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Structural Transitions of Satellite Tobacco Mosaic Virus Particles

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Satellite tobacco mosaic virus (STMV) can undergo at least two physical transitions that significantly alter its mechanical and structural characteristics. At high pH the 17-nm STMV particles expand radially by about 5 Å to yield particles having diameters of about 18 nm. This pH-induced transition is further promoted by aging of the virions and degradation of the RNA, so that swollen particles ultimately appear even at neutral pH. While the native 17-nm particles crystallize as orthorhombic or monoclinic crystals which diffract to high resolution (1.8 Å), the enlarged 18-nm particles crystallize in a cubic form which diffracts to no better than 5 Å. In the transition, not only do the capsid protein subunits move radially outward, but the helical RNA segments with which they interact do as well. This is noteworthy because it demonstrates that the RNA and the protein shell are capable of coordinated movement, and that neither structure is rigidly defined or independent of the other. Using atomic force microscopy, it can be shown that STMV particles, upon drying, lose their mechanical rigidity and undergo deformation. Virions initially 17 nm in diameter shrink to more uniform final sizes than do 18 nm, initially swollen particles. This transition appears to be irreversible, as the particles do not reassume their former size nor structural rigidity upon rehydration. Evidence is also presented that preparations of native virus and their crystals are naturally somewhat heterogeneous and contain a variety of particles of anomalous size.

INTRODUCTION

Satellite tobacco mosaic virus (STMV) is a T = 1 particle of about 17 nm diameter containing a single-stranded RNA genome of 1058 bases (Mirkov et al., 1989) inside a capsid composed of 60 identical copies of a coat protein having 159 amino acids and $M_r = 17,542$ (Valverde and Dodds, 1986, 1987; Valverde et al., 1991).

The structure of STMV was solved by X-ray crystallography and reported at 1.8 Å resolution (Larson et al., 1998) for the orthorhombic form. The coat protein was shown to be a "Swiss roll" beta barrel of 123 carboxyl-terminal residues and a long, extended amino terminal strand of 36 amino acids. The first 12 amino terminal residues were not visible and apparently in the interior of the virus.

An unanticipated feature of the STMV structure was the appearance of nearly 45% of the total viral RNA in the electron density maps. This was visualized as 30 double-helical segments of seven base pairs with an additional base stacked at either 3′ terminus (Larson et al., 1993a, b). The double-helical RNA segments were closely associated with dimers of coat protein and were oriented so that viral twofold axes were perpendicular to the helical axes of the segments and coincident with their central dyad. Thus the RNA segments were disposed in a manner consistent with the icosahedral symmetry of the particle (Casper and Klug, 1962; Horne, 1974). From the 1.8 Å resolution, refined STMV structure (Larson et al., 1998), it was possible, with a high degree of certainty, to describe the distribution of solvent molecules both inside and outside the capsid and to define their roles in maintaining intermolecular interactions. In addition, the structural association between the capsid protein and the visible portions of the RNA were delineated in detail.

In the course of additional X-ray diffraction and atomic force microscopy (AFM) studies of STMV, it became evident that the virus particles could undergo certain structural transitions. These occurred as a consequence of age, alteration of the pH, and as a consequence of drying. The transitions in structure are accompanied by changes in the mechanical properties of the particles as well as their sizes. Structural changes of this nature have long been known to occur in T = 3 icosahedral plant viruses (Kaper, 1975).

RESULTS

If freshly prepared STMV samples are deployed for crystallization at 12–20% saturated ammonium sulfate using vapor diffusion below pH 8.0, they consistently form orthorhombic crystals above 12°C, and monoclinic crystals at colder temperatures. Above pH 8.0, at any temperature greater than 2°C, cubic crystals grow. Orthorhombic crystals of STMV diffract to at least 1.8 Å resolution, and the structure of STMV was refined to that resolution limit (Larson et al., 1998). The monoclinic STMV crystals diffract to about 2.7 Å, possibly higher.
FIG. 2. Central cross section of STMV based on the model refined at 1.8 Å resolution by X-ray crystallography (Larson et al., 1998). This model is derived from the orthorhombic crystal form which yields a 17-nm particle by AFM and quasi elastic light scattering. The protein is shown in ribbon format in red, and the RNA as all atoms in white. Tabulated below are relevant values for the native and swollen particles.

