A single point mutation disrupts the capsid assembly in Sesbania Mosaic Virus resulting in a stable isolated dimer

Anju Pappachan a, Subashchandrabose Chinnathambi b, P.S. Satheshkumar b, H.S. Savithri b, M.R.N. Murthy a,⁎

a Molecular Biophysics Unit, Indian Institute of Science, Bangalore-560 012, India
b Department of Biochemistry, Indian Institute of Science, Bangalore-560 012, India

ARTICLE INFO

Article history:
Received 11 May 2009
Returned to author for revision 31 May 2009
Accepted 29 June 2009
Available online 30 July 2009

Keywords:
Virus capsid
Virus assembly
Virus stability
Ca2+
Protein–protein interactions
X-ray crystallography

ABSTRACT

Protein–protein interactions play a crucial role in virus assembly and stability. With the view of disrupting capsid assembly and capturing smaller oligomers, interfacial residue mutations were carried out in the coat protein gene of Sesbania Mosaic Virus, a T=3 ss (+) RNA plant virus. A single point mutation of a Trp 170 present at the five-fold interface of the virus to a charged residue (Glu or Lys) arrested assembly of virus like particles and resulted in stable soluble dimers of the capsid protein. The X-ray crystal structure of one of the isolated dimer mutants — rCPΔN65W170K was determined to a resolution of 2.65 Å. Detailed analysis of the dimeric mutant protein structure revealed that a number of structural changes take place, especially in the loop and interfacial regions during the course of assembly. The isolated dimer was “more relaxed” than the dimer found in the T=3 or T=1 capsids. The isolated dimer does not bind Ca2+ ion and consequently four C-terminal residues are disordered. The FG loop, which interacts with RNA in the virus, has different conformations in the isolated dimer and the intact virus suggesting its flexible nature and the conformational changes that accompany assembly. The isolated dimer mutant was much less stable when compared to the assembled capsids, suggesting the importance of inter-subunit interactions and Ca2+ mediated interactions in the stability of the capsids. With this study, SeMV becomes the first icosahedral virus for which X-ray crystal structures of T=3, T=1 capsids as well as a smaller oligomer of the capsid protein have been determined.

© 2009 Elsevier Inc. All rights reserved.

Introduction

Sesbania Mosaic Virus (SeMV) is a plant virus with a positive sense single-stranded RNA genome and belongs to the Sobemovirus genus. The protein and nucleic acids of SeMV can be separated and reassembled in vitro. Also, expression of the coat protein (CP) gene of SeMV in Escherichia coli leads to the formation of virus like particles (VLPs). Therefore, SeMV is an excellent model system to study the assembly pathways that lead to the formation of complex virus shells. Earlier structural and functional studies on the native virus, the recombinant coat protein and its various mutants have revealed the following: SeMV (~30 nm in diameter) is a T=3 virus with chemically identical A-, B- and C-subunits occupying quasi-equivalent positions in the icosahedral asymmetric unit of the virus particle. The A-type subunits form pentamers at the five-fold, and the B- and C-type subunits form hexamers at the icosahedral three-fold axes. The subunit arrangement of T=3 and T=1 capsids is shown in Fig. 1. The amino terminus of the polypeptide is ordered from residue 72 in the A- and B-subunits whereas it is ordered from residue 44 in the C-subunit (Bhuvaneshwari et al., 1995). The disordered segment in all the subunits has an arginine rich motif (N-ARM). The segment ordered only in C-subunits contains a β-annulus structure formed by extensive hydrogen bonding interactions of residues 48–54 from one C-subunit with residues 55–59 of the neighboring C-subunit at the icosahedral three-fold (quasi six-fold) and a β-segment (iαA). The virus is stabilized by protein–protein, protein–RNA and Ca2+ mediated protein–protein interactions. Virus like particles (VLPs) formed by the expression of full-length CP encapsidate 23 S E. coli tRNA and CP mRNA (Lokesh et al., 2002). Expression of a deletion mutant lacking the N-terminal 65 residues (rCPΔN65) which results in the removal of the N-ARM, the β-annulus and the iαA leads to the formation of stable T=1 particles (Lokesh et al., 2002). The β-annulus, which was earlier believed to be an important molecular switch controlling the assembly of T=3 VLPs was found to be dispensable (Pappachan et al., 2008; Satheshkumar et al., 2005) The N-ARM, though important for RNA encapsidation, was not essential for capsid assembly (Satheshkumar et al., 2005). Depletion of Ca2+ ions led to slight swelling of virus particles and significantly reduced stability (Satheshkumar et al., 2004). Extensive studies on the VLPs suggested that the assembly is most likely initiated by the dimers of
the capsid protein. Treatment with ethylene diamine tetraacetate followed by incubation in 4 M LiCl results in the dissociation of particles into dimers (Erickson and Rossmann, 1982; Lokesh G.L. unpublished).

