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Structural studies on recombinant *T* = 3 capsids of Sesbania mosaic virus coat protein mutants

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When expressed in *Escherichia coli*, the recombinant coat protein (rCP) of Sesbania mosaic virus (SeMV) was shown to self-assemble into $T = 3$ capsids encapsidating CP mRNA and 23S rRNA derived from the host. Expression of CP-P53A, in which a conserved proline at position 53 in the β-annulus was substituted by alanine (CP-P53A), also produced similar capsids. Purified rCP and CP-P53A particles were crystallized and X-ray crystal structures of their mutant capsids were determined to resolutions of 3.6 and 4.1 Å, respectively. As in the native viral CP, the CPs in these recombinant capsids adopt the jelly-roll β-sandwich fold. The amino-terminal residues of the *C* subunits alone are ordered and form the β-annulus structure at the quasi-sixfold axes. A characteristic bend in the β-annulus remains unaffected in CP-P53A. The quasi-threefold interfaces of the capsids harbour calcium ions coordinated by ligands from the adjacent threefold-related subunits in a geometry that is analogous to that observed in the native capsid. Taken together with studies on deletion and substitution mutants of SeMV CP, these results suggest the possibility that the β-annulus and nucleic acid-mediated interactions may be less important for the assembly of sobemoviruses than previously envisaged.

1. Introduction

Sesbania mosaic virus (SeMV) is a single-stranded RNA virus belonging to the family Sobemoviridae (Tamm & Truve, 2000). The coat protein (CP) adopts an eight-stranded antiparallel β-barrel motif and is arranged in a $T = 3$ icosahedral lattice. The CP subunits occur in three quasi-equivalent environments designated *A*, *B* and *C* (Bhuvaneshwari *et al.*, 1995; Murthy *et al.*, 1997). Calcium ions that enhance the stability of the capsids are present at the interfaces of these subunits. At the N-terminal end of the CP there is an arginine-rich motif (ARM, residues 28–36) that is believed to be involved in RNA binding and a β-annulus (residues 48–58) formed by the ordered amino-terminal arms of the *C* subunits at the quasi-sixfold axes. The polypeptide backbone makes a turn near Pro53 to enable interaction between adjacent *C* subunits related by icosahedral threefold symmetry. The proline residue is conserved in CPs belonging to the sobemoviruses and also in tomato bushy stunt virus (TBSV; Harrison *et al.*, 1978), carnation mottle virus (Morgunova *et al.*, 1994) and tobacco necrosis virus (TNV; Oda *et al.*, 2000) where the β-annulus is observed, suggesting that the presence of this residue is probably important for the formation of the β-annulus.

When the gene coding for SeMV CP (rCP) was expressed in *Escherichia coli*, virus-like particles (VLPs) were produced that co-migrated with the native SeMV CP in SDS–PAGE and

Table 1
Data-reduction statistics of recombinant and mutant $T = 3$ capsids.

Values in parentheses refer to the last resolution shell.

Parameter	rCP	CP-P53A
Space group	*R*3	*C*2
Unit-cell parameters		
a (Å)	291.3	471.5
b (Å)	291.3	330.1
c (Å)	291.3	351.5
α (°)	61.8	90.0
β (°)	61.8	131.1
γ (°)	61.8	90.0
Resolution limits (Å)	15.0–3.6 (3.7–3.6)	20.0–4.1 (4.3–4.1)
Total No. of observations	318497	324695
No. of unique reflections	191916	301529
$I/\sigma(I)$	5.3 (2.4)	2.48 (1.1)
Completeness (%)	75.6 (62.2)	92.9 (74.8)
$R_{\rm merge}$ (%)	15.5 (36.3)	17.8 (44.1)

† $R_{\rm merge} = \sum_{hkl} |I - \langle I \rangle| / \sum I$, where I is the observed intensity and $\langle I \rangle$ is the average intensity from observations of symmetry-related reflections, respectively.

Table 2
Refinement statistics of recombinant $T = 3$ capsids of SeMV.

