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Crystallization and preliminary X-ray diffraction analysis of the TetR-family transcriptional repressor YhgD from *Bacillus halodurans*

YhgD is a member of the TetR-family transcription factors, which regulate genes encoding proteins involved in multidrug resistance, virulence, osmotic stress and pathogenicity. YhgD from the alkaliphilic bacterium *Bacillus halodurans* was cloned and overexpressed in *Escherichia coli*. YhgD (Bh2145) from *B. halodurans* is composed of 193 amino-acid residues with a molecular mass of 21 853 Da. YhgD was crystallized at 296 K using ethylene glycol as a precipitant by the sitting-drop vapour-diffusion method. The crystal diffracted to 1.9 Å resolution and belonged to the apparent triclinic space group *P*1, with unit-cell parameters $a = 37.22$, $b = 47.85$, $c = 54.15$ Å, $\alpha = 92.75$, $\beta = 107.9$, $\gamma = 90.27^\circ$. The asymmetric unit is likely to contain two molecules of monomeric YhgD, giving a crystal volume per mass (V_M) of $2.05 \text{ Å}^3 \text{ Da}^{-1}$ and a solvent content of 40.2%.

1. Introduction

A living organism must adapt rapidly to environmental changes for its survival. In many cases, the adaptation of an organism is mediated by gene expression and/or transcriptional regulation. The TetR family of transcriptional regulators control genes encoding proteins involved in multidrug resistance, virulence, osmotic stress and pathogenicity of Gram-negative and Gram-positive bacteria (Ramos *et al.*, 2005). The TetR protein regulates the expression of the *tet* genes, the products of which possess resistance to tetracycline (Hinrichs *et al.*, 1994). Most members of the TetR family have two domains with a highly homologous N-terminal domain of ~45 residues, a DNA-binding helix–turn–helix (HTH) motif that transduces the signals and a signal-receiving effector domain (Schumacher *et al.*, 2002).

Sequence similarity suggests that YhgD (Bh2145) from *Bacillus halodurans* is a member of the TetR-family transcription factors (Pfam PF00440). The N-terminal domain (residues 9–55) of the *B. halodurans* YhgD protein shows significant sequence similarity to the N-terminal HTH domain of the TetR family of transcriptional regulators. These include *Escherichia coli* TetR (Swiss-Prot ID P04483; 26% sequence identity for residues 9–55), *Staphylococcus aureus* QacR (P0A0N4; 36% sequence identity for residues 7–53), *Vibrio cholerae* HapR (Q1WDD2; 30% sequence identity for residues 22–68), *Vibrio vulnificus* SmcR (Q9L8G8; 30% sequence identity for residues 21–67) and *E. coli* RutR (P0ACU2; 45% sequence identity for residues 23–69). Several structures of TetR-family proteins have been determined, including those of *E. coli* TetR, *S. aureus* QacR, *V. cholerae* HapR, *Mycobacterium tuberculosis* EthR, *V. vulnificus* SmcR and *E. coli* RutR (Hinrichs *et al.*, 1994; Schumacher *et al.*, 2001; De Silva *et al.*, 2007; Dover *et al.*, 2004; Kim *et al.*, 2010). The structures of TetR-family proteins from different species have revealed that no sequence conservation exists outside the HTH domain, although the three-dimensional structures of TetR-family proteins are almost identical.

B. halodurans is an alkaliphilic bacterium that grows well above pH 9.5 and its genome has been completely sequenced (Takami *et al.*, 2000). The *yhgd* gene (Bh2145) from *B. halodurans* encodes a protein of 193 amino-acid residues with 35% sequence identity to *B. subtilis* YhgD and 27% sequence identity to *E. coli* RutR based on the BLAST program. *E. coli* RutR, which is encoded by the *ycdC*

gene, regulates genes associated with pyrimidine and purine catabolism, the synthesis of glutamine and the transport of glutamate (Shimada *et al.*, 2007, 2008; Nguyen Ple *et al.*, 2010). The crystal structure of *E. coli* RutR revealed that it is a homodimer, and one uracil molecule was incorporated at the C-terminal domain in each subunit. Because functional or structural studies of YhgD have not been reported, it would be interesting to determine the structure of YhgD from *B. halodurans*. As a first step, we report here the cloning, purification, crystallization and preliminary X-ray diffraction data of *B. halodurans* YhgD.