In SeMV, the capsid is held together not only by many hydrophobic and hydrogen bonding interactions between the subunits but also by protein–RNA interactions mediated by the arginine rich ARM. Therefore, it might be possible to generate smaller oligomeric states of SeMV protein by disruption of either protein–protein or protein–RNA interactions. Although the quaternary organization of the T = 3 and T = 1 capsids of SeMV are different, the nature of contacts at several interfaces are conserved. The contacts at the five-fold and three-fold interfaces of T = 1 particles closely resemble those across five-fold and quasi three-fold axes, respectively of T = 3 particles. Both in T = 3 and T = 1 capsids, the five-fold interface is the most stable due to tight hydrogen bonding. There are many polar residues (majority serines) lining this interface. At the icosahedral five-fold axes, towards its outer opening is a cluster of five tryptophans (residue 170 in SeMV; Fig. 2).

This residue is conserved across the Sobemoviridae family. Mutation of Trp 170 to Ala, Cys, Lys and Glu in the full-length rCP and rCPΔN65 genes was carried out to break the interactions at the five-fold interface. Mutation to Ala or Cys did not lead to breakdown of particle assembly. However, it was found that rCPW170E, rCPW170K and rCPΔN65W170E and rCPΔN65W170K lead to the formation of stable dimers. Attempts were made to determine the structure of these dimers with the view of ascertaining the conformational changes in the subunit structure upon particle assembly. In this paper, we provide a detailed account of the crystal structure analysis of the rCPΔN65W170K mutant dimer and comparison of its structure with the dimers of native virus, T = 3 and T = 1 VLPs.

**Results**

**Gel filtration and stability studies**

rCPW170A, rCPW170C, rCPW170E, rCPW170K mutants and the corresponding mutants in rCPΔN65 were generated using rCP and rCPΔN65 as template respectively with the appropriate primers as described in Materials and methods. The mutants were over expressed in E. coli BL21 (DE3) cells and purified as described earlier (Lokesh et al., 2002). The wild type and the mutant proteins were subjected to gel filtration analysis using a Superdex S200 analytical gel filtration column (Fig. 3). The mutants were over expressed in E. coli BL21 (DE3) cells and purified as described earlier (Lokesh et al., 2002). The wild type and the mutant proteins were subjected to gel filtration analysis using a Superdex S200 analytical gel filtration column (Fig. 3). rCPW170E and rCPW170K eluted with a mass of ~60 kDa and rCPΔN65W170E and rCPΔN65W170K eluted with a mass of ~50 kDa. These correspond to the molecular mass of dimeric forms of the full length and deletion mutants of CP, respectively. The intact capsids eluted with the void fraction, rCPW170A and rCPW170C also eluted with the fraction corresponding to that of capsids showing that these mutations did not affect capsid assembly.

The stability of the isolated dimer was very low compared to the intact virus. Thermal melting studies using CD showed a Tm ~ 45 °C for the dimer where as for the full capsid it is around 90 °C.

