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Crystallization and preliminary X-ray crystallographic analysis of BxlE, a xylobiose transporter from *Streptomyces thermoviolaceus* OPC-520

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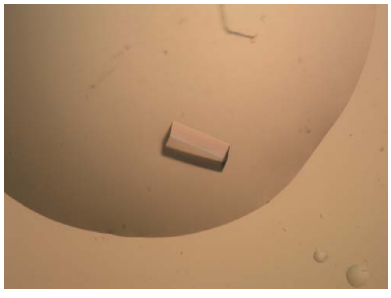
Together with the integral membrane proteins BxlF and BxlG, BxlE isolated from *Streptomyces thermoviolaceus* OPC-520 forms an ATP-binding cassette (ABC) transport system that mediates the uptake of xylan. To clarify the structural basis of sugar binding by BxlE at the atomic level, recombinant BxlE was crystallized using the hanging-drop vapour-diffusion method at 290 K. The crystals belonged to the monoclinic space group $P2_1$, with unit-cell parameters $a = 44.63$, $b = 63.27$, $c = 66.40$ Å, $\beta = 103.05^\circ$, and contained one 48 kDa molecule per asymmetric unit (V_M = 1.96 Å^3 Da^{-1}). Diffraction data collected to a resolution of 1.65 Å using a rotating-anode X-ray source gave a data set with an overall R_{merge} of 2.6% and a completeness of 91.3%. A data set from a platinum derivative is being used for phasing by the SAD method.

1. Introduction

Streptomyces bacteria are Gram-positive and produce a number of secondary metabolites and extracellular proteins, including enzymes that hydrolyze various types of polysaccharides such as xylan, cellulose and chitin (McCarthy & Williams, 1992). *S. thermoviolaceus* OPC-520 secretes two types of xylanases (StxI and StxII), an acetyl xylan esterase (StxIII) and an α-L-arabinofuranosidase (StxIV) in the presence of xylan (Tsujibo *et al.*, 1997, 2002). The xylan-degradation products (mainly xylobiose) produced by the action of these enzymes enter the cell and are then degraded to xylose by an intracellular β-xylosidase (BxlA; Tsujibo *et al.*, 2001). A variety of enzymes that can degrade xylans have been identified and the corresponding genes have been cloned from saprophytic prokaryotes (Gilbert *et al.*, 1988; Irwin *et al.*, 1994; Luthi *et al.*, 1990; Shareck *et al.*, 1991) and eukaryotes (Gielkens *et al.*, 1997; Margolles-Clark *et al.*, 1997; van Peji *et al.*, 1997). However, little is known about the uptake system for xylan-degradation products and the molecular mechanisms of gene regulation in *Streptomyces* species.

Genetic analysis of the xylanolytic system of *S. thermoviolaceus* OPC-520 revealed a gene cluster consisting of four different open reading frames organized in the order *bxlE*, *bxlF*, *bxlG* and *bxlA* which is transcribed as a polycistronic mRNA (Tsujibo *et al.*, 2004). The soluble BxlE, the gene product of *bxlE*, shows a high affinity for xylobiose ($K_d = 8.75 \times 10^{-9}$ *M*) and xylotriose ($K_d = 8.42 \times 10^{-8}$ *M*); BxlF and BxlG, the gene products of *bxlF* and *bxlG*, were predicted to be integral membrane proteins (Tsujibo *et al.*, 2004). These proteins showed similarity to components of the bacterial ATP-binding cassette (ABC) transport system, although the gene for the ATP-binding protein was not linked to the *bxl* operon.

On the basis of the above insights, the major role of BxlE in the ABC transporter system is now believed to be catching xylooligosaccharides such as xylobiose and transferring them into the cell by virtue of BxlF and BxlG. However, the mechanism of the xylobiose–xylotriose import system of *S. thermoviolaceus* OPC-520 and other Gram-positive bacteria is poorly understood at present. In order to reveal the structural scaffold of BxlE and the mechanism of transport of xylooligosaccharides at the atomic level, we initiated X-ray structural analysis of this protein.



