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Crystallization and preliminary X-ray crystallographic studies of DnaJ from *Streptococcus pneumoniae*

DnaJ, cooperating with DnaK and GrpE, promotes the folding of unfolded hydrophobic polypeptides, dissociates protein complexes and translocates protein across membranes. Additionally, DnaJ from *Streptococcus pneumoniae* (SpDnaJ) is involved in the infectious disease process and is being developed as a potential vaccine to prevent bacterial infection. Here the expression, purification, crystallization and preliminary crystallographic analysis of SpDnaJ are reported. The crystals belong to space groups *I*222 or *I*2₁2₁ and the diffraction resolution is 3.0 Å with unit-cell parameters *a* = 47.68, *b* = 104.45, *c* = 234.57 Å. The crystal most likely contains one molecule in the asymmetric unit, with a *V*_M value of 3.24 Å³ Da⁻¹ and a solvent content of 62.1%.

1. Introduction

Streptococcus pneumoniae causes serious diseases, including bacterial pneumonia, febrile bacteraemia, meningitis and other related diseases worldwide. Because of antibiotic overuse, multi-antibiotic-resistant strains are becoming a common and serious problem in the clinical management of pneumococcal infection (O'Brien *et al.*, 2009; WHO Publication, 2012). Therapeutic vaccines have been shown to be a promising approach in the treatment of antibiotic-resistant pneumococcal diseases. Recently, studies have shown that DnaJ of *S. pneumoniae* (SpDnaJ) could enhance the IgG titres, lymphocyte proliferation, IL-2 and γ-IFN in mice with mucosal and intra-peritoneal immunization (Khan *et al.*, 2006; Cui *et al.*, 2011). In addition, experimental mice immunized with SpDnaJ have a significant decline in *S. pneumoniae* colonization in nasal and lung tissues and consequently lower mortality than that of the unimmunized group, implying that the SpDnaJ is a protein vaccine candidate to pneumococcal disease.

DnaJ, also known as HSP40 (heat shock protein 40 kDa), is found ubiquitously in eukaryotic organisms, bacteria and other species (Hahn *et al.*, 1999; Martinez-Yamout *et al.*, 2000; Li *et al.*, 2003). DnaJ consists of multiple functional domains, including a highly conserved J-domain (Cheetham & Caplan, 1998; Szyperski *et al.*, 1994). According to the composition of domains, DnaJ can be divided into three groups, including type I (containing a J-domain, a G/F-domain, a zinc-binding domain and a conserved C-terminal domain), type II (N-terminal J- and G/F-domains) and type III (J-domain only) (Walsh *et al.*, 2004). As a molecular chaperone, DnaJ, cooperating with the co-chaperones, DnaK and GrpE, facilitates folding of some hydrophobic polypeptides, protein complex dissociation and protein translocation across membranes (Bukau & Horwich, 1998; Jensen & Johnson, 1999; Lemmon, 2001). Moreover, DnaJ could recognize and deliver substrate to HSP70 and stimulate its ATPase activity (Liberek *et al.*, 1990, 1991; Langer *et al.*, 1992). In addition, DnaJ could even perform chaperone function independently of DnaK (Wall *et al.*, 1994).

To date, the crystal structures of HSP40 Ydj1 from yeast (PDB code: 1nlt, Li *et al.*, 2003), DnaJ from *Klebsiella pneumoniae* (PDB code: 3lz8, Midwest Center for Structural Genomics, unpublished work) and *Caenorhabditis elegans* (PDB code: 2och, Midwest Center for Structural Genomics, unpublished work) have been reported, whereas there is no report on the structure of full-length SpDnaJ (Li *et al.*, 2003). In order to gain further insight into the function of

SpDnaJ (type I), we have initiated the determination of its three-dimensional structure by X-ray crystallography. Here, the crystallization and preliminary X-ray studies of DnaJ from *S. pneumoniae* TIGR4 are reported. Structure determination will be pursued using experimental phasing methods.

2. Materials and methods

2.1. Protein expression and purification

The gene encoding SpDnaJ (SP0519, residues 1–372) was amplified from *S. pneumoniae* strain TIGR4 genomic DNA. The PCR product was digested by *Bam*HI and *Sal*I restriction enzymes (TaKaRa) and was cloned into vector pET28a (Novagen) to generate pET28a-DnaJ with an N-terminal His-tag sequence (residues 1–372 from SpDnaJ with the N-terminal tag: MGSSHHHHHHSSGGLVPRGSHMAS-MTGGQQMGRGS). The inserted DNA sequence was confirmed by DNA sequence analysis. The expressed plasmid was transformed into *Escherichia coli* BL21 cells in LB nutrient medium containing kanamycin sulfate (50 µg ml⁻¹) at 310 K. When the OD₆₀₀ reached 0.6–0.8, induction was performed with 0.2 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) overnight at 293 K and then the cells were harvested by centrifugation at 5000g for 10 min.

