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Crystallization and preliminary X-ray diffraction analysis of bacteriophage φ12 packaging factor P7

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Bacteriophage φ12 protein P7 is a structural component of the polymerase complex and ensures stable packaging of the genomic RNA. φ12 P7 has been cloned, purified and crystallized. Crystals belong to space group $P3_221$, with unit-cell parameters a = 75.7, b = 75.7, c = 45.2 Å, $\alpha = 90$, $\beta = 90$, $\gamma = 120^\circ$, and diffract beyond 2.0 Å. Multiple anomalous dispersion data have been collected from crystals of selenomethionylated P7. Mass spectroscopy showed proteolysis of the crystallized protein and a truncated form, P7ΔC, gave crystals of similar morphology. Cross-linking experiments implicated the N-terminal domain of P7 as being essential for dimerization.

1. Introduction

Cystoviruses (φ6–φ14) are enveloped icosahedral bacteriophages with a double-stranded RNA genome consisting of three segments enclosed within the polymerase complex (PC), which is coated by a nucleocapsid protein shell and a lipid envelope. The PC is composed of 120 copies of the major structural protein P1, 12 monomers of the RNA-dependent RNA polymerase P2, 12 hexamers of the packaging motor P4 and 30 dimers of the structural protein P7 (Poranen & Tuma, 2004).

P7 participates in PC assembly (Poranen & Tuma, 2004) and is essential for RNA packaging and transcription regulation (Juuti & Bamford, 1997). P7 associates directly with the inner surface of the P1 shell (Pirttimaa *et al.*, 2002) at an average radius of 160 Å (Ikonen *et al.*, 2003). Although P7 proteins from different cystoviruses exhibit only limited sequence identity, they seem to form elongated dimers (Juuti & Bamford, 1997; Kainov *et al.*, 2003). In this study, we present a preliminary biochemical and crystallographic analysis of P7 from bacteriophage φ12 (Gottlieb *et al.*, 2002), which exhibits limited sequence similarity to rotavirus protein NSP5 (Taraporewala & Patton, 2004).

2. Materials and methods

2.1. Cloning, expression and purification of φ12 P7 and P7ΔC

The φ12 P7 expression plasmid (pPG29) was prepared by PCR amplification of the P7 gene from the pP12L1 (Gottlieb *et al.*, 2002) template with recombinant *Pfu* DNA polymerase (Stratagene) using the oligonucleotides 5′-GGTAA**CCATGG**ACTTCATTACTGAC-3′ and 5′-A**GGATCC**TTATTCGTCGGCATGAT-3′ as upstream and downstream primers, respectively. The PCR fragment was digested with *Nco*I and *Bam*HI and inserted into the pET-21-d vector (Novagen) at *Nco*I–*Bam*HI sites (in bold).

To produce the C-terminal deletion mutant (P7ΔC) missing residues 130–169, the 5′-terminal region of P7 gene was PCR-amplified from the template pPG29 with *Pfu* DNA polymerase (Stratagene) using the oligonucleotides 5′-GCGA**CATATG**GACTTCATTACTGACA-3′ and 5′-CGCT**GAATTC**TCACATGTTCGCTTTG-3′ as upstream and downstream primers, respectively, and inserted into the plasmid pT7-7 (Tabor & Richardson, 1990) at the *Nde*I–*Eco*RI sites (in bold), giving plasmid pVS1.

Soluble φ12 P7 and P7ΔC were produced in *Escherichia coli* strain BL21 (DE3) and purified as described for φ12 P4 (Mancini *et al.*, 2004).

Selenomethionylated proteins were expressed in MOPS minimal medium containing 50 μg ml^{-1} L-selenomethionine (Nanduri *et al.*, 2002) and purified in the same way as the native protein. Purified protein was stored in TNM buffer at 277 K (20 m*M* Tris–HCl pH 8.0, 50 m*M* NaCl, 7.5 m*M* $MgCl_2$).

2.2. Crystallization, data collection and processing

Crystallization conditions were identified with sitting-drop vapour-diffusion sparse-matrix screens (Jancarik & Kim, 1991).