**Crystallization, data collection and quality of the structure**

All the four dimeric mutants were used for crystallization trials. One of the mutants (rCPΔN65W170K) crystallized under oil in the presence of 0.1 M Bis Tris (pH 6.5) and 28% PEG 2000. X-ray diffraction data were collected at 100 K to a resolution of 2.65 Å using a single crystal. The crystal belonged to P1 space group and contained two monomers in the asymmetric unit. Table 1 lists unit cell parameters and data collection statistics. Approximate phases of reflections were determined by molecular replacement using a monomer of rCPΔN65 (PDB code 1vak) as the phasing model. Iterative cycles of model building and refinement improved the quality of the map as revealed by R_free. No electron density was found for the C-terminal 4 residues in
both monomers. There was a break in the main chain density in the external loop (exposed to solvent in the intact virus) spanning residues 131–134. The internal loop (facing RNA in the intact virus) spanning residues 177–185 was disordered in one of the monomers (monomer A) while it was reasonably ordered in the other monomer (monomer B). Residues for which no clear side chain density was observed were truncated to alanines. 99.4% of the residues were in the allowed regions of Ramachandran plot (Ramachandran and Sasisekharan, 1968). Refinement statistics and quality of the structure are shown in Table 2.

### Subunit structure

The structure of the A-subunit superposed reasonably well with the T = 3 and T = 1 monomers with r.m.s.d.s of 0.73 Å and 0.66 Å, respectively. The residual differences in Cα position after the superposition of the backbone structures of the native and rCPΔN65 monomers with the A-subunit of the isolated dimer are shown in Fig. 4A. The deviations are maximum for the internal loop (177–185), the external surface loop (209–225), the Ca2+ binding loop (145–150), the residues which are involved in five-fold and quasi six-fold interactions (156–159) and the N- and C-termini. Most of these segments also have high B-factors (Fig. 4B).

### Dimeric structure

The asymmetric unit of the crystal (the unit cell in P1 space group) contained two monomers. However, the two subunits of the asymmetric unit did not resemble the structure of dimers in the virus capsids. Therefore, to identify the actual dimer, neighboring subunits were generated by translation. When one of the subunit was translated along crystallographic-b, it resulted in an oligomeric structure that closely resembled the dimeric structure of the capsids. An area of 883 Å² is buried in the interface of the two subunits. This is comparable to the area buried in the A/B subunit interface of rCP (852 Å²) and more extensive than the interface of rCPΔN65 dimer (683 Å²). The contacts at the interface also resembled those of A- and B-subunits of virus particles. However, the monomers are closer in the present structure when compared to rCPΔN65. There is a water bridge that connects the two strands across the dimeric interface in rCPΔN65 (Sangita et al., 2004). This water bridge was absent in the present structure. Although the resolution of structure determination in the

---

**Table 1**

Data collection statistics.

<table>
<thead>
<tr>
<th>Unit cell parameters</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>a (Å)</td>
<td>35.88</td>
<td></td>
</tr>
<tr>
<td>b (Å)</td>
<td>41.95</td>
<td></td>
</tr>
<tr>
<td>c (Å)</td>
<td>68.65</td>
<td></td>
</tr>
<tr>
<td>α (°)</td>
<td>81.07</td>
<td></td>
</tr>
<tr>
<td>β (°)</td>
<td>75.00</td>
<td></td>
</tr>
<tr>
<td>γ (°)</td>
<td>84.25</td>
<td></td>
</tr>
</tbody>
</table>

| Space group          | P1 |
| Resolution range (Å) | 41.34–2.65 (2.79–2.65) |
| Total number of reflections | 28,903 |
| No. of unique reflections | 10,322 |
| Mean (I)/(σ(I))      | 9.9 (2.2) |
| R(merge)             | 0.095 (0.469) |
| Completeness (%)     | 93.2 (92.0) |
| Protein subunits in the asymmetric unit | 2 |
| Solvent content (%)  | 38.2 |

---

**Table 2**

Refinement statistics.

