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Expression, purification and crystallization of two major envelope proteins from white spot syndrome virus

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White spot syndrome virus (WSSV) is a major virulent pathogen known to infect penaeid shrimp and other crustaceans. VP26 and VP28, two major envelope proteins from WSSV, have been identified and overexpressed in *Escherichia coli*. In order to facilitate purification and crystallization, predicted N-terminal transmembrane regions of approximately 35 amino acids have been truncated from both VP26 and VP28. Truncated VP26 and VP28 and their corresponding SeMet-labelled proteins were purified and the SeMet proteins were crystallized by the hanging-drop vapour-diffusion method. Crystals of SeMet-labelled VP26 were obtained using a reservoir consisting of 0.1 *M* citric acid pH 3.5, 3.0 *M* sodium chloride and 1%(*w*/*v*) polyethylene glycol 3350, whereas SeMet VP28 was crystallized using a reservoir solution consisting of 25% polyethylene glycol 8000, 0.2 *M* calcium acetate, 0.1 *M* Na HEPES pH 7.5 and 1.5%(*w*/*v*) 1,2,3-heptanetriol. Crystals of SeMet-labelled VP26 diffract to 2.2 Å resolution and belong to space group *R*32, with unit-cell parameters $a = b = 73.92$, $c = 199.31$ Å. SeMet-labelled VP28 crystallizes in space group $P2_12_12_1$, with unit-cell parameters $a = 105.33$, $b = 106.71$, $c = 200.37$ Å, and diffracts to 2.0 Å resolution.

1. Introduction

White spot syndrome virus (WSSV) is a virulent pathogen that is known to infect penaeid shrimp and several crustacean species such as crabs, spiny lobsters, freshwater shrimps and crayfish (Lo *et al.*, 1996; Huang *et al.*, 2001). Clinical signs of infection by this virus include a red colour on the entire body and obvious white spots on the carapace and appendages. WSSV is becoming a great threat not only to the shrimp aquaculture industry but also to marine ecology in general. Previous electron-microscopy studies showed that the intact WSSV virion is a non-occluded enveloped particle with an olive-like shape (Durand *et al.*, 1997; Lu *et al.*, 1997). The complete genome of WSSV is a double-stranded circular DNA of 305 107 bp (Yang *et al.*, 2001). Based on its uniqueness, WSSV is classified into a new virus family, Nimaviridae (van Hulten *et al.*, 2001).

Knowledge of the structures of the viral envelope proteins would contribute to an understanding of their functional roles in host–pathogen interaction. Envelope proteins are believed to be the first molecules to come into contact with the host and are consequently important in cell targeting as well as in triggering host defences (Tsai *et al.*, 2006). VP26 and VP28 are the two most abundant WSSV envelope proteins, accounting for approximately two-thirds of the viral envelope. Expression and purification trials on full-length VP26 and VP28 resulted in the production of insoluble proteins. Sequence analysis using the DAS server (Stockholm University) indicated the presence of a transmembrane region (TM) at the N-terminal region of each of these two proteins. We postulated that removal of the predicted transmembrane region would increase the solubility of the protein constructs and enhance their suitability for crystallization trials. Here, we describe the expression, purification and crystallization of the N-terminal TM-truncated VP26 and VP28 of WSSV, termed rVP26 and rVP28, respectively.

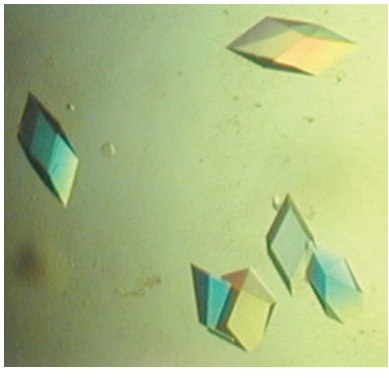


Table 1
Data-collection statistics of SeMet rVP26 and SeMet rVP28.

Values in parentheses are for the last resolution shell.

