 2
Crystals of SgGlkA in complex with glucose.

Table 1
Summary of data-collection statistics.

Values in parentheses are for the highest resolution shell.

Beamline	PF BL-17A
Wavelength (Å)	1.0000
Space group	$P6_222$ or $P6_422$
Unit-cell parameters	
$a = b$ (Å)	108.19
c (Å)	141.18
Resolution range (Å)	20.0–1.84 (1.89–1.84)
Multiplicity	9.7 (5.3)
Completeness (%)	99.8 (98.9)
R_{merge}† (%)	4.1 (33.0)
$\langle I/\sigma(I)\rangle$	32.89 (4.31)

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

2.2. Data collection and processing

The crystals were mounted on cryoloops and flash-cooled at 95 K in a nitrogen stream for data collection. For cryoprotection, the crystals of SgGlkA in complex with glucose were soaked in reservoir solution supplemented with 20%(*v*/*v*) ethylene glycol for a few seconds. An X-ray diffraction data set was collected on the BL-17A beamline at the Photon Factory (PF; Tsukuba, Japan) using an ADSC Quantum 270 CCD detector and an X-ray wavelength of 1.0000 Å. The data set consisted of 180 frames with an oscillation range of 0.5°. The diffraction data were indexed, integrated and scaled with *XDS* (Kabsch, 2010). A self-rotation function was calculated using the program *POLARRFN* from the *CCP*4 suite (Winn *et al.*, 2011).

3. Results and discussion

Recombinant SgGlkA was expressed in *E. coli* and purified by two column-chromatography steps. SDS–PAGE analysis revealed that the purity of SgGlkA was greater than 95% (Fig. 1). The best crystal of SgGlkA in complex with glucose was obtained using a reservoir

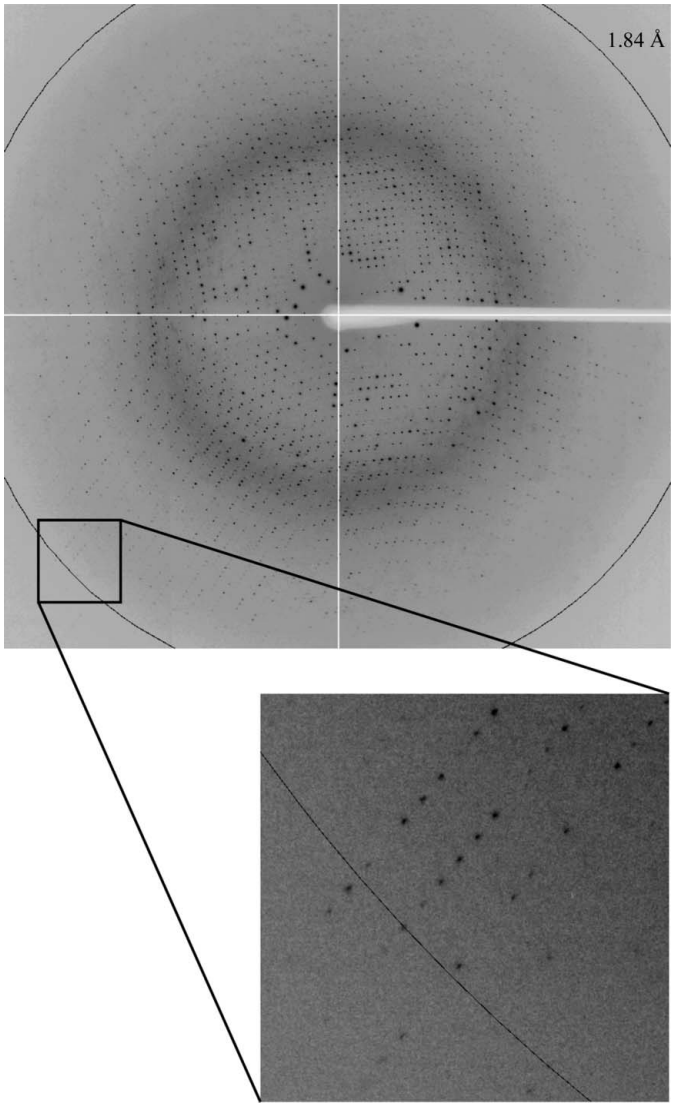

Figure 3
X-ray diffraction image of SgGlkA in complex with glucose. The circle indicates a resolution of 1.84 Å.

solution consisting of 0.9 *M* sodium/potassium tartrate, 0.2 *M* NaCl, 0.1 *M* imidazole pH 8.1. Fig. 2 shows a typical crystal (0.10 × 0.10 × 0.07 mm). The best crystal diffracted X-rays to 1.84 Å resolution (Fig. 3). The space group of the crystal was determined to be $P6_222$ or $P6_422$, with unit-cell parameters $a = b = 108.19$, $c = 141.18$ Å. Statistics of the data collection are summarized in Table 1. Analysis of the self-rotation function did not reveal any significant peaks corresponding to noncrystallographic symmetry. Assuming the presence of one SgGlkA molecule per asymmetric unit, the Matthews coefficient of the crystal was 3.69 Å^3 Da^{-1} (Matthews, 1968). These results show that the SgGlkA crystal contains one SgGlkA molecule in the asymmetric unit. Structure determination by the molecular-replacement method using the coordinates of a putative *N*-acetylmannosamine kinase (PDB code 2aa4; 35% sequence identity to SgGlkA; Y. Patskovsky & S. C. Almo, unpublished work) as a template model is currently under way.

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