2. Materials and methods

2.1. Expression and purification

The expression plasmid pGstBxlE coding for BxlE was constructed as follows. Two oligonucleotide primers (FwGstBxlE, 5′-CGGCTC-G**GGATCC**ACGATCAC-3′; RvGstBxlE, 5′-GCGGTGGTG**CTCG-AG**GTCACTT-3′) were synthesized with *Bam*HI and *Xho*I restriction sites (bold) to facilitate cloning in frame into the glutathione *S*-transferase (GST) fusion protein expression vector pGEX-6P-1. PCR was performed with the plasmid pThiHis-BxlE (Tsujibo *et al.*, 2004) as template for 30 cycles (367 K for 15 s, 330 K for 30 s and 341 K for 30 s). The amplified DNA was digested by *Bam*HI and *Xho*I and the resulting fragment (1194 bp) was inserted into the corresponding sites of pGEX-6P-1. *Escherichia coli* BL21(DE3) cells harbouring pGstBxlE were induced with 0.1 m*M* isopropyl *β*-D-thiogalactopyranoside (IPTG) at the mid-exponential growth phase (OD_{600} = 0.5) and incubated for a further 1.5 h at 310 K. Cells were harvested by centrifugation, washed and resuspended in 50 m*M* Tris–HCl buffer pH 7.0. The cells were disrupted by sonication and the lysate was centrifuged at 10 000*g* for 10 min. The supernatant fraction was applied onto a column packed with Q-Sepharose Fast Flow (GE Healthcare) and the GST-fusion protein (GSTBxlE) was eluted with a linear gradient of NaCl from 0 to 0.5 *M* in 50 m*M* HEPES–NaOH pH 7.5. Fractions containing GSTBxlE were loaded onto a glutathione-Sepharose 4B column equilibrated with 50 m*M* Tris–HCl pH 7.0 containing 150 m*M* NaCl. After washing the column with the same buffer, PreScission protease dissolved in cleavage buffer (50 m*M* Tris–HCl pH 7.0, 150 m*M* NaCl, 1 m*M* EDTA and 0.01% Triton X-100) was loaded onto the column to remove the GST tag. BxlE was eluted from the column with cleavage buffer and dialysed overnight into 10 m*M* Tris–HCl pH 7.0. The sample was concentrated to approximately 10 mg ml^{-1} using Centricon centrifugal concentrators (Amicon). The purity of the final preparation was estimated to be greater than 95% by SDS–PAGE. The size distribution of protein molecule in solution was shown to be monodisperse by dynamic light-scattering measurements (DynaPro).

2.2. Crystallization

Initial crystallization screening took place using the hanging-drop vapour-diffusion method at 290 K and crystallization kits from Hampton Research. Crystallization drops consisting of 2 µl protein solution and 2 µl reservoir solution were equilibrated against 0.5 ml reservoir solution. Thin plate-shaped crystals were obtained in condition No. 40 [25%(*w*/*v*) PEG 3350 and 0.1 *M* citric acid pH 3.5] of the Index screen. Subsequent optimization screening yielded diffraction-quality crystals using 24–27%(*w*/*v*) PEG 3350 and 0.1 *M* citrate buffer pH 3.2. Crystals grew within a week to maximum dimensions of approximately 0.5 × 0.3 × 0.1 mm (Fig. 1). Platinum derivatives were prepared by adding 1 µl 10 m*M* K_2PtCl_4 to the reservoir solution of a crystallization drop. Crystals were soaked for 1 d at 290 K.

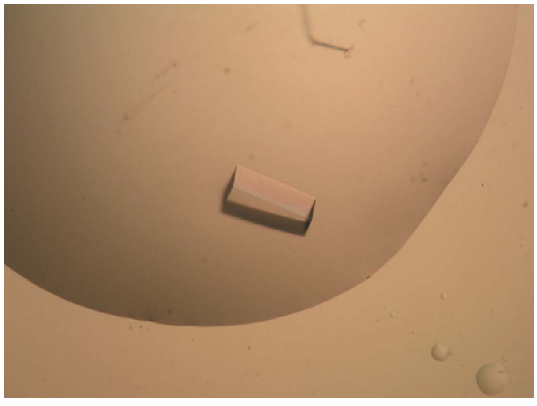


Figure 1
Native crystal of recombinant BxlE. The crystal has approximate dimensions of 0.5 × 0.3 × 0.1 mm.

2.3. Data collection and processing

A BxlE crystal was transferred to a cryoprotectant solution consisting of reservoir solution containing 30% glycerol in three steps (10%, 20% and 30% glycerol) prior to flash-cooling. Native data were collected at 100 K using Cu *Kα* radiation from an FR-E rotating-anode X-ray generator (Rigaku Corp.) equipped with CMF optics (Osmic Inc.) and an R-AXIS VII detector (the crystal-to-detector distance was 135 mm). 360° of data were collected as 720 diffraction images using a 0.5° oscillation angle and an exposure time of 60 s (Fig. 2). Pt-derivative data were collected using Cr *Kα* radiation under the same conditions in order to take advantage of the enhanced anomalous signal from Pt atoms at the longer wavelength. All data were processed using the program *CrystalClear* (Rigaku MSC; Pflugrath, 1999).