2. Materials and methods

2.1. Expression and purification

The gene encoding YhgD (Bh2145) was amplified by the polymerase chain reaction using the genomic DNA of *B. halodurans* as template. It was inserted into the *NdeI/XhoI*-digested expression vector pET-28b(+) (Novagen, Germany) containing a hexahistidine tag at its N-terminus. The recombinant *B. halodurans* YhgD protein was expressed in *E. coli* BL21(DE3) Star pLysS cells (Invitrogen, USA). The cells were grown at 310 K to an OD_{600} of ~ 0.5 in Luria–Bertani medium supplemented with $30\ \mu\text{g ml}^{-1}$ kanamycin and chloramphenicol. Protein expression was induced by 1.0 mM isopropyl β -D-1-thiogalactopyranoside (IPTG). Cell growth continued at 303 K for 4 h after IPTG induction and the cells were harvested by centrifugation at 7000g for 10 min at 277 K.

The cell pellet was resuspended in lysis buffer [20 mM Tris–HCl pH 8.0, 0.5 M NaCl, 10% (v/v) glycerol, 1 mM phenylmethylsulfonyl fluoride] and homogenized with an ultrasonic processor. The insoluble fraction including cellular debris was removed by centrifugation at 40 000g for 60 min at 277 K and the recombinant protein in the supernatant fraction was purified using three chromatographic steps at 277 K. The first step was metal-chelate chromatography on Ni–NTA resin (GE Healthcare, UK). The His-tagged YhgD protein was eluted with buffer A [20 mM Tris–HCl pH 8.0, 0.5 M NaCl, 10% (v/v) glycerol] containing 300 mM imidazole followed by enzymatic removal of the His-tag by overnight incubation with homemade PreScission protease. The PreScission protease recognizes the sequence Leu–Phe–Gln/Gly–Pro. Three additional residues (Gly–Pro–His) remained at the N-terminus. The uncleaved His-tagged protein and PreScission protease were removed from the target YhgD by application onto an Ni–NTA affinity column. The next step

was gel filtration on a Superdex 200 column (GE Healthcare, UK) employing an elution buffer consisting of 0.2 M NaCl, 20 mM Tris–HCl pH 8.0, 1 mM DTT, 5 mM MgCl_2 , 5% (v/v) glycerol. The purified protein was concentrated to $30\ \text{mg ml}^{-1}$ using Centricon YM-10 (Millipore) and aliquots of the protein were stored at 193 K.

2.2. Crystallization

Crystallization conditions for YhgD were screened with a Mosquito Crystallization Robot (TTP LabTech, UK) using various commercial screening solution kits (Hampton Research, USA; Qiagen, Germany; Axygen, USA; Emerald Biosystems, USA). Crystals were obtained by the sitting-drop vapour-diffusion method using 96-well Crystal Quick plates (Greiner Bio-One, Germany); each crystallization drop was prepared by mixing 0.2 μl reservoir solution and 0.2 μl protein solution. Crystals were initially obtained in 25% (v/v) ethylene glycol, which was optimized to 22.5% (v/v) ethylene glycol, 0.015 mM CYMAL-7 by the addition of additive solutions according to the manufacturer's instructions (Hampton Research, USA). The crystals of *B. halodurans* YhgD used for data collection were grown by mixing equal volumes (0.5 μl) of protein and reservoir solution and 0.1 μl of additive solution and equilibrating the mixed solutions against 100 μl reservoir solution in a 96-well plate at 296 K.

2.3. X-ray data collection

Crystals were transferred into a cryoprotectant solution containing 30% (v/v) ethylene glycol and 10% (v/v) glycerol and immediately flash-cooled in liquid nitrogen. X-ray diffraction data were collected at 100 K on an ADSC Quantum 210 CCD image-plate detector using synchrotron radiation on beamline 7A of the Pohang Accelerator Laboratory, Pohang, Republic of Korea. The data were collected using a 1° oscillation per image with a crystal-to-detector distance of 200 mm. The crystal was exposed to X-rays for 1 s per image and a

Figure 1
Triclinic crystals of *B. halodurans* YhgD. Their approximate dimensions are $0.05 \times 0.05 \times 0.15\ \text{mm}$.