The cell pellet was resuspended in buffer A (300 mM NaCl, 20 mM Tris–HCl pH 8.0) and then lysed by sonication. All purification steps were performed at 277 K. The solution was subsequently centrifuged at 14 000 rev min⁻¹ to remove cell debris. The collected supernatant was applied onto a Ni–NTA affinity column (GE Healthcare) and then the protein-bound beads were extensively washed with buffer A plus 40 mM imidazole to remove contaminating proteins. Subsequently, the target protein was eluted by buffer B (500 mM imidazole, 20 mM Tris–HCl pH 8.0) (Fig. 1). Finally, the eluted protein was loaded onto a DEAE–Sephacrose fast-flow column (GE Healthcare) pre-equilibrated with buffer C (20 mM Tris–HCl pH 8.0) and was eluted with a linear gradient from 0 to 500 mM NaCl plus 20 mM Tris–HCl pH 8.0. The purity of SpDnaJ for subsequent crystallization was about 90% as assessed by SDS–PAGE. The final purified protein concentration was adjusted to 15 mg ml⁻¹ for crystallization trials.

2.2. Crystallization

Initial crystallization screening was carried out at 293 K by the sitting-drop vapour-diffusion method in 48-well plates, using Index and PEG/Ion Screen kits (Hampton Research). Each drop consisted

Figure 1

The SDS–PAGE gel of DnaJ from *S. pneumoniae*. The proteins were separated by 15% SDS–PAGE. Lane 1, the molecular-mass markers; lane 2, total proteins of induced *E. coli*; lane 3, supernatant after ultrasonic crash of induced *E. coli*; lane 4, flow through after Ni–NTA; lanes 5, 6 and 7, eluate washed by 40 mM imidazole; lane 8, eluate washed by 500 mM imidazole.

of 1 µl protein solution and 1 µl reservoir solution, and the drop was vapour-equilibrated against 80 µl reservoir solution. Crystals of SpDnaJ were obtained in a number of conditions containing Jeffamine M-600 within 7–15 d. Further optimization of the conditions, varying the concentrations of the organic compound and the pH, gave good diffraction-quality crystals at 25–40% Jeffamine M-600, 0.1 M HEPES pH 5.5–8.5. Crystals appeared after 10 d equilibration against the crystallization solution and grew to full size (0.1 × 0.1 × 0.15 mm) in 20 d (Fig. 2). Data were collected from a crystal grown using HEPES at pH 8.0.

2.3. Data collection and processing

Crystals of SpDnaJ were transferred to a reservoir solution augmented with 20%(v/v) PEG 200 and immediately placed in a 100 K nitrogen gas stream. X-ray diffraction data were collected from a single crystal on beamline BL17U1 of the Shanghai Synchrotron Radiation Facility (SSRF). The crystal-to-detector distance was 280 mm. The oscillation range was 1° per image and a total of 180° of data were collected (Fig. 3). The images were processed using *HKL-*

Figure 2

The crystal of DnaJ from *S. pneumoniae*.

Figure 3

X-ray diffraction pattern of the SpDnaJ crystal.

Table 1

Data-collection and processing statistics.

Values in parentheses are for the highest-resolution shell.

Wavelength (Å)	0.9792
Space group	<i>I</i> 222 or <i>I</i> 2 ₁ 2 ₁ 2 ₁
Unit-cell parameters (Å)	<i>a</i> = 47.68, <i>b</i> = 104.45, <i>c</i> = 234.57
Resolution range (Å)	50–3.0 (3.16–3.0)
Total reflections	82629
Unique reflections	12096
Completeness (%)	99.5 (91.2)
<i>I</i> / <i>σ</i> (<i>I</i>)	15.2 (5.6)
Multiplicity	6.8 (6.3)
<i>R</i> _{merge} [†] (%)	9.0 (32.0)

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

2000 (Otwinowski & Minor, 1997). The final data-collection and processing statistics of SpDnaJ are presented in Table 1.

3. Results and discussion

A complete diffraction data set was collected to 3.0 Å resolution from a single crystal and the data-collection statistics are reported in Table 1. A total of 82 629 measured reflections were merged into 12 096 unique reflections with an R_{merge} of 9.0%. The merged data were 99.5% complete to the resolution range 50–3.0 Å. The SpDnaJ crystal belonged to the space group *I*222 or *I*2₁2₁2₁, with unit-cell parameters *a* = 47.68, *b* = 104.45 and *c* = 234.57 Å. Based on the molecular weight of SpDnaJ (about 41.3 kDa) and space group, solvent-content analysis indicated that one molecule could be accommodated per asymmetric unit, suggesting a V_M value of 3.24 Å³ Da^{−1} and a solvent content of 62.1% (Matthews, 1968). Molecular replacement using *AMoRe* (Navaza, 2001), *Phaser* (McCoy *et al.*, 2005) and *MOLREP* (Vagin & Teplyakov, 2000) was carried out using the HSP40 Ydj1 from yeast (PDB code: 1nlt; Li *et al.*, 2003) as a search model which shares 27% sequence identity to the C-terminal domains (104–332 residues) of SpDnaJ. However, structure determination by molecular replacement using the Ydj1 coordinates was unsuccessful. In order to solve the structure of SpDnaJ by multi-wavelength anomalous

dispersion methods, a selenomethionyl derivative of SpDnaJ, which contains three methionine residues, is being prepared.

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