Parameters	rCP	CP-P53A
No. of protein atoms per subunit		
Subunit *A*	1447	1419
Subunit *B*	1447	1408
Subunit *C*	1632	1616
No. of ions per asymmetric unit	3	3
$R_{\rm work}$ (final) (%)	23.2	26.8
$R_{\rm free}$ (final) (%)	23.4	27.0
Total No. of reflections	159147	297206
R.m.s.d.s		
Bond lengths (Å)	0.008	0.008
Bond angles (°)	1.3	2.8
Dihedral angles (°)	25.3	25.7
Improper angles (°)	0.92	3.13
Ramachandran statistics (%)		
Most favoured region	83.6	76.7
Additionally allowed region	16.0	21.6
Generously allowed region	0.2	1.5
Disallowed region	0.2	0.2

enclosed *E. coli* 23S ribosomal RNA and CP mRNA. Site-specific mutation of Pro to Ala at position 53 in the β-annulus (CP-P53A) also led to the formation of $T = 3$ capsids. The stability properties of rCP and CP-P53A were similar to those of the native capsids.

In this communication, we present the X-ray structure of rCP and CP-P53A and discuss the results in the light of observations on the assembly characteristics of other mutants.

2. Materials and methods

2.1. Crystallization

The mutant capsids were purified using previously described protocols (Lokesh *et al.*, 2002). The best crystals of rCP were obtained using the hanging-drop method when the capsid at a concentration of 10 mg ml^{-1} was precipitated with 4% PEG 3350, 0.1 *M* $MgCl_2$, 5% isopropanol and 0.05 *M* HEPES pH 7.5. For CP-P53A, a combination of 10–12%(*w*/*v*) PEG 3350 with 0.1–0.3 *M* Li_2SO_4, 5% isopropanol and 0.05 *M* HEPES pH 7.5 gave good crystals. The capsids crystallized in space group *R*3 for rCP and *C*2 for CP-P53A. The unit cells of rCP and CP-P53A are compatible with one and two VLPs, respectively.

2.2. Data collection and processing

X-ray diffraction data were collected to 3.6 and 4.1 Å resolution from single crystals of rCP and CP-P53A, respectively, at liquid-nitrogen temperature. The crystals were soaked in 20% glycerol for 1 min prior to the start of exposure. The frames were indexed using *DENZO* and the processed frames were scaled and post-refined using *SCALEPACK* (Otwinowski & Minor, 1997). The data-processing statistics are listed in Table 1.

2.3. Structure determination of recombinant $T = 3$ capsids

The structure solution of rCP was achieved using molecular replacement starting with the native SeMV capsid as the phasing model. The orientation of the rCP particles in the unit cell was determined by locked self-rotation functions (Tong & Rossmann, 1990). The cell origin was taken as the centre of the virus particle, as the rhombohedral cell is compatible with only one virus particle. The values obtained from the locked self-rotation function correspond to an orientation of the icosahedral symmetry axes in the *R*3 orthogonal system such that two mutually perpendicular twofolds are at $(\Phi, \psi) = (20.30, 69.09^\circ)$ and $(\Phi, \psi) = (90.00, 50.30^\circ)$ and a fivefold is at $(\Phi, \psi) = (20.30, 37.38^\circ)$, suggesting that the particle orientation is close to that of the particles in the wild-type crystals. The NCS matrices corresponding to this orientation were used for electron-density averaging using locally developed programs (Bhuvaneshwari *et al.*, 1995). The *R* factor and correlation coefficient at the end of averaging were 28.7 and 73.8%, respectively {*R* is defined as $100(|F_o - F_c|)/\sum F_o$ and the correlation coefficient as $\sum(F_o - \langle F_o \rangle) \times (F_c - \langle F_c \rangle) / [\sum(F_o - \langle F_o \rangle)^2 \times \sum(F_c - \langle F_c \rangle)^2]^{1/2}$}.