<table>
<thead>
<tr>
<th>Parameters</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Rwork (%)</td>
<td>22.4</td>
<td></td>
</tr>
<tr>
<td>Rfree (%)</td>
<td>29.4</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Model quality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of atoms</td>
</tr>
<tr>
<td>Protein atoms</td>
</tr>
<tr>
<td>Water</td>
</tr>
<tr>
<td>Mean B-factors (Å²)</td>
</tr>
<tr>
<td>Protein atoms</td>
</tr>
<tr>
<td>Water</td>
</tr>
<tr>
<td>RMS deviation from ideal value</td>
</tr>
<tr>
<td>Bond length (Å)</td>
</tr>
<tr>
<td>Bond angle (°)</td>
</tr>
<tr>
<td>Residues in Ramachandran plot (%)</td>
</tr>
<tr>
<td>Most allowed region</td>
</tr>
<tr>
<td>Allowed region</td>
</tr>
<tr>
<td>Generously allowed region</td>
</tr>
<tr>
<td>Disallowed region</td>
</tr>
</tbody>
</table>

---

![Fig. 3. Gel filtration chromatographic profile of rCPW170E monitored at 280 nm in a Superdex S200 analytical column precalibrated with known molecular weight standards. AU, absorbance units. The protein peak corresponds to a molecular weight of ~60 kDa. The absorbance peaks corresponding to the molecular weight standards (Tyroglobulin (669 kDa), Ferritin (441 kDa), Aldehyde dehydrogenase (150 kDa), Bovine serum albumin (66 kDa), Carbonic anhydrase (29.0 kDa) and Cytochrome c (12 kDa) respectively, from left to right) are also shown in the figure.](image-url)
present case and for \( T = 1 \) VLPs is comparable, the electron density of the latter is superior due to non-crystallographic symmetry averaging. It is possible to build water molecules at the interface of the isolated dimer without steric clashes. Therefore, the absence of density for water molecules in the present case could also be due to limited resolution of the structure. Superposition of the Cα atoms of the isolated dimer on the corresponding atoms of intact virus A/B dimer and rCP\( \Delta N65 \) dimer resulted in r.m.s.d.s of 1.36 Å and 1.53 Å, respectively. Interestingly, there was a noticeable change in the organization of the subunits of the dimer (Fig. 5). The isolated dimer appears to be less bent and more relaxed than the other two dimers. The matrix obtained by the superposition of subunit A of the isolated dimer and the A-subunit of the dimer found in the virus or rCP\( \Delta N65 \) was used for transformation of both A- and B-subunits. Residual rotations required for the superposition of the transformed B-subunit of the isolated dimer on the B-subunit of the \( T = 3 \) and \( T = 1 \) structures were 5.437° and 3.652°, respectively.

**Calcium binding**

Ca\(^{2+}\) ions are present at the A/B, A/C and B/C interfaces of the native virus and various \( T = 3 \) VLPs (Bhuvaneshwari et al., 1995) and at an equivalent environment in the \( T = 1 \) VLPs (Sangita et al., 2004). The metal ion is liganded by oxygen atoms of the carboxylate groups of Asp 146 and Asp 148 from one subunit and of the main chain carbonyl group of Tyr 207, side chain carbonyl group of Asn 267 and of C-terminal carboxyl group from the neighboring subunit related by quasi three-fold. Since this interface is not present in the structure of the isolated dimer, Ca\(^{2+}\) ions are absent. Consequently, the C-terminal residues Asn 267 and Asn 268, which were involved in Ca\(^{2+}\) binding are disordered. No electron density could be observed for the four C-terminal residues. However, Asp 146 and Asp 149, which are also ligands of Ca\(^{2+}\) in the VLPs, are ordered in the isolated dimer structure. This is probably due to new hydrogen bonding interactions between these residues as well as between these and other residues and two water molecules in the vicinity. The main chain corresponding to the Ca\(^{2+}\) binding loop is displaced by \( \sim 1.5 \) Å in the isolated dimer mutant with respect to wild type.

