Assembly into single-shelled virus-like particles by major capsid protein VP1 encoded by genome segment S1 of Bombyx mori cypovirus 1

Kyoji Hagiwara and Hisashi Naitow

1Laboratory of Virology, National Agricultural Research Center, Tsukuba, Ibaraki 305-8666, Japan
2Division of Bio-Crystallography Technology, RIKEN Harima Institute, Sayo-gun, Hyogo 679-5148, Japan

The major capsid protein VP1 encoded by genome segment S1 of Bombyx mori cypovirus 1 was expressed in a baculovirus system. In the absence of any other capsid proteins, VP1 was found to assemble into single-shelled virus-like particles. The VP1 particles were more sensitive to acidic conditions than were intact particles.

Viruses in the family Reoviridae have a capsid made up of concentric layers of proteins that are organized as one, two or three distinct capsid shells. Among the nine established genera, namely Orthoreovirus, Orbivirus, Rotavirus, Coltivirus, Aquareovirus, Cypovirus, Fijivirus, Phytoreovirus and Orzavirus (Mertens et al., 2000), and one recently proposed genus, Seadornavirus (Attoui et al., 2000), only cypoviruses have single-shelled particles. The three-dimensional structure of cypovirus particles has been determined using cryo-electron microscopy (Hill et al., 1999; Zhang et al., 1999) and a recent publication suggested that the turret (spike) plays an important role in maintaining capsid integrity (Zhang et al., 2002). However, it is not known whether the major capsid protein of cypoviruses has the ability to assemble into single-shelled particles. Bombyx mori cypovirus 1 (BmCPV-1), a type member of the genus Cypovirus, has six structural proteins, VP1, VP2, VP3, VP4, VP6 and VP7, encoded by segments S1, S2, S3, S4, S6 and S7, respectively (Hagiwara & Matsumoto, 2000; Hagiwara et al., 2002). In this study, we expressed VP1 in insect cells using a baculovirus system to determine the ability of VP1 to assemble into virus-like particles.

The cDNA of genome segment S1 of BmCPV-1 cloned into pBluescript KS+ (Hagiwara et al., 2002) was subcloned into the pBlueBacIII transfer vector for baculovirus expression. Restriction enzyme analysis and DNA sequencing were performed to confirm that the coding sequence of the S1 segment was correctly oriented with the baculovirus polyhedrin promoter. The resulting transfer plasmid and linearized AcNPV DNA were co-transfected into Sf9 cultured cells using CellFECTIN, according to the manufacturer’s instructions (Invitrogen), and recombinant VP1 was expressed in the insect cells.

To detect expression of VP1, antibody against intact virus was produced. BmCPV-1 particles were purified from polyhedra and two BALB/c mice were immunized with 100 µg of these particles, as described previously (Hagiwara et al., 1998). The serum was collected 4 days after the last immunization. In SDS-PAGE and Western blot analysis using BmCPV-1-specific antibody, major bands were detected in recombinant baculovirus-infected Sf9 cells (Fig. 1, lane 3) at the same position as VP1 of purified BmCPV-1 particles (Fig. 1, lane 1), indicating that the major capsid protein VP1 was correctly expressed by the baculovirus system.

Recombinant baculovirus-infected Sf9 cells were treated with BugBuster Protein Extraction Reagent (Novagen) and supernatant was collected after centrifugation at 30,000 g for 5 min. After 10–40% sucrose density gradient centrifugation of the supernatant at 94,500 g for 60 min, the band material was collected and pelleted by centrifugation at 155,000 g for 60 min. Many single-shelled virus-like particles were observed in this fraction after expression of VP1 alone (Fig. 2b-1). To confirm that the virus-like particles were constructed from VP1 protein, immunogold staining of the particles was performed using BmCPV-specific antibody against intact particles according to the method described by Lin (1984). As shown by electron microscopy [Fig. 2(a-2 and b-2)], intact BmCPV-1 and VP1 particles were specifically labelled with gold particles. SDS-PAGE and Western blot analyses of purified VP1 protein (Fig. 1, lane 4) also confirmed that the particles were composed of expressed VP1. These results indicate that VP1 protein has the ability to form a single-shelled particle without the assistance of any of the other structural proteins of BmCPV-1.