A self-rotation function computed for CP-P53A for $\kappa = 72$, 120 and 180° using reflections in the resolution range 12–5.5 Å confirmed the icosahedral symmetry of the capsids. As anticipated for a particle sitting on a crystallographic twofold, only one set of icosahedral peaks were observed in the rotation functions. The structure solution was obtained using *AMoRe* (Navaza & Saludjian, 1997) with the full capsid of $T = 3$ native SeMV as the search model. The program placed the particle on the twofold at (1/2, 0, 1/2). The correlation coefficient and *R* values at the end of *AMoRe* structure solution were 49.9 and 34.9%, respectively.

2.4. Refinement and analysis of $T = 3$ recombinant capsid structures

The model was refined using *CNS* v.1.1 (Brünger *et al.*, 1998). Rounds of refinement were interspersed with manual rebuilding into difference Fourier and Fourier maps using the interactive graphics program *O* (Jones *et al.*, 1991). Strict icosahedral symmetry was imposed at all stages of refinement.

The details of refinement statistics are presented in Table 2. The electron-density map was further improved by cyclic 60-fold NCS averaging for rCP.

The quality of the final model was assessed using the program *PROCHECK* (Laskowski *et al.*, 1993). The structures were superposed using the program *ALIGN* (Cohen, 1997). The buried surface areas and the association energies were evaluated from the VIPER website (Reddy *et al.*, 2001). *MOLSCRIPT* (Kraulis, 1991) and *BOBSCRIPT* (Esnouf, 1997) were used for generating the figures and *RASTER3D* (Merritt & Bacon, 1997) was used for rendering them to TIF format.

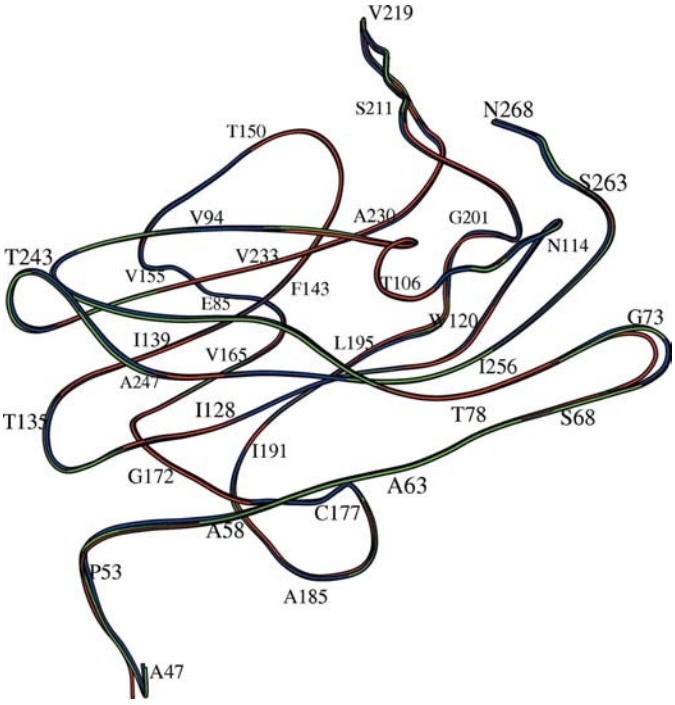

Figure 1
Superposition of the *C* subunits of native SeMV (blue), rCP (red) and CP-P53A (green).

3. Results

The CPs of the native and recombinant capsids have a similar organization of secondary-structural elements, with the core consisting of a β-sandwich jelly-roll motif. There is a disulfide bridge between Cys177 and Cys184 that stabilizes the internal loop in each of the subunits in all the capsids. There are three salt bridges, two connect strand βI to βB (Glu85 and Arg249) and βD (Lys117 and Glu260), and the third of which connects αD to the adjacent loop (Asp212 and Lys202). The buried residues in each subunit form two clusters, of which cluster 1 comprises the conserved residues Met141, Tyr127 and Val169 while cluster 2 is composed of Val99, Val123, Phe143 and Cys252. The rCP and CP-P53A superpose well with the native CP with an overall r.m.s.d. of 0.3 Å (Fig. 1). The maximum deviation (~0.8 Å) is observed in the regions located near the N-terminus and the internal and external loop regions.