Figure 2
X-ray diffraction image from a crystal of *B. halodurans* YhgD. The crystal-to-detector distance was 200 mm and the wavelength was 0.9793 Å. The black circle is drawn at the resolution of the detector edge (1.90 Å).

Table 1

Data-collection statistics for the *B. halodurans* YhgD crystal.

Values in parentheses are for the highest resolution shell.

Wavelength (Å)	0.9793
Temperature (K)	100
Oscillation range (°)	1.0
Resolution range (Å)	30.0–1.90 (1.94–1.90)
No. of observations	73734 (5093)
Unique reflections	26074 (1774)
Space group	<i>P</i> 1
Unit-cell parameters (Å, °)	<i>a</i> = 37.22, <i>b</i> = 47.85, <i>c</i> = 54.15, <i>α</i> = 92.75, <i>β</i> = 107.9, <i>γ</i> = 90.27
Data completeness (%)	93.5 (94.8)
Multiplicity	2.8 (2.9)
Average <i>I</i> / <i>σ</i> (<i>I</i>)	13.6 (4.4)
<i>R</i> _{merge} [†] (%)	5.9 (23.5)

[†] $R_{\text{merge}} = \frac{\sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl) \rangle|}{\sum_{hkl} \sum_i I_i(hkl)}$, where $I(hkl)$ is the intensity of reflection hkl , $\sum_{hkl}$ is the sum over all reflections and $\sum_i$ is the sum over *i* measurements of reflection hkl .

total of 270 frames were recorded. Data were processed with *MOSFLM* through the *iMOSFLM* interface (Leslie, 2006; Battye *et al.*, 2011) and scaled with *SCALA* (Evans, 2006) using the *CCP4i* interface (Potterton *et al.*, 2003).

3. Results and discussion

The recombinant *B. halodurans* YhgD protein was expressed in an *E. coli* system and purified to give a final yield of ~18 mg per litre of culture. The protein was purified by three steps of chromatography to homogeneity as estimated by 12% SDS–PAGE. The crystals were obtained reproducibly by sitting-drop vapour diffusion at 296 K from optimized reservoir solution consisting of 22.5% (v/v) ethylene glycol, 0.015 mM CYMAL-7. They grew to dimensions of approximately 0.05 × 0.05 × 0.15 mm within 2 d (Fig. 1). A total of 26 151 unique reflections were measured with a redundancy of 2.8 (Fig. 2). The merged data set was 93.5% complete to 1.90 Å resolution and gives an *R*_{merge} (on intensity) of 5.9%. The crystals belonged to the triclinic space group *P*1, with unit-cell parameters *a* = 37.22, *b* = 47.85, *c* = 54.15 Å, *α* = 92.75, *β* = 107.9, *γ* = 90.27°. Self-rotation functions were calculated using the programs *MOLREP* and *POLARREN* from the *CCP4* suite (Winn *et al.*, 2011). They consistently showed a moderate peak with 38–47% of the original peak in the *κ* = 180° section, while the *κ* = 120° section did not show any significant peaks. The self-rotation peak indicated the existence of noncrystallographic twofold symmetry. The asymmetric unit is likely to contain two molecules of monomeric YhgD, giving a crystal volume per mass (*V*_M) of 2.05 Å³ Da^{−1} and a solvent content of 40.2% (Matthews, 1968; Kantardjieff & Rupp, 2003). Table 1 summarizes the statistics of data collection.

Molecular replacement (MR) was attempted using several YhgD-homologue structures (*E. coli* RutR, PDB entry 3loc, 27% sequence

identity, Y. V. Patskovsky, V. Mennella & S. C. Almo, unpublished work; *Pectobacterium atrosepticum* Eca1819, PDB entry 2hyt, 26% sequence identity, Joint Center for Structural Genomics, unpublished work; *S. aureus* QacR, PDB entry 3bqz, 24% sequence identity, B. E. Brooks, K. M. Hardie, M. H. Brown, R. A. Skurray & R. G. Brennan, unpublished work) with the program *Phaser* (Storoni *et al.*, 2004). We attempted MR searches with both monomeric and dimeric models, without success. To determine the crystal structure, selenomethionine-substituted *B. halodurans* YhgD has been purified and crystallized. We are presently attempting to collect MAD data.

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