	SeMet rVP26			SeMet rVP28		
	Peak	Inflection	Remote	Peak	Inflection	Remote
Unit-cell parameters (Å)	$a = b = 73.915$, $c = 199.313$			$a = 105.33$, $b = 106.71$, $c = 200.37$		
Space group	$R32$			$P2_12_12_1$		
Beamline at BNL	X12C			X29		
Wavelength (Å)	0.9782	0.9787	0.9600	0.9796	0.9799	0.9600
Oscillation range (°)	1	1	1	1	1	1
Resolution range (Å)	50–2.2	50–2.6	50–2.7	50–2.0	50–2.1	50–2.1
Observed *hkl*	187787	188815	120101	1818612	1608124	774774
Unique *hkl*	10798	10841	11519	152547	132247	131772
Overall $I/\sigma(I)$	23	19.1	12.3	15.3	15.9	15.5
Redundancy	17.4 (13.9)	17.4 (13.5)	10.4 (10.1)	11.9 (9.7)	12.2 (11.2)	5.9 (5.4)
Completeness (%)	99.9 (99.4)	99.7 (97.4)	99.8 (98.8)	100 (99.7)	100 (100)	99.7 (99.5)
R_{sym}† (%)	9.0 (32.7)	8.8 (32.2)	8.2 (25.9)	8.0 (20.9)	7.2 (17.9)	7.3 (18.1)

† $R_{sym} = \sum_h \sum_i |I_i(h) - \langle I(h)\rangle| / \sum_h \sum_i I_i(h)$.

2. Materials and methods

2.1. Cloning, expression and purification

Genes coding for the transmembrane region-truncated constructs of rVP26 (Asn35–Lys204) and rVP28 (Asn31–Glu204) were amplified from the WSSV genome by PCR using specific primers (for VP26, CGC GGA TCC ATG AAC ACA CGT GTT GGA AGA and CCG GAA TTC TTA CTT CTT CTT GAT TTC GTC CTT; for VP28, CGC GGA TCC AAC ACT GTG ACC AGG ACC ATC GAA and CCG GAA TTC TTA CTC GGT CTC AGT GCC AGA). The PCR products were digested with *Bam*HI and *Eco*RI and further cloned into pGEX6P-1 (GE Healthcare). Overexpression was performed by induction of mid-log phase *Escherichia coli* BL21 (DE3) cells (Novagen) with 0.4 m*M* isopropyl β-D-thiogalactopyranoside (IPTG) for 6–7 h at 298 K. The overexpressed GST-rVP26 and GST-rVP28 proteins (rVP26 and rVP28 proteins with N-terminal GST tags) were purified using Glutathione Sepharose 4B (GE Healthcare), followed by cleavage of the GST tags with PreScission protease (GE Healthcare). The detagged proteins were further purified by size-exclusion chromatography (SEC) using Superdex75 (GE Healthcare) equilibrated in a buffer consisting of 20 m*M* Tris pH 7.4, 150 m*M* NaCl, 10 m*M* DTT and 5% glycerol. The separately purified rVP26 and rVP28 protein fractions were collected and concentrated to final concentrations of 8–10 mg ml^{-1} using a Vivaspin 20 (Vivasciences). Approximately 3 mg of each protein could be purified per litre of culture medium. The selenomethionine (SeMet) substituted rVP26 and rVP28 were expressed in LeMaster medium (Hendrickson *et al.*, 1990) using the methionine-auxotrophic strain DL41. Purification and concentration steps were the same as for the corresponding native proteins. The purification-step results, shown by SDS–PAGE, are given in Fig. 1.