**Simulation of the quasi three-fold interface**

There is no interface in the crystal structure of the isolated dimer equivalent to the quasi three-fold interface of virus particles. In the native virus, the residues involved in Ca\(^{2+}\) binding and Lys 208, Asp 212 and Asn 222 are involved in hydrogen bonds across the quasi three-fold interface. In order to examine the compatibility of the polypeptide fold of the isolated dimer for the formation of the quasi three-fold interface, the A-subunits of the isolated dimer were superposed on the subunits related by the quasi three-fold axes. The three subunits of such a simulated interface are more widely separated and hence many of the interactions observed across the interface in the virus are now not possible. The main chain is shifted by almost 1.8 Å in the region spanning residues 209 to 225, which

---

**Fig. 4.** (A) Plot showing the deviation of Cα atoms of rCP\( \Delta N65W170K \) mutant from the corresponding atoms of the A-subunit of the native \( T = 3 \) virus structure after superposition. The deviations (Å) are plotted against the respective residue numbers. The ovals indicate the regions of maximum deviation. (B) Plot showing the B-factors of Cα atoms of rCP\( \Delta N65W170K \). The ovals indicate the regions of high B-factor.

**Fig. 5.** The rCP\( \Delta N65W170K \) (green) dimer superposed over the A/B dimer of the native virus (orange). The isolated dimer is less bent or more relaxed than the native dimer.
includes the αD helix (210–219) when compared to the virus. This region faces the exterior of the capsid. A new set of interactions between Lys 208 with Pro 206 are observed in the polypeptide structure of the isolated dimer that are absent in the virus. There is a large shift in the main chain at Asp 212 and its side chain forms new interactions with Thr 209. The main chain interaction between Asn 222 O and Thr 225 N within the same subunit is lost in the isolated dimer. The conformation of Arg 162 side chain is also different from that of the virus. These observations suggest that a series of conformational changes take place in the polypeptide upon formation of the quasi three-fold interface.

The FG loop

The stretch of residues which connect the two strands β-F and β-G is termed as the FG loop. This is an internal loop of the virus and faces RNA. Many of the residues in this region are involved in interactions at the five-fold interface in the virus particles. There is a disulphide bond between Cys 177 and Cys 184 in the native virus particle. This region is highly disordered in the A-subunit of the isolated dimer and electron density is absent for some residues. In the B-subunit, this region is more ordered. Therefore, the following description is based on the conformation of the loop in the B-subunit. There is a large change in the conformation of this loop (Fig. 6) in the isolated dimer when compared to that of the virus. A series of new intrasubunit contacts are observed as a result of this. The orientations of the sulphhydril groups involved in the disulphide bond formation in the virus have changed and hence the disulphide bond is absent in the B-subunit of the isolated dimer. Cys 177 forms a new set of interactions with Ile 179, Asn 180 and Thr 182.