All diffraction data were collected at 100 K. For data collection, crystals were cryoprotected with well buffer supplemented with 30% glycerol and frozen directly in the gas stream. A selenomethionine three-wavelength MAD experiment was performed at beamline BM-14-UK, ESRF. Data were collected on a 133 mm MAR CCD detector and reduced and

scaled with *HKL*-2000 (Otwinowski & Minor, 1997).

2.3. Mass spectrometry, gel filtration and crosslinking of φ12 P7 and P7ΔC

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI–MS; Voyager DE-STR, Applied Biosystems) was performed on both native and labelled protein from crystals dissolved in water.

Gel filtration was performed at 293 K on an analytical Superdex-75 10/30 column (Amersham Biosciences) in TNM buffer (1 ml min^{-1}).

Table 1
Data-collection statistics for φ12 P7.

Data were processed with the *HKL* program suite (Otwinowski & Minor, 1997). Values in parentheses are for the highest shell. Wavelengths were selected on the basis of the X-ray fluorescence spectrum of the crystal with the program *CHOOCH* (Evans & Pettifer, 2001).

	Peak	Inflection	Remote
Wavelength (Å)	0.978663	0.979325	0.918398
Resolution (Å)	20.0–2.20 (2.28–2.20)	20.0–2.20 (2.28–2.20)	20.0–2.20 (2.28–2.20)
No. observed reflections	63551	40438	40179
No. unique reflections	7541 (291)	7278 (135)	7452 (297)
Completeness (%)	92.0 (35.5)	89.0 (16.6)	90.9 (36.3)
R_{sym}† (%)	6.2 (26.3)	5.1 (30.6)	5.5 (33.2)
$I/\sigma(I)$	19.7 (5.0)	18.8 (4.8)	18.1 (5.0)

† $R_{sym} = \sum_h \sum_i |I_{h,i} - \overline{I}_h| / \sum_h \sum_i I_{h,i}$, where $I_{h,i}$ is the intensity of a measured reflection i and $\overline{I}_h$ is the average intensity for this reflection.