2.2. Crystallization

Purified rVP26 and rVP28 proteins were crystallized by the hanging-drop vapour-diffusion method at 298 K. Initial crystallization screening was performed using the sparse-matrix approach with screening solutions from Hampton Research (Crystal Screens 1 and 2 and Index). In the crystallization screening experiments, 1 µl protein solution was mixed with 1 µl reservoir solution and equilibrated against 500 µl reservoir solution. Crystallization attempts using the native rVP26 and rVP28 proteins were unsuccessful. However, the SeMet-substituted rVP26 and rVP28 proteins readily gave diffraction-quality crystals. These crystals started to appear in 24 h and grew to maximum dimensions of 0.2 × 0.1 × 0.2 mm for rVP26 and 0.3 × 0.1 × 0.1 mm for rVP28 within two weeks.

2.3. Data collection

Prior to data collection, the SeMet-substituted crystals of rVP26 and rVP28 were briefly soaked for 10 s in cryoprotectant solution consisting of their respective reservoir solutions (see §3) supplemented with 30% glycerol. Crystals were picked up in a nylon loop

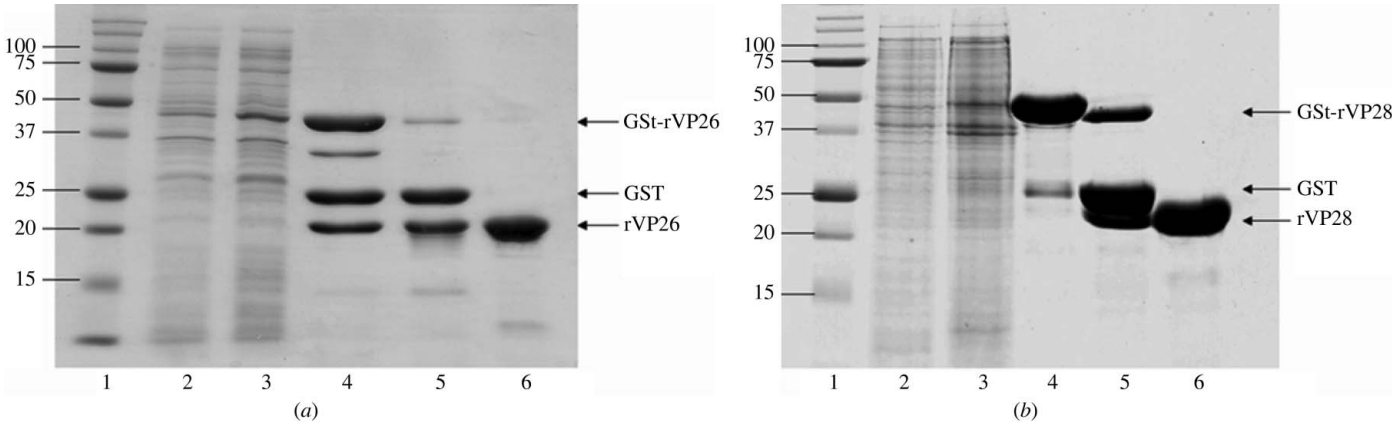
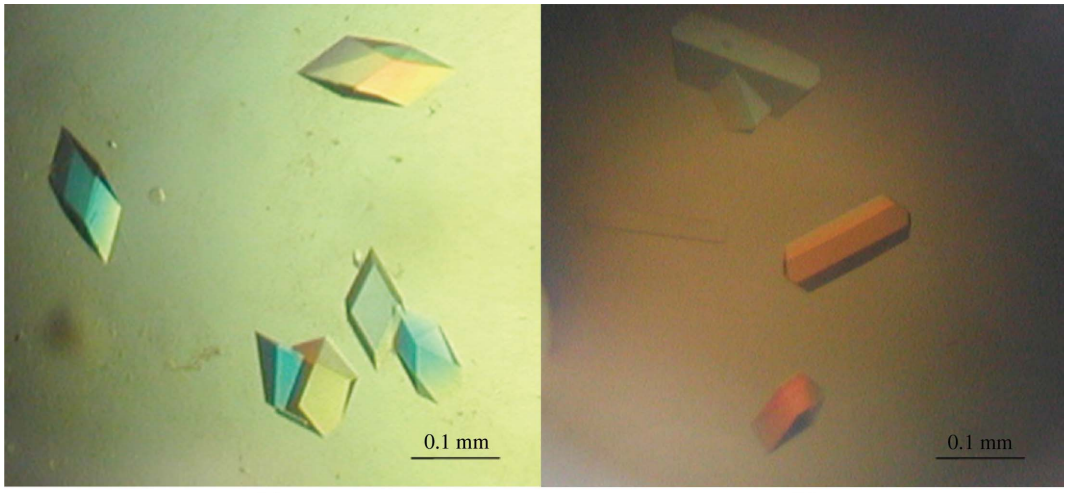


