Envelope protein VP24 from *White spot syndrome virus*: expression, purification and crystallization

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White spot syndrome virus (WSSV) is a major shrimp pathogen known to infect penaeid shrimp and other crustaceans. VP24 is one of the major envelope proteins of WSSV. In order to facilitate purification, crystallization and structure determination, the predicted N-terminal transmembrane region of approximately 26 amino acids was truncated from VP24 and several mutants were prepared to increase the proportion of selenomethionine (SeMet) residues for subsequent structural determination using the SAD method. Truncated VP24, its mutants and the corresponding SeMet-labelled proteins were purified, and the native and SeMet proteins were crystallized by the hanging-drop vapour-diffusion method. Crystals of VP24 were obtained using a reservoir consisting of 0.1 M Tris-HCl pH 8.5, 2.75 M ammonium acetate with a drop volume ratio of two parts protein solution to one part reservoir solution. Notably, ATP was added as a critical additive to the drop with a final concentration of 10 mM. Crystals of SeMet-labelled VP24 mutant diffracted to 3.0 Å resolution and those of the native diffracted to 2.4 Å resolution; the crystals belonged to space group $I2_13$, with unit-cell parameters $a = b = c = 140$ Å.

1. Introduction

White spot syndrome virus (WSSV) is a major pathogen in cultured shrimp and has caused huge economic losses to the world's shrimp-farming industry (Chen *et al.*, 1997; Wang *et al.*, 2000). The virus has a wide host range, infecting penaeid shrimps and several crustacean species such as crabs, spiny lobsters, freshwater shrimps and crayfish (Flegel, 1997; van Hulten, Westenberg *et al.*, 2000). WSSV is becoming a great threat not only to the shrimp aquaculture industry but also to marine ecology in general (Escobedo-Bonilla *et al.*, 2008). Electron-microscopy studies have shown that WSSV is an enveloped and rod-shaped virus of approximately 275 nm in length and 120 nm in width with a tail-like appendage at one end (Wongteerasupaya *et al.*, 1995; Tsai *et al.*, 2006). It has been identified as a novel type species which describes the *Whispovirus* genus and the *Nimaviridae* family, of which it is the only member described to date (van Hulten *et al.*, 2001; Chen *et al.*, 2002; van Hulten, Vlak *et al.*, 2000; Yang *et al.*, 2001; Leu *et al.*, 2009).

Detailed structural interpretations of the viral structural proteins would provide valuable information for understanding their functional roles in host-pathogen interaction. Among the viral structural proteins, the envelope proteins are believed to be the first molecules to interact with the host and consequently play critical roles in cell targeting as well as in triggering host defences (Xie *et al.*, 2006; Chang *et al.*, 2010;

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Li *et al.*, 2011; Wu *et al.*, 2005; Sritunyalucksana *et al.*, 2006). Previous studies have shown that VP28, VP26, VP24 and VP19 are the main components of the virus envelope (van Hulten, Westenberg *et al.*, 2000; van Hulten, Vlak *et al.*, 2000; Xie *et al.*, 2006; Zhou *et al.*, 2009). VP24 may interact with another envelope protein VP28 during WSSV infection and functions at an early stage in virus infection (Xie & Yang, 2006; Li, Chen *et al.*, 2015). To date, only two three-dimensional structures of the WSSV structural proteins VP26 (PDB entry 2edm) and VP28 (PDB entry 2ed6) have been presented (Intorasoot *et al.*, 2007). Expression of and purification trials on full-length VP24 resulted in the production of insoluble protein. Sequence analysis using the *TMHMM* server indicated the presence of a transmembrane region (TM; residues 4–26) at the N-terminal region of VP24 (Krogh *et al.*, 2001; Xie & Yang, 2006). Despite the removal of the predicted transmembrane region to increase the solubility of VP24 (Li, Chen *et al.*, 2015; Xie & Yang, 2006; Li, Li *et al.*, 2015), the

Table 1

Macromolecule-production information.

DNA source	WSSV
Forward primer	GCCTCATATGAACATAGAACTTAAC
Reverse primer	GCCCTCGAGTTTTTCCCAACC
Expression vector	pET-21b
Expression host	<i>E. coli</i>
Complete amino-acid sequence of the construct produced†	(MHMWGVYAAILAGLTLILVVISIVVT)MNIELNKKLDKKDKDAYPVESEIINLTINGVARGNHFN-FVNGTLQTRNYGKVVVAGQGTSDSELVKKKGDIILTSLLGDGHTLNVNKAESKELEYARVYN-NTKRDITVDSVSLSPGLNATGREFSANKFVLY-FKPTVLKKNRINTLVFGATFDEIDDTNRHYL-LSMRFPSPGNDLFKVGKELEHHHHHH

† The sequence in parentheses is the removed TM domain. Mutants are produced using the underlined L or I residues; selenomethione is represented by an underlined M.

solubility of the protein remained limiting for crystallization trials. Here, we describe the expression, purification and crystallization of N-terminally TM-truncated VP24 of WSSV.

