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Crystallization and initial X-ray diffraction studies of scaffolding protein (gp7) of bacteriophage φ29

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The *Bacillus subtilis* bacteriophage φ29 scaffolding protein (gp7) has been crystallized by the hanging-drop vapour-diffusion method at 293 K. Two new distinct crystal forms that both differed from a previously crystallized and solved scaffolding protein were grown under the same conditions. Form I belongs to the primitive tetragonal space group $P4_12_12$, with unit-cell parameters $a = b = 77.13$, $c = 37.12$ Å. Form II crystals exhibit an orthorhombic crystal form, with space group $C222$ and unit-cell parameters $a = 107.50$, $b = 107.80$, $c = 37.34$ Å. Complete data sets have been collected to 1.78 and 1.80 Å for forms I and II, respectively, at 100 K using Cu $K\alpha$ X-rays from a rotating-anode generator. Calculation of a V_M value of 2.46 Å^3 Da^{-1} for form I suggests the presence of one molecule in the asymmetric unit, corresponding to a solvent content of 50.90%, whereas form II has a V_M of 4.80 Å^3 Da^{-1} with a solvent content of 48.76% and two molecules in the asymmetric unit. The structures of both crystal forms are being determined by the molecular-replacement method using the coordinates of the published crystal structure of gp7.

1. Introduction

In the structural assembly of double-stranded (ds) DNA viruses such as the herpes viruses (Lee & Guo, 1995), adenoviruses (D'Halluin *et al.*, 1978), T3 (Carazo *et al.*, 1996), T4 (Driedonks *et al.*, 1981), T7 (Kocsis *et al.*, 1995), λ (Kochan & Murialdo, 1984), φ29 (Anderson & Reilly, 1993; Guasch *et al.*, 1998; Valle *et al.*, 1999) and P22 (Bazinet & King, 1985) viruses, the proheads, the site of DNA encapsidation, are the first intermediate structures to be formed. The formation of these precursor capsids require proteins that are not present in the mature virions, known as scaffolding proteins. Scaffolding proteins have been hypothesized to play various vital complex roles including initial prohead assembly, DNA packaging and ejection and determination of the final structural forms of proheads (Kellenberger, 1991; Guo *et al.*, 1991; Anderson & Reilly, 1993; Green & King, 1996).

In the studies of the tailed bacteriophage φ29 of *Bacillus subtilis*, 180 copies of the 11.2 kDa gene product (gp7) are required in the initial capsid assembly *in vivo* (Nelson *et al.*, 1976). The prolate capsid of φ29 is also composed of two other essential structural proteins, the connector protein (gp10) and the shell protein (gp8), which interact during prohead formation (Bazinet & King, 1986; Casjens & Hendrix, 1998). Earlier data on φ29 gp7 have indicated that gp7 may act as a linker between the portal vertex and capsid protein (gp8), which is crucial in regulating the structural form of the prohead (Guo *et al.*, 1991). Subsequently, the NMR structure of the scaffolding protein of P22, which exhibits a helix–loop–helix motif, showed the terminal regions of the scaffolding protein to be responsible for binding to the coat protein (Sun *et al.*, 2000). More recently, the X-ray crystal structure of φ29 gp7 (Morais *et al.*, 2003) has confirmed its structural identity with the scaffoding protein of P22, with the 33 N-terminal amino-acid residues of P22 being similar to those of the φ29 scaffolding protein, suggesting common functions for the two proteins. The structure of φ29 gp7 is the first structure of an intact phage scaffolding protein and has shown homodimers that resemble arrows, with a helix bundle composing the arrowhead domain and a coiled coil forming the tail domain. In this paper, we describe crystallization conditions that yield well ordered crystals of φ29 gp7 that diffract to high resolution and their preliminary X-ray characterization. The

X-ray structure solutions of these crystals are in progress by molecular replacement using the atomic coordinates of a homologous gp7 (Morais *et al.*, 2003; PDB code 1noh) as a search model. Heavy-atom derivative data sets have also been collected. The high-resolution X-ray structures of these crystals and their comparison with the previously determined scaffolding protein structure will be reported elsewhere.