The native and recombinant capsids are ~288 Å in diameter and their shells have a thickness of ~20 Å. The diameters of the openings at the icosahedral fivefold and threefold are ~8 and ~7 Å, respectively. These openings span a length of 27 and 22 Å at the fivefold and threefold axes, respectively. The threefold opening is continuous in rCP and CP-P53A from the inner end to the outer end, while in the native capsid it is obstructed by an ion near the inner end (Fig. 2).

Figure 2
(*a*) Top view of the threefold axis in the native capsid on looking down the quasi-threefold axis. (*b*) Top view of the threefold axis in the rCP capsid on looking down the quasi-threefold axis.

The intersubunit interactions in the recombinant capsids match well those of the native capsids. The icosahedral fivefold interface has the largest interacting surface area and the most favourable association energy owing to the large number of polar residues. There is a salt bridge between Asp238 of one subunit and Arg249 of the fivefold partner in both the native and recombinant capsids. In addition to many hydrogen bonds, the quasi-threefold interface houses a calcium ion that adopts octahedral coordination with ligands Asp146 and Asp149 from one subunit and Tyr207, Asn267 and Asn268 from another subunit. The contact distances of the ion and its ligands are slightly higher in rCP and CP-P53A capsids compared with the native capsid. The twofold interface is the least extensive of all the interfaces. The quasi-twofold has fewer contacts than the icosahedral twofold interface, mainly owing to the ordering of the βA arms.

The conformation of the β-annulus is similar in rCP and native capsids. The residues Gly48–Ala58 contribute to the formation of the β-annulus *via* hydrogen bonding. Among these, a conserved proline residue at position 53 introduces a bend in the native polypeptide. In CP-P53A, the substitution of the conserved proline with alanine at position 53 does not seem to affect either the bend or the conformation of the β-annulus. The hydrogen-bonded β-annulus structure may be a highly preferred state and imposes the bend required for its formation at the alanine residue.

4. Discussion

Assembly in sobemoviruses is known to occur only in the presence of the nucleic acid and there have been no reports of the occurrence of empty capsids. Therefore, it was anticipated that the absence of genomic RNA would affect the capsid structure of the virus. The non-specific encapsidation of 23S rRNA in rCP produced no substantial changes in either the stability properties (Satheshkumar *et al.*, personal communication) or the structure of the recombinant capsids, which were similar to those of the native virus. However, in an earlier study it was shown that the absence of calcium-mediated interactions affected the stability of the capsid profoundly and led to drastic changes in the conformations of residues at the calcium-binding site (Sangita *et al.*, 2004; Satheshkumar *et al.*, 2004). Thus, ion-mediated interactions seem to be of greater importance for the structural stability of the capsids.

The substitution of a conserved Pro by Ala at a crucial bend in the β-annulus (position 53) produced no noticeable effect in either the structure or assembly of the mutant capsids and the β-annulus remained intact. In an independent study, mutational analysis of the residues that form the β-annulus showed that even complete deletion of all the residues did not abolish capsid assembly and the mutant capsids showed stability characteristics that were similar to those of the wild-type capsids (Satheshkumar *et al.*, personal communication). Therefore, either a different segment compensates for the deletion or the β-annulus does not play a predominant role in the capsid assembly. The presence of the β-annulus in the CP-P53A mutant, similar to that seen in the native capsid, suggests that the bending of the polypeptide chain is indeed conferred by the hydrogen-bonding interactions of the residues that form the β-annulus and not by the proline residue alone. Nonetheless, it is important to note that the proline residue is invariant in all the known homologous viruses. The structures of both rCP and CP-P53A capsids indicate that despite the differences in the nucleic acid that is encapsidated and the manipulation of the crucial proline at the β-annulus, the core of the CP and the interactions at various interfaces remain largely unaffected.

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