2. Materials and methods

2.1. Cloning, expression and purification

The gene coding for the transmembrane region-truncated construct of VP24 (residues 27–208; Table 1) was amplified from the WSSV genome by PCR using specific primers: forward primer GCCTCATATGAACATAGAACTTAAC and reverse primer GCCCTCGAGTTTTTCCCAACC. The PCR products were digested with *Nde*I and *Xho*I and further cloned into pET-21b, resulting in a C-terminal hexahistidine tag for purification. The mutants (L69M, L88M, L121M, I133M and L69M/L121M) were generated by site-directed mutagenesis using the pET-21b-VP24 vector as the template and were confirmed by sequencing. All recombinant plasmids were transformed into *Escherichia coli* BL21 (DE3) cells. Overexpression was performed by the induction of mid-log-phase *E. coli* BL21 (DE3) cells with 0.3 mM isopropyl β -D-1-thiogalactopyranoside (IPTG) for 12–16 h at 16°C. The

Figure 1

Expression and purification of VP24. Lane 1, total cell lysate; lane 2, supernatant from the cell lysate; lane 3, pellet from the cell lysate; lane 4, flowthrough of the supernatant from an NTA affinity column; lanes 5 and 6, 20 and 50 mM imidazole washes from the NTA affinity column, respectively; lane 7, 500 mM imidazole eluate from the NTA affinity column; lane 8, molecular-weight markers (labelled in kDa).

Figure 2

Purification of VP24 using size-exclusion chromatography (a) and analysis using SDS-PAGE (b). The eluate containing VP24 from the NTA affinity column was concentrated to a volume of ~5 ml and was then subjected to gel filtration on a Superdex 200 column (GE Healthcare). VP24 was mainly eluted in peak 2. It showed perfect purity after fraction 18, which ran as a single band in the SDS-PAGE without any extra bands.

overexpressed VP24-His₆ was purified using Ni-NTA affinity chromatography (GE Healthcare) followed by gel filtration using Superdex 200 HR 16/60 (GE Healthcare) equilibrated in a buffer consisting of 25 mM Tris-HCl pH 7.0, 200 mM NaCl, 5% glycerol. Purified protein fractions were made 0.5 M in the nondetergent sulfobetaine 3-(1-pyridino)-1-propanesulfonate (NDSB-201; Sigma) and then concentrated using centrifugal concentrators to a final concentration of 2.5–3.5 mg ml⁻¹; the concentration was measured using a Bradford Protein Assay kit (Bio-Rad). Selenomethionine (SeMet)-labelled proteins were produced by inhibiting endogenous methionine biosynthesis in M9 minimal medium supplemented with specific amino acids as well as SeMet (Qoronfleh *et al.*, 1995; Hendrickson *et al.*, 1990). The purification and concentration steps were the same as for the corresponding native protein. The purification-step results, shown by SDS-PAGE, are shown in Figs. 1 and 2. Macromolecule-production information is summarized in Table 1.

2.2. Crystallization

Purified VP24 protein was crystallized by the hanging-drop vapour-diffusion method at 16°C. Initial crystallization screening was performed using the sparse-matrix approach

with screening solutions from crystallization screening kits (The JCSG Core I–IV Suites; Qiagen) by the sitting-drop method. In the initial crystallization screening experiments, 0.5 µl protein solution (approximately 3.5 mg ml⁻¹) was mixed with 0.5 µl reservoir solution and equilibrated against 70 µl reservoir solution. Thin needle crystals were obtained (Fig. 3*a*) using the condition 0.1 M Tris-HCl pH 8.5, 25% PEG 400, 0.1 M Li₂SO₄. The initial condition was further optimized and Additive Screen (Hampton Research) was also used to optimize the crystals (Fig. 3*b*). The best crystals of VP24 were found using a reservoir solution consisting of 0.1 M Tris-HCl pH 8.5, 2.75 M ammonium acetate with a drop volume ratio of two parts protein solution (approximately 2.5 mg ml⁻¹) to one part reservoir solution using the hanging-drop vapour-diffusion method (Fig. 3). At the same time, ATP was added as a critical additive to the drop with a final concentration of 10 mM. Crystals of mutants and their SeMet variants were obtained using similar conditions to the native protein.

