Shaocheng Zhang,^{a,b} Xun Min,^b
 Zhen Guo,^a Hongpeng Zhang,^b
 Ailong Huang,^a Yibing Yin^b and
 Deqiang Wang^{a,b*}

^aKey Laboratory of Molecular Biology on
 Infectious Disease, Chongqing Medical
 University, YiXueYuanlu-1, Chongqing 400016,
 People's Republic of China, and ^bDepartment of
 Laboratory Medicine, Chongqing Medical
 University, YiXueYuanlu-1, Chongqing 400016,
 People's Republic of China

Correspondence e-mail: wangd@ustc.edu

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Cloning, purification, crystallization and preliminary X-ray studies of the putative transcriptional regulator SPD0280 from *Streptococcus pneumoniae* D39

Streptococcus pneumoniae SPD0280 is a hypothetical protein that has been putatively identified as a transcriptional regulator. However, it has very low sequence identity to other well characterized transcriptional regulators. Determination of the three-dimensional structure may provide information for the characterization of proteins; therefore, it was decided to use X-ray diffraction analysis to learn more about this protein. Here, the expression, purification, crystallization and preliminary crystallographic analysis of SPD0280 from *S. pneumoniae* are reported. The crystals belonged to space group $P2_12_12_1$, with unit-cell parameters $a = 45.886$, $b = 66.785$, $c = 150.050$ Å, and diffracted to a resolution of 2.5 Å. The crystals are likely to contain one molecule in the asymmetric unit, with a V_M value of 2.06 Å³ Da⁻¹.

1. Introduction

Streptococcus pneumoniae is an important human opportunistic pathogen which causes several systemic diseases such as otitis media, pneumonia, bacteraemia and meningitis (Zhao *et al.*, 2013; Gupta *et al.*, 2013). Globally, *S. pneumoniae* is one of the leading causes of mortality (responsible for 3–5 million deaths annually) from respiratory and invasive infections in children and the elderly (Berkley *et al.*, 2005; Luby *et al.*, 2009). Antibiotic-resistant strains of *S. pneumoniae* have increased dramatically over the past few years (Mackenzie *et al.*, 2009). In recent years, a strain resistant to the last-resort antibiotic vancomycin has emerged (Wang & Lipsitch, 2006).

Transcriptional regulators (TRs), which are the key regulators of gene expression, play crucial roles in living organisms. For example, KynR, a Lrp/AsnC-type transcriptional regulator, directly regulates the kynurenine-pathway genes (Knoten *et al.*, 2011). Rgg (also known as RopB), a transcriptional regulator in Gram-positive bacteria, participates in pathogen virulence and regulates the expression of the annotated chromosomal genes involved in nonglucose carbohydrate metabolism, DNA recombination, protein biosynthesis and bacterial defence mechanisms (Zheng *et al.*, 2011; Anbalagan *et al.*, 2012).

SPD0280, a hypothetical transcriptional regulator of *S. pneumoniae* D39, is a novel gene related to the virulence of *S. pneumoniae* (Tong *et al.*, 2001; Long *et al.*, 2004; McKessar & Hakenbeck, 2007; Blomberg *et al.*, 2009). To gain further insight into the function of SPD0280, we initiated determination of its three-dimensional structure by X-ray crystallography. Here, we report the crystallization and preliminary X-ray study of SPD0280 from *S. pneumoniae* D39. Structure determination will be pursued using experimental phasing methods.

2. Materials and methods

2.1. Cloning and expression

The coding sequence for SPD0280 (amino acids 5–495) was amplified by polymerase chain reaction (PCR) from *S. pneumoniae* D39 genomic DNA using primers which contained *Bam*HI and *Xho*I restriction sites. The amplified product was digested using the *Bam*HI and *Xho*I endonucleases and was cloned into a pET-28a vector (previously digested with the same restriction enzymes) to generate pET-28a-SPD0280-His₆. The complete nucleotide sequence of the

Table 1

Data-collection and processing statistics.

Values in parentheses are for the outermost resolution shell.

Wavelength (Å)	0.9792
Space group	$P2_12_12_1$
Unit-cell parameters (Å)	$a = 45.886, b = 66.785, c = 150.050$
Resolution range (Å)	50–2.5 (2.64–2.50)
Total reflections	52380 (7752)
Unique reflections	15778 (2317)
Completeness (%)	96.7 (94.6)
$\langle I/\sigma(I) \rangle$	5.4 (3.5)
Multiplicity	7.7 (7.4)
$R_{\text{merge}}^{\dagger}$ (%)	9.8 (27.2)

$\dagger R_{\text{merge}} = \sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl) \rangle| / \sum_{hkl} \sum_i I_i(hkl)$, where $I_i(hkl)$ is the intensity of the i th measurement of an equivalent reflection with indices hkl .

insertion was confirmed by DNA-sequence analysis. The recombinant plasmid was transformed into *Escherichia coli* BL21 (DE3) cells to express the fusion protein. The cells were grown (at 310 K) with shaking in 1.2 l LB medium containing 50 mg l⁻¹ kanamycin. When the OD₆₀₀ reached 0.6, protein expression was induced by the addition of 0.5 mM isopropyl β -D-1-thiogalactopyranoside (IPTG) at 293 K. 9 h later the cells were harvested by centrifugation for 10 min at 5000g and 277 K.