2. Materials and methods

2.1. Purification

The cloning and overexpression of *B. subtilis* bacteriophage φ29 scaffolding protein (gp7) has been described elsewhere (Guo *et al.*, 1991). A 50 ml culture of transformed cells was grown overnight in Terrific Broth (12 g Bacto-tryptone, 24 g yeast extract, 2 g KH_2PO_4/K_2HPO_4) with 50 µg ml^{-1} ampicillin at 307 K. Cultures were diluted to 20:1 in the same medium to about 5×10^8 ml^{-1} until an OD_{600} of 0.5–0.7 was reached. Isopropyl-β-D-thiogalactopyranoside (IPTG) was added to a final concentration of 5 m*M* at the same temperature and the cells was induced for an additional 3 h. The cells were harvested by centrifugation and the normal yield was 40 g wet cells per litre. The cell pellet was suspended in 30 ml buffer *A* [50 m*M* Tris–HCl pH 8.0, 2 m*M* dithiothreitol (DTT), 5% glycerol, 50 m*M* NaCl] and the cells were lysed by passing twice through a French press at 69 MPa. The cell debris was removed by centrifugation at 10 000*g* for 30 min and the supernatant containing the scaffolding protein was precipitated with ammonium sulfate to a final concentration of 50% and the pellet was resuspended and dialyzed overnight in buffer *A*. The protein solution was run through a DEAE-Sephadex A-50 ion-exchange column equilibrated with buffer *A* and eluted with an NaCl gradient (50 m*M*–1 *M*) in the same buffer at a rate of 1 ml min^{-1}. The peak pool was dialyzed with 3 × 1000 ml sample buffer (50 m*M* Tris–HCl pH 8.0, 50 m*M* NaCl) and concentrated to a final concentration of 7–10 mg ml^{-1} for crystallization experiments.

2.2. Crystallization

Crystallization of gp7 was achieved using Hampton Research Crystal Screen I and the hanging-drop vapour-diffusion method at 293 K. Initially, 5 µl of 5 mg ml^{-1} protein solution was mixed with an equal volume of reservoir solution in the hanging drop. Several of the drops, including the solutions that yielded crystals, formed a slight precipitate upon addition of the reservoir solution. Small crystals appeared after six to seven months in Crystal Screen condition Nos. 18, 22, 28 and 46. These conditions contain ethylene glycol 4000 and 8000 as precipitants and the salts magnesium acetate, sodium acetate, sodium citrate and calcium acetate, respectively. Similar Crystal Screen conditions without salts did not produce crystals, an indication that salts are required to obtain the scaffolding-protein crystals. Crystallization conditions were optimized by increasing both the protein concentration to 7.5–10 mg ml^{-1} and the polyethylene glycol 4000 and 8000 concentration to 35–38%. These conditions yielded crystals with two different morphologies: form I crystals were tetragonal, while form II crystals were much longer and rectangular in shape (Fig. 1).

Figure 1
The lower crystal is a form I tetragonal crystal grown by the hanging-drop method using 35% PEG 4000 in 0.1 *M* sodium cacodylate pH 7.0 in the presence of 0.2 *M* magnesium acetate. The crystal is about 0.15 mm in thickness and 0.02 mm in length. The upper crystal belonging to form II was grown in the same crystallization conditions and drop. The crystal is approximately 0.20 mm in length and 0.015 mm in diameter.

Table 1
Summary of data-collection and processing statistics.

Values in parentheses refer to the highest resolution data shell.

	Form I	Form II
Unit-cell parameters (Å)		
a	77.13	107.50
b	77.13	107.68
c	37.34	37.12
Space group	$P4_12_12$	$C222$
Resolution range	30–1.78 (1.80–1.70)	30–1.80 (1.9–1.7)
Total No. of reflections	99150	113545
No. of unique reflections	18734	15503
Data completeness (%)	95.5 (92.1)	97.5 (94.9)
R_{merge} (%)	5.5 (8.7)	8.8 (12.2)
Average $I/\sigma(I)$	2.3 (1.50)	3.2 (1.7)
Redundancy	5.1 (6.7)	5.0 (4.7)
Mosaicity (°)	0.7	1.0
Exposure time (min)	5.0	5.0