3. Results and discussion

Recombinant BxlE protein was crystallized by the vapour-diffusion method, yielding monoclinic crystals belonging to space group $P2_1$ with unit-cell parameters a = 44.63, b = 63.27, c = 66.40 Å, β = 103.05°. The asymmetric unit provides space for only a single monomer, giving a V_M value of 1.96 Å^3 Da^{-1} and a solvent content of about 37% (Matthews, 1968). These values are within the range usually observed in protein crystals.

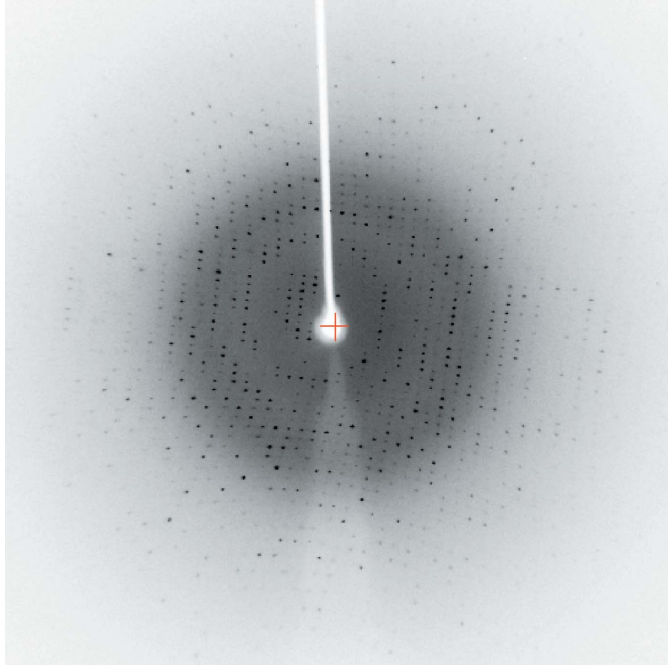


Figure 2
0.5° oscillation image of a native BxlE crystal.

Table 1
Statistics for the native and Pt-derivative BxlE diffraction data sets.

Values in parentheses are for the highest resolution shell.

Data set	Native	Pt derivative
Space group	$P2_1$	$P2_1$
Unit-cell parameters		
a (Å)	44.63	44.06
b (Å)	63.27	61.38
c (Å)	66.40	67.22
β (°)	103.05	103.43
Resolution range (Å)	34.16–1.65 (1.71–1.65)	44.75–2.70 (2.80–2.70)
No. of unique reflections	39659 (3690)	9512 (971)
Average redundancy	3.59 (3.58)	7.28 (7.05)
Completeness (%)	91.3 (88.4)	97.9 (95.0)
$R_{\rm merge}$†	0.026 (0.201)	0.060 (0.087)
Average $I/\sigma(I)$	20.8 (4.0)	23.5 (9.0)

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i - I_m| / \sum_{hkl}\sum_i I_i$, where I_i and I_m are the observed intensity and mean intensity of related reflections, respectively.

A high-quality diffraction data set was obtained, containing 39 659 unique reflections to 1.65 Å resolution with 91.3% completeness and an $R_{\rm merge}$ of 2.6% (Table 1).

Initial phases were determined by the SAD method using the anomalous signal from Pt atoms and Cr $K\alpha$ radiation. Prominent Pt–Pt self-vectors were observed in an anomalous difference Patterson map on the Harker section at $y = 1/2$. Using $|F_{\rm o}(+) - F_{\rm o}(-)|$ amplitudes, *SHELXS* (Sheldrick, 1997) located three platinum sites and initial phases were calculated by the *CCP*4 program *OASIS* (Collaborative Computational Project, Number 4, 1994; Hao *et al.*, 2000) using these platinum positions as the substructure. Following solvent flattening by *DM* (Cowtan, 1994), *RESOLVE* (Terwilliger, 2003) was able to build 50% of the entire structure. Further model building and refinement against the high-resolution native data set is currently in progress and is expected to reveal the oligosaccharide-binding mechanism of BxlE at the atomic level.

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