2.2. Purification

The cell pellet was resuspended in 100 ml lysis buffer (20 mM Tris–HCl pH 8.5, 300 mM sodium chloride) and lysed by sonication on ice. All purification steps were performed at 277 K. The lysate was centrifuged at 12 000g for 30 min. The supernatant was applied onto an Ni²⁺–NTA affinity column (GE Healthcare) equilibrated with lysis buffer. Nonspecifically bound proteins were eluted by washing with 200 ml lysis buffer containing 30 mM imidazole and the target protein was eluted in about 60 ml elution buffer (lysis buffer containing a constant concentration of 300 mM imidazole). Subsequently, the eluted protein was loaded onto a DEAE–Sephacrose Fast Flow column (GE Healthcare) equilibrated with buffer A (20 mM Tris–HCl pH 8.5) and eluted using buffer A containing a linear gradient of 0–400 mM sodium chloride. Finally, fractions containing the protein were combined, concentrated, buffer-exchanged into the final buffer (5 mM Tris–HCl pH 8.5) using a 10 kDa cutoff Millipore Amicon concentrator and stored at 193 K for crystallization studies. The final purified protein concentration was about 7.8 mg ml⁻¹ and the purity was determined by SDS–PAGE to be about 90%.

Figure 1

Crystals of SPD0280 from *S. pneumoniae* D39. The dimensions of the crystals are 0.02 × 0.02 × 0.5 mm.

2.3. Crystallization

The protein sample was centrifuged at 12 000g and 277 K for 15 min to clarify the solution before initiating crystallization trials. Screening was performed by the sitting-drop vapour-diffusion method using 48-well vapour-diffusion plates with reservoirs filled with 100 μ l of each screening condition. Trials were performed with the Crystal Screen, Crystal Screen 2 and PEG/Ion screening kits (Hampton Research). In each sitting drop, 1.0 μ l protein solution was mixed with an equal amount of reservoir solution from the reservoir and incubated at 293 K. After 15 d, crystals of SPD0280 were observed in a condition with a reservoir solution consisting of 20% (w/v) polyethylene glycol (PEG) 10 000, 0.1 M MES pH 6.5. Further optimization of this condition by varying the PEG 10 000 concentration and the pH yielded good diffraction-quality crystals from 22–24% PEG 10 000, 0.1 M MES pH 7.0. Crystals appeared after 7 d of equilibration against the crystallization solution and grew to full size (0.02 × 0.02 × 0.5 mm) in 30 d (Fig. 1).

2.4. Data collection and processing

X-ray diffraction data were collected from a single crystal using a MAR CCD 245 image-plate detector on beamline BL17U1 of the Shanghai Synchrotron Radiation Facility (SSRF). The crystals were mounted in a nylon-fibre loop and flash-cooled in a nitrogen-gas stream at 100 K with reservoir solution containing 30% (v/v) ethylene glycol. The crystal-to-detector distance was 200 mm. The oscillation range was 1° per image and a total of 157° of data were collected (see Fig. 2). The data were processed using the HKL-2000 program package (Otwinowski & Minor, 1997). The final data-collection and processing statistics are shown in Table 1.

3. Results and discussion

The plasmid (pET-28-SPD0280-His₆) was constructed and the recombinant protein was expressed in *E. coli* strain BL21, yielding about 7.8 mg purified protein per litre of bacterial culture. The recombinant protein was prone to precipitation during desalting and

Figure 2

X-ray diffraction pattern of a crystal of SPD0280 from *S. pneumoniae* D39 obtained at the Shanghai Synchrotron Radiation Facility (SSRF). The circle indicates 3.5 Å resolution.

buffer exchange at pH 8.0; the pH of the final buffer was therefore adjusted to 8.5 to improve the stability of the protein in solution.

A complete diffraction data set was collected from a single crystal to 2.5 Å resolution and the data-collection statistics are reported in Table 1. A total of 52 380 measured reflections in the resolution range 50–2.5 Å were merged into 15 778 unique reflections with an R_{merge} of 9.8%. From an analysis of the diffraction intensities, the most plausible space group of the SPD0280 crystal was orthorhombic $P2_12_12_1$, with unit-cell parameters $a = 45.886$, $b = 66.785$, $c = 150.050$ Å. Based on the amino-acid sequence (5–495) of the N-terminus of SPD0280 and space group $P2_12_12_1$, it was presumed that the crystal contained one molecule per asymmetric unit, with a Matthews coefficient V_M of $2.06 \text{ Å}^3 \text{ Da}^{-1}$ and a solvent content of 40.45% (Matthews, 1968).

Despite the collection of several full sets of diffraction data from the SPD0280 crystals, we have unfortunately so far been unable to determine the structure of SPD0280 by the molecular-replacement method using the structure of the PRD domain of the transcriptional anti-terminator protein LicT from *Bacillus subtilis* (PDB entry 1tlv; Graille *et al.*, 2005) as a model. The PRD domain has about 26% sequence identity to the central domain (residues 214–394) of SPD0280 from *S. pneumoniae* D39. In order to solve the structure of SPD0280, the multiple-wavelength anomalous dispersion (MAD) or single-wavelength anomalous dispersion (SAD) methods will be used; the preparation and crystallization of selenomethionine-derivatized protein are currently in progress.

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