Simulation of five-fold and the quasi six-fold interfaces

In order to examine the compatibility of the structure of isolated dimer for the formation of the five-fold and quasi six-fold interfaces, the subunits forming these interfaces were replaced by the B-subunit of the dimeric structure. The FG loop, residues 156–159, 162–168 and 130–135 are involved in these interfaces. The conformation of many of these residues and the associated main chain are different between the virus and the isolated dimer. The mutated residue Trp 170 is present in the five-fold interface of the virus. This residue is involved in many interactions with Gln 168 at the A1–A5 interface of the five-fold and the C1–B5 interface of the quasi six-fold. With the mutation of Trp 170 to Lys 170, these interactions are disrupted and hence Gln 168 adopts a different side chain conformation in the mutant. Trp 170 interacts with residues 131–133 in the A1–A5 five-fold interface of the native virus. Gln 168 NE2 also strongly hydrogen bonds with Cys 131 O at the five-fold and with Pro 129 at the quasi six-fold interface in the virus structure. As these interactions are absent in the isolated dimer, the main chain of this region shows significant deviations from the wild type. In the A-subunit, no significant electron density is observed for residues 131–134. But this region is ordered in the B-subunit probably due to the interactions made by Pro 131 with Ser 173 of its crystallographic neighbor. The residues 164–167 are involved in the formation of a β-structure with the residues 60–63 of the C-subunit in the native virus and the absence of these interactions probably results in the displacement of the main chain in this region. Steric clashes are observed at the five-fold and six-fold interfaces generated using the B-subunits of the isolated dimer, suggesting that the formation of these interfaces induces conformational changes in the polypeptide.

Discussion

Coat proteins of small isometric viruses assemble into precise capsid structures of a well-defined triangulation number. Although the three-dimensional structures of several icosahedral viruses have been determined, the precise pathway of capsid assembly is not self evident from the final structures. Understanding the mechanism of self-assembly would need the structures of assembly intermediates. It is difficult to trap these intermediates for structural studies due to the cooperative nature of assembly. Only infrequently T = 1 capsid structures for a few viruses with T = 3 wild type architecture have been determined (Erickson et al., 1985; Larson et al., 2005; Sangita et al., 2004). Even more infrequently, structures of small oligomeric assemblies of the coat protein have been determined (Ni et al., 1995, 1996). Achievement of quasi-equivalence in protein–protein interactions of the capsid probably depends on several factors such as interactions between the protein subunits, interactions of capsid protein with the viral genome and on metal ion mediated interactions. Several molecular switches believed to play a crucial role in successful self-assembly of viruses have been proposed in different viruses. In several single-stranded T = 3 RNA viruses such as Sobemovirus and Tombusvirus, the flexible N-terminal arm with an ARM, the β-annulus and the β-A strand are believed to play a crucial role in self-assembly. This segment of the polypeptide is ordered in the C/C dimeric interface but not in the A/B dimeric interface. However, earlier studies on SeMV assembly have suggested that the ordering of the amino terminus may not be a prerequisite for assembly and instead may be a result of assembly in the present manuscript, we have reported the structure of an isolated dimer and the structural alterations that result upon assembly.

With the view of obtaining smaller oligomers for structural studies, several interfacial residues were targeted by site-specific mutagenesis. Several of the mutations tested did not affect the assembly of T = 3 particles. However, mutation of a single Tryptophan (Trp 170) to a charged residue (Lys or Glu) resulting in total disruption of virus assembly and resulted in the formation of stable soluble dimers. These Trp residues contribute to the hydrophobic association of the subunits at the icosahedral five-fold axes (Fig. 2). The electrostatic repulsion between the five charged residues (Lys or Glu) resulting from the mutation was sufficient to disrupt the assembly. It has been proposed that a pentamer of five dimers initiates the assembly of SeMV particles.

Fig. 6. Superposition of monomers of the native virus (orange), rCPΔN65 (grey) and rCPΔN65W170K (green). The region showing the FG loop is circled in the figure. The FG loop has a distinctly different conformation in the isolated rCPΔN65W170K.
Interfacial residue mutations have been carried out in many viruses though there are only a few examples where it has resulted in stable soluble smaller oligomers of the coat protein. In Physalis Mottle Virus (PhMV), certain mutations of interfacial residues in the rCP led to partially folded large oligomers in solution (Umashankar et al., 2003). In the bacteriophage MS2, mutation of a Trp to an Arg prevented assembly beyond the dimeric state (Ni et al., 1995). In the bacteriophage GA, mutations at four sites in the capsid protein led to the formation of dimers (Ni et al., 1996). In Murine Leukaemia Virus, mutations which reversed the charge of residues that mediate the inter-hexamer contacts blocked particle formation (Mortuza et al., 2008). Mutations in the interfacial residues carried out in other viruses have resulted in defects of assembly. Point mutations near the cleavage site in Nudaurelia capensis virus (NoV) VLP disrupted both folding and assembly (Taylor and Johnson, 2005).