2.3. Data collection

Prior to data collection, crystals were briefly soaked in a cryoprotectant solution consisting of 30% glycerol, picked up in a cryoloop and flash-cooled in liquid nitrogen. The X-ray

Figure 3

Crystals of VP24. (*a*) Initial crystals from the condition 0.1 M Tris-HCl pH 8.5, 25% PEG 400, 0.1 M Li₂SO₄. (*b*) The initial condition was optimized using the Additive Screen kit (Hampton Research). ATP was screened as a critical additive to the drop with a final concentration of 10 mM. In the presence of ATP as an additive, the crystallization condition was further optimized to 0.1 M Tris-HCl pH 8.5, 2.25–2.75 M ammonium acetate using the sitting-drop vapour-diffusion (*c*) and hanging-drop vapour-diffusion (*d*) methods.

diffraction data sets were collected with single anomalous diffraction (SAD) on beamline BL17U at Shanghai Synchrotron Radiation Facility (SSRF), Shanghai, People's Republic of China using a charge-coupled device (CCD) detector. The data sets were processed and scaled using the *HKL*-2000 (Otwinowski & Minor, 1997) and *CCP4* suites (Winn *et al.*, 2011). The data-collection statistics are shown in Table 2.

3. Results and discussion

Transmembrane region-truncated VP24 was successfully expressed in *E. coli* BL21 (DE3) cells and purified. However, when the concentration of VP24 exceeded 0.8 mg ml^{-1} the protein was unstable and produced a large amount of precipitation. This concentration is not sufficient for subsequent crystallization trials. With the addition of the nondenaturing protein-solubilizing agent NDSB-201, soluble VP24 could reach a concentration of 3.5 mg ml^{-1} and yield crystals.

Diffraction-quality crystals of VP24 and its SeMet variants were obtained in a reservoir solution consisting of 0.1 M Tris-HCl pH 8.5, 2.75 M ammonium acetate with a protein concentration of 2.5 mg ml^{-1} and with 10 mM ATP in the drop. Recombinant SeMet variants were produced in order to determine the structure using the SAD method. However, only two Met sites (Met1 and Met194) are present in the truncated VP24 sequence; Met1 at position 1 in particular would be flexible and would have poor electron-density in the structure. Therefore, we produced several mutants (L69M, L88M, L121M, I133M and L69M/L121M) to increase the proportion of SeMet residues for subsequent structural determination using the SAD method. During purification, the

Table 2

Data collection and processing.

Values in parentheses are for the outer shell.

	SeMet L69M/L121M VP24	Native VP24
Diffraction source	BL17U, SSRF	BL17U, SSRF
Wavelength (Å)	0.97915	0.97915
Temperature (K)	100	100
Detector	ADSC Q315R	ADSC Q315R
Crystal-to-detector distance (mm)	400	350
Rotation range per image (°)	1	1
Total rotation range (°)	60	360
Exposure time per image (s)	1	1
Space group	<i>I</i> ₂ 3	<i>I</i> ₂ 3
<i>a</i> , <i>b</i> , <i>c</i> (Å)	140, 140, 140	140, 140, 140
α , β , γ (°)	90, 90, 90	90, 90, 90
Resolution range (Å)	44–2.80 (2.85–2.80)	50–2.40 (2.44–2.40)
Total No. of reflections	162323	1363134
No. of unique reflections	11194	19582
Completeness (%)	99.8 (100)	100 (100)
Mean <i>I</i> / σ (<i>I</i>)	32.6 (3.03)	51 (1.65)
<i>R</i> _{merge} (%)	10 (77.6)	7.6 (94)
Multiplicity	7.3 (7.5)	20.6 (20.4)
Wilson <i>B</i> factor (Å ²)	71.49	72.74

I133M mutant was not stable. The L69M, L121M and L69M/L121M mutants diffracted to a resolution of 3.0 Å . The L88M mutant diffracted to only 6.0 Å resolution. Therefore, the SeMet derivative of the L69M/L121M mutant was prepared for SAD data collection. The crystals belonged to the cubic space group *I*₂3, with unit-cell parameters $a = b = c = 140 \text{ Å}$, and contained two molecules in the asymmetric unit ($V_M = 2.7 \text{ Å}^3 \text{ Da}^{-1}$). The native crystals diffracted to 2.4 Å resolution. Figs. 3 and 4 shows the crystals and the diffraction of

Figure 4

Diffraction images of the VP24 crystals. (a) SeMet L69M/L121M VP24; (b) native VP24. The diffraction resolution of the SeMet L69M/L121M variant was 3.0 Å , while that of the native was approximately 2.4 Å .

VP24. Using data sets collected from the SeMet-substituted crystals, the structure of VP24 has successfully been determined.

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