Figure 1
Expression in *E. coli* and purification of (*a*) rVP26 and (*b*) rVP28. Lane 1, molecular-weight markers (kDa). Lane 2, non-induced cells. Lane 3, induced cells. Lane 4, purified GST-fusion protein (GST-rVP26 and GST-rVP28). Lane 5, GST-fusion protein after cleavage by PreScission protease. Lane 6, purified rVP26/rVP28 protein.



(*a*) (*b*)

Figure 2
Crystals of SeMet-labelled (*a*) rVP26 and (*b*) rVP28.

and flash-cooled at 100 K in a cold nitrogen-gas stream (Oxford Cryosystems, Oxford, England). Complete three-wavelength data sets for multiple-wavelength anomalous diffraction (MAD) were collected for rVP26 and rVP28 at beamlines X12C and X29, respectively, at the National Synchrotron Light Source (NSLS), Brookhaven National Laboratory using a CCD detector (Area Detector Systems Corp., Poway, CA, USA). Data sets were processed and scaled using *HKL*-2000 (Otwinowski & Minor, 1997). The data-collection statistics are given in Table 1.

3. Results and discussion

The purified N-terminally truncated VP26 and VP28 proteins (rVP26 and rVP28) consist of 175 and 179 residues, respectively. Both constructs include an additional five amino-acid residues at their N-termini resulting from the GST cleavage sites. Gel-filtration (SEC) profiles as well as dynamic light-scattering (DLS) data showed that rVP26 and rVP28 exist as monomers in solution. For both proteins, the size homogeneity was assayed at various protein concentrations by native gel electrophoresis and DLS. The protein concentration giving the best size-homogeneity profile in the SEC buffer was found to be 8–10 mg ml^{-1}. All crystallization trials were therefore conducted using this protein concentration.

SeMet rVP26 was crystallized using a reservoir solution of 0.1 *M* citric acid pH 3.5, 3.0 *M* sodium chloride and 1%(*w*/*v*) polyethylene glycol 3350. These crystals belong to the rhombohedral system, space group *R*32, with unit-cell parameters $a = b = 73.92$, $c = 199.31$ Å, and contain one molecule in the asymmetric unit ($V_M = 3.00$ Å^3 Da^{-1}). The crystals diffract to 2.2 Å resolution. Crystals of SeMet rVP28 were obtained with a reservoir solution consisting of 25% polyethylene glycol 8K, 0.2 *M* calcium acetate, 0.1 *M* Na HEPES pH 7.5 and 1.5%(*w*/*v*) 1,2,3-heptanetriol. These crystals are orthorhombic, space group $P2_12_12_1$, with unit-cell parameters $a = 105.33$, $b = 106.71$, $c = 200.37$ Å, and contain 12 molecules in the asymmetric unit. The calculated V_M was 2.55 Å^3 Da^{-1} (Matthews, 1968) and corresponds to a solvent content of 51.78%. Using a synchrotron-radiation source, the diffraction of these crystals extends to a resolution of approximately 2.0 Å. Fig. 2 shows the crystals of SeMet rVP26 and SeMet rVP28. Using data sets collected from the SeMet-substituted crystals, the structures of both rVP26 and rVP28 have been successfully determined (Tang *et al.*, 2007)

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