3. Data collection

For data collection, a single crystal was looped out of the hanging drop and placed in reservoir solution containing an increasing amount of glycerol to a maximum of 25%(*v*/*v*) over a period of hours. The crystal was then flash-cooled in a liquid-nitrogen stream at 100 K generated by an Oxford Cryosystems cryocooling device. X-ray diffraction data sets for both form I and II crystals were collected on an in-house X-ray Rigaku Rotaflex rotating-anode generator operating at 50 kV and 100 mA (Cu $K\alpha$, 1.548 Å) equipped with an R-AXIS VI^{++} image-plate detector at a crystal-to-detector distance of 160 mm using an oscillation width of 1° per frame. Form I and II crystal data sets were collected to 1.78 and 1.80 Å resolution, respectively, and were indexed, integrated and scaled using *d*TREK* (Pflugrath, 1999) and *TRUNCATE* from the *CCP*4 suite (Collaborative Computational Project, Number 4, 1994). There were 99 150 measurements which reduced to 18 734 unique reflections with an R_{merge} of 5.5% on intensities for form I and the data set was 95.5% complete. A form II data set was collected to 1.8 Å. There were 113 545 reflections which reduced to 15 503 unique reflections with an R_{merge} of 8.8 on intensities. The data set was 97.5% complete. Data-collection statistics are summarized in Table 1.

4. Results and discussion

After purification by ion-exchange DEAE-Sephadex chromatography, a single band was observed on 8–16% SDS Pre-cast gel (Criterion, Bio-Rad), corresponding to the 11.2 kDa molecular weight of φ29 scaffolding protein. Crystallization trials were performed using Hampton Research Crystal Screen, which produced initial crystals. A fine screen that included variations in both protein and precipitant concentrations yielded well ordered diffraction-

quality crystals. Two distinct crystal forms were observed to grow in the same drop. Form I grew as typical tetragons, while form II crystals were rectangular in shape. Both crystal forms were soaked in reservoir solutions containing up to 20–25% glycerol prior to X-ray data collection.

The φ29 bacteriophage gp7 crystals were found to be very stable at low salt concentration (0.2 *M*), unlike the connector protein (gp10), the other important structural protein of φ29 bacteriophage, the crystallization of which is highly salt-dependent (0.4 *M*; Guasch *et al.*, 1998; Badasso *et al.*, 2000). However, like connector protein, salts such as magnesium, calcium and sodium are required in order to obtain crystals of gp7 that are suitable for X-ray diffraction analysis. The new gp7 crystals were extremely stable to X-ray radiation and diffracted to a much higher resolution of 1.78 Å, compared with the 2.2 Å obtained for the previous crystal structure (Morais *et al.*, 2003). Form I crystals belong to space group $P4_12_12$, with unit-cell parameters $a = b = 77.13$, $c = 37.34$ Å. The Matthews coefficient (V_M; Matthews, 1968) of form I is 2.46 Å^3 Da^{-1}, corresponding to a solvent content of 50.9% with one molecule in the asymmetric unit. Form II crystals belong to space group *C*222, with unit-cell parameters $a = 107.50$, $b = 107.68$, $c = 37.12$ Å and a Matthews coefficient of 2.40 Å^3 Da^{-1}, corresponding to a solvent content of 48.76%. There are two molecules in the asymmetric unit. Attempts to solve the crystal structures of both forms I and II by molecular replacement using the atomic coordinates of a homologous scaffolding protein (gp7; Morais *et al.*, 2003; PDB code 1noh) as a model are under way. The rotation function from *AMoRe* (Navaza, 1994) generates a solution in space group $P4_12_12$, different from the model space group $P2_12_12$ with tetramers in the asymmetric unit. Optimization of the translation function yielded an *R* factor of 0.434 and a correlation coefficient of 0.690 in the resolution range 15.0–6.0 Å. Subsequent annealing refinement using the computer package *CNS* (Brünger *et al.*, 1998) appeared promising, with an overall *R* factor for test and working data sets of 0.364 and 0.332, respectively, for data in the resolution range 30.0–2.2 Å. The unit cell also packs correctly with one molecule of scaffolding protein in space group $P4_12_12$.

The high-resolution X-ray crystal structure of φ29 scaffolding protein (gp7) should reveal the complete electron density for all 98 amino-acid residues of gp7. In the previous structure (Morais *et al.*, 2003) only 74 amino-acid residues were defined and the terminal 15 residues were also disordered. The present well ordered crystals grew in two different space groups, as shown above, both of which differed from the previous gp7 space group $P2_12_12$. The complete X-ray crystal structure of gp7 will hence exhibit additional new structural details that might further describe the structure and function of this protein.

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