To compare the stabilities of the particles at low- and high-pH, intact BmCPV-1 and VP1 particles were subjected to various pH treatments. Intact and broken particles were observed using electron microscopy and were counted. After treatment with 0.2 M NaH2PO4 at pH 5.0 at 37°C for
5 min, broken particles began to appear. Many particles were broken by treatment with 0·2 M NaH$_2$PO$_4$, pH 4·2, at 0°C (on ice) for 5 min, although a few retained their structural integrity (Table 1). VP1 particles were completely broken down by pH 4·2 at 37°C for 5 min, while intact particles were completely broken down by pH 3·7 at 37°C for 5 min (Table 1). Under alkaline conditions the stability of the two particle types was almost the same: both BmCPV and VP1 particles retained their structural integrity when incubated in 20 mM PBS, pH 12·3, at 37°C for 5 min (Table 1). These results suggest that other structural proteins play an important role in stabilizing the particles under acidic conditions.

Analysis of the three-dimensional structure of cypovirus particles by cryogenic electron microscopy has shown that

---

**Fig. 1.** SDS-PAGE and Western blot analyses of VP1 protein expressed by baculovirus. Recombinant VP1 was expressed in Sf9 cells and purified by 10–40% sucrose density gradient centrifugation. Samples were separated by SDS-PAGE (a) and detected by Western blotting using a BmCPV-1-specific antibody (b). Lane 1, purified BmCPV-1 particles; lane 2, uninfected Sf9 cells; lane 3, recombinant baculovirus-infected Sf9 cells; lane 4, purified virus-like particles composed of expressed VP1.

---

**Fig. 2.** Electron micrographs of uranyl acetate-stained BmCPV-1 and VP1 particles. (a) BmCPV-1 particles; (b) VP1 particles. Panels labelled 1 display the purified particles in 20 mM PBS, pH 7·3; panels labelled 2 show immunogold labelling of the particles. Bar, 100 nm.
there are two spike molecules and transcription enzyme complexes along the icosahedral fivefold axes both inside and outside the particles (Zhang et al., 1999). Our study has demonstrated that these exterior and interior proteins are not required for construction of the VP1 shell, but that they may influence the stability of virus-like particles, suggesting that attachment of these proteins on the fivefold axes may play an important role in maturation of the virus particles. It seems that both exterior (spikes) and interior proteins (such as RNA polymerase) recognize the icosahedral fivefold symmetry when they attach to the shell. Thus, the ability of VP1 to form a single-shelled particle independent of other structural proteins is an extremely important function for assembly of cypovirus particles.

**REFERENCES**


**Table 1.** Stabilities of BmCPV and VP1 particles following under high- and low-pH treatments

Double (++) and triple (+++) plus symbols indicate that more than 50% and 80% of the particles remained, respectively, while a single plus (+) symbol indicates that most of the particles were broken down (less than 20% remaining). A minus sign (−) indicates that the particles were completely broken down.

<table>
<thead>
<tr>
<th></th>
<th>PBS</th>
<th>PBS</th>
<th>PBS</th>
<th>PBS</th>
<th>PBS</th>
<th>PBS</th>
<th>PBS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12·3</td>
<td>11·9</td>
<td>5·5</td>
<td>5·0</td>
<td>4·7</td>
<td>4·2</td>
<td>3·7</td>
</tr>
<tr>
<td>BmCPV</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
</tr>
<tr>
<td>On ice</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
</tr>
<tr>
<td>At 37 °C for 5 min</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
</tr>
<tr>
<td>VP1 particles</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
</tr>
<tr>
<td>On ice</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
</tr>
<tr>
<td>At 37 °C for 5 min</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
</tr>
</tbody>
</table>