The dimer that resulted from W170K mutation resembles the A/B dimer and not the C/C dimer of the virus particle. Assembly of SeMV and a few other viruses appears to be initiated by the A/B dimers (Krol et al., 1999; Rossmann et al., 1983; Satheshkumar et al., 2005). Although the isolated dimer structure is similar to the A/B dimer of the intact virus, there are some significant differences. The dimeric interfaces are similar. However, there are larger variations towards the edge of the dimer. The isolated dimer can be considered “more relaxed” than the relaxed A/B dimer of the assembled capsid. Perhaps the dimer attains the final conformation found in the virus only after assembly and interactions with RNA and Ca$^{2+}$. Extensive efforts made for the crystallization of the isolated full-length CP dimer were unsuccessful. This might be due to the inherent disorder of the amino terminal segment.

Ca$^{2+}$ is bound in the quasi three-fold interfaces of the capsid. The present study shows that Ca$^{2+}$ binding is necessary for the ordering of the C-terminus. The C-terminus was also disrupted in mutant capsids of SeMV in which the two aspartates involved in Ca$^{2+}$ binding were mutated to asparagines (Sangita et al., 2004). The regions involved in the quasi three-fold contacts exhibit the largest differences between the isolated dimer and the intact virus. These large differences are probably due to the quasi three-fold interactions present in the virus but not in the dimers and also due to the absence of bound Ca$^{2+}$ in the dimers.

The FG loop residues, which face RNA, have relatively high B-factors even in the native virus, which implies that this region is flexible. In the isolated dimer, this region is either disordered (subunit A) or shows a significant change in conformation (subunit B). If a five-fold or a quasi six-fold protomer is generated using the B-subunit of the isolated dimer, steric clashes are observed between the FG loops in both five-fold and six-fold interfaces. Similar results were reported in the case of the isolated MS2 dimer (Ni et al., 1995). Certain mutations in the RNA interacting domain of BMV had debilitating effects on the conformation and RNA packaging in BMV (Calhoun and Rao, 2008). Conformational flexibility of this region may be required for proper binding of the RNA and for the formation of stable capsids.

The stability of the virus particles is due both to the intrinsic stability of the polypeptide and the packing interactions of the capsid. Studies on association energies of protein–protein interactions of viral capsids have suggested their importance for pathways of capsid assembly (Shepherd and Reddy, 2005). These interactions must be strong enough to hold the capsid during viral transmission while it must be flexible enough to allow uncoating and release of genome upon infection. The present work shows the importance of protein–protein interactions in the stability of SeMV capsids. The stability of the isolated dimer is only ~45 °C while that of the intact capsids is ~90 °C. The results of the present investigations suggest that local interactions at inter-subunit interfaces play an important role in capsid assembly. A single point mutation at the five-fold interface affected local folding in the nearby segments, the impact of which was transferred to loops and other interfaces preventing capsid assembly and resulting in the formation of soluble dimers. These results reflect the requirement for precise inter-subunit interactions and conformational flexibility of loops and interfaces for the correct assembly of viral capsids.

### Materials and methods

#### Site directed mutagenesis

Site directed mutagenesis was carried out in the rCP gene and the rCPΔN65 gene using PCR based sense and antisense approach (Weiner et al., 1994) with overlapping sense and antisense mutant oligonucleotide primers (Table 3). The primers were designed with Kpn21 restriction site for easy screening of the mutant clones. The PCR was carried out using Deep Vent DNA polymerase (New England Biolabs) with appropriate primers and template. The PCR product was digested with Dpn1 enzyme to remove the template, following which it was transformed to E. coli DH5α cells. The plasmids were isolated from the colonies and screened for mutation using restriction digestion and the mutations were confirmed by DNA sequencing.