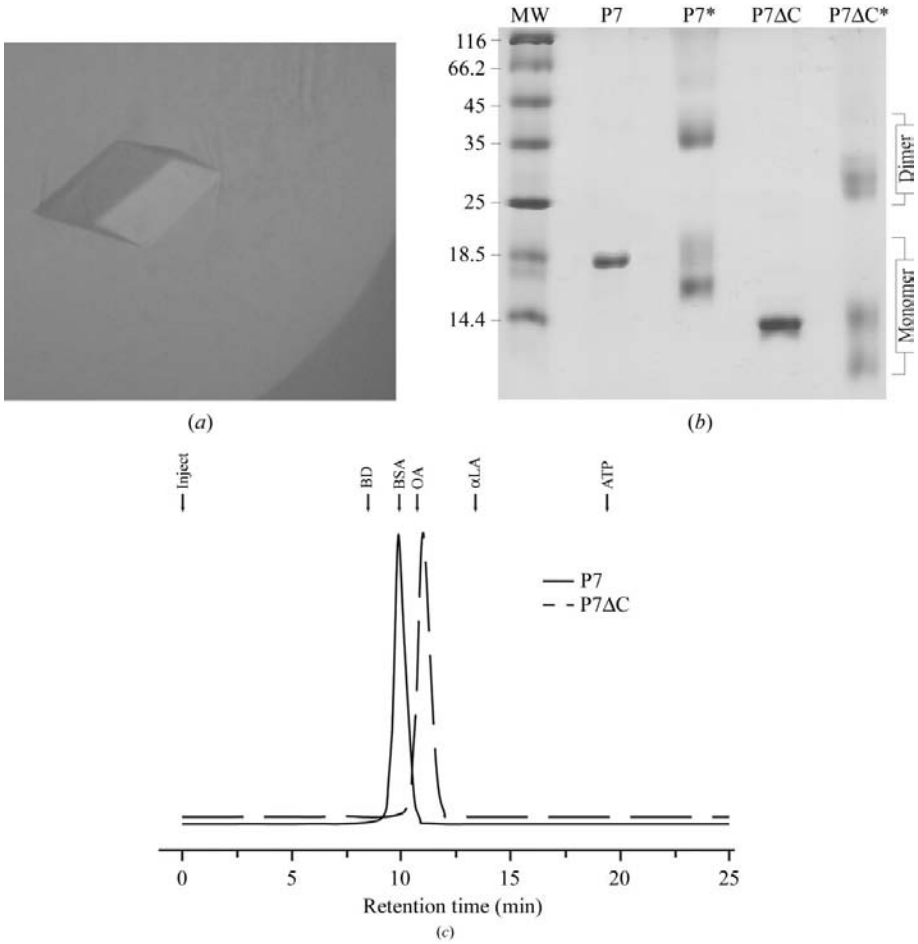

Figure 1
(*a*) Crystal of φ12 P7 in polarized light measuring ∼0.4 × ∼0.4 × ∼0.4 mm. (*b*) Cross-linking of φ12 P7 and P7ΔC proteins. Lanes P7 and P7ΔC contain purified proteins and lanes P7* and P7ΔC* contain cross-linked products. The migration of P7 and P7ΔC monomers and dimers is shown on the right. Lane MW contains molecular-weight standards (kDa). (*c*) Purified φ12 P7 and P7ΔC proteins were analyzed on a Superdex-75 gel-filtration column. Traces represent absorbance at 280 nm. Arrows indicate the injection time (inject) and positions of molecular-weight standards (Sigma): BD, Blue Dextran (2000 kDa); BSA, bovine serum albumin (67 kDa); OA, ovalbumin (45 kDa); αLA, α-lactalbumin (14.2 kDa); ATP, adenosine triphosphate (0.6 kDa).

Crosslinking of P7 or P7ΔC was performed on ice for 30 min in 0.5%(*v*/*v*) glutaraldehyde with 75 µg of protein and analyzed by SDS–PAGE.

3. Results and discussion

Conditions of 25% PEG 1500 in 100 m*M* sodium acetate pH 5.0 with a 1:1 ratio of P7 protein (4 mg ml^{-1}) to well solution yielded large single rhomboid crystals (typical dimensions 0.4 × 0.4 × 0.4 mm) at 277 K within two weeks (Fig. 1*a*). Selenomethionylated P7 crystals were obtained under the same conditions. Crystals diffracted beyond 2.0 Å resolution with synchrotron radiation and belonged to space group $P3_221$, with unit-cell parameters a = 75.7, b = 75.7, c = 45.2 Å, α = 90, β = 90, γ = 120°. Data-collection statistics are shown in Table 1.

The MALDI–MS mass of the crystallized P7 (14 066 compared with the theoretical 18 519) indicated the proteolytic removal of 40 C-terminal residues during crystallization. The selenomethionylated P7 mass (14 440) indicated the labelling of all eight methionine residues. P7ΔC was constructed and purified. Purified P7ΔC yielded diffracting crystals from the same conditions as full-length P7. They had the same morphology and unit-cell parameters (data not shown). Assuming a full-length protein, the calculated V_M of 2.02 Å^3 Da^{-1} implies the presence of one P7 monomer per asymmetric unit, with a solvent content of 41%. For P7ΔC the calculated V_M of 2.78 Å^3 Da^{-1} implies the presence of one P7ΔC monomer per asymmetric unit, with a solvent content of 56%. Phasing, model building and refinement of the P7 and P7ΔC structures are in progress.

Cross-linking showed that P7 can form dimers (Fig. 1*b*). The gel-filtration profile indicates P7 to be an elongated dimer (Fig. 1*c*). To determine the P7 region involved in dimerization, the P7ΔC construct was examined by the same methods. Both cross-linking and gel-filtration showed (Figs. 1*b* and 1*c*) that the C-terminus is not involved in dimer formation; rather, it is the N-terminus that is important.

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