### Expression and purification

The mutant clones were transformed in E. coli BL21 DE3 cells containing the helper plasmid pSBET A for efficient expression (Lokesh et al., 2002). Single colonies of the transformed cells were initially grown in 25 mL of LB medium and transferred to 500 mL of Terrific Broth medium. Proteins were expressed by inducing the culture with 0.3 mM IPTG for 12 h at 20 °C when the absorbance was ~0.6 OD at 660 nm. Cells were harvested by centrifugation, resuspended in lysis buffer containing 50 mM Tris pH 8, 200 mM NaCl, 2% Triton X100 and 30% glycerol and lysed by sonication on ice. The supernatants obtained after centrifugation of the cell lysate were

### Table 3

<table>
<thead>
<tr>
<th>Name</th>
<th>5′-3′ Sequence</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP W170E-s</td>
<td>TCTGGGCCAGGCTGAGTCCGGATCTGCA</td>
<td>Primers used for W170E mutation in CP gene.</td>
</tr>
<tr>
<td>CP W170E-a</td>
<td>TGACAATCCGGCCACCTCAATCTGACCA</td>
<td>Kpn21site created is underlined.</td>
</tr>
<tr>
<td>CP W170K-s</td>
<td>TCTGGCGAGGCTGAGTCCGGATCTGCA</td>
<td>Primers used for W170K mutation in CP gene.</td>
</tr>
<tr>
<td>CP W170K-a</td>
<td>TGACAATCCGGCCACCTCAATCTGACCA</td>
<td>Kpn21site created is underlined.</td>
</tr>
<tr>
<td>CP W170A-s</td>
<td>TCTGGCGAGGCTGAGTCCGGATCTGCA</td>
<td>Primers used for W170A mutation in CP gene.</td>
</tr>
<tr>
<td>CP W170A-a</td>
<td>TGACAATCCGGCCACCTCAATCTGACCA</td>
<td>Kpn21site created is underlined.</td>
</tr>
<tr>
<td>CP W170C-s</td>
<td>TCTGGCGAGGCTGAGTCCGGATCTGCA</td>
<td>Primers used for W170C mutation in CP gene.</td>
</tr>
<tr>
<td>CP W170C-a</td>
<td>TGACAATCCGGCCACCTCAATCTGACCA</td>
<td>Kpn21site created is underlined.</td>
</tr>
<tr>
<td>CPAN65 W170E-s</td>
<td>TCTGGCGAGGCTGAGTCCGGATCTGCA</td>
<td>Primers used for W170E mutation in CPAN65gene.</td>
</tr>
<tr>
<td>CPAN65 W170E-a</td>
<td>TGACAATCCGGCCACCTCAATCTGACCA</td>
<td>Kpn21site created is underlined.</td>
</tr>
<tr>
<td>CPAN65 W170K-s</td>
<td>TCTGGCGAGGCTGAGTCCGGATCTGCA</td>
<td>Primers used for W170K mutation in CPAN65gene.</td>
</tr>
<tr>
<td>CPAN65 W170K-a</td>
<td>TGACAATCCGGCCACCTCAATCTGACCA</td>
<td>Kpn21site created is underlined.</td>
</tr>
</tbody>
</table>
MrN and HSS thank DST and DBT for financial support. AP and PSS acknowledge the Council for Scientific and Industrial Research (CSIR), Government of India for the award of senior research fellowships.

References


Leslie, A.G.W., 1992. Recent Changes to the MOSFLM Package for Processing Film and Image Plate Data.


Taylor, D.J., Johnson, J.E., 2005. Folding and particle assembly are disrupted by single-point mutations near the autocatalytic cleavage site of Nduaurel cpps. Biochemistry 44 (2), 401–408.