<table>
<thead>
<tr>
<th>State of Virion</th>
<th>Maximum Radius</th>
<th>Minimum Radius</th>
<th>Mean Radius</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native Protein</td>
<td>90.73 Å</td>
<td>52.71 Å</td>
<td>73.08 Å</td>
<td>5.68 Å</td>
</tr>
<tr>
<td>Swollen Protein</td>
<td>95.06 Å</td>
<td>57.95 Å</td>
<td>78.76 Å</td>
<td>5.73 Å</td>
</tr>
<tr>
<td>Native RNA</td>
<td>64.69 Å</td>
<td>43.14 Å</td>
<td>54.39 Å</td>
<td>5.73 Å</td>
</tr>
<tr>
<td>Swollen RNA</td>
<td>70.48 Å</td>
<td>48.89 Å</td>
<td>60.11 Å</td>
<td>6.25 Å</td>
</tr>
<tr>
<td>SO₄ in Native</td>
<td></td>
<td></td>
<td>73.74 Å</td>
<td></td>
</tr>
<tr>
<td>SO₄ in Swollen</td>
<td></td>
<td></td>
<td>79.99 Å</td>
<td></td>
</tr>
</tbody>
</table>

FIG. 1. (a) Variation of the correlation coefficient (CC) and R value as a function of radial displacement from the particle center based on 5–20 Å data cut at F ≥ 5σᵣ. The darker curves are for the orthorhombic STMV model placed in the cubic cell and rotated 10.8° about the body diagonal and then expanded in 10 Å by 0.1 Å increments. Clearly, the optimum radial expansion is slightly more than 5 Å. For comparison (light curves), a similar expansion of the STMV model in the orthorhombic cell was performed starting from a particle shrunken by 5 Å and then expanded 10 Å by 0.1 Å increments. (b) Variation of CC and R as a function of the rotation angle about the body diagonal of the cubic cell using 10–20 Å data (dark curves) and 5–20 Å data (light curves), both with F ≥ 5σᵣ. The particle was expanded by 5 Å from the original orthorhombic model and then rotated 120° in 0.1° increments about the diagonal. The optimum rotation is about 10.8°, which further refinement showed to be 11.19°. (c) Variation of CC and R as a function of particle translation along the body diagonal using 5–20 Å data cut at F ≥ 5σᵣ. The particle was expanded by 5.38 Å and rotated by 11.11° before the 5 Å translation along the diagonal was executed using 0.1 Å increments. Clearly the correct particle position is the origin.
and their structure is now also solved (unpublished data). Cubic STMV crystals, however, diffract to no more than 5 Å resolution even in the best of cases (Koszelak et al., 1995).

As the STMV ages through storage or standing, however, the pH at which cubic crystals appear decreases, so that after several years, when the RNA is fragmented, as clearly shown by polyacrylamide gel electrophoresis, cubic crystals appear even at pH 7. Often in aged preparations, near neutral pH, both orthorhombic and cubic crystals coexist in the same crystallization samples. The RNA of STMV, although it does eventually break down, is remarkably stable. If, for example, STMV particles are exposed to pH 9.0 for 24 to 36 h, their RNA, when analyzed by agarose gel electrophoresis, is almost entirely intact. Other experiments (Day et al., 2001) show that even heating to 90°C produces little disruption of the encapsidated RNA conformation in particles extensively digested with proteinase K.

Cubic STMV crystallizes in space group $P2_13$ with $a = 257.25$ Å. This implies that the STMV particle must have its center on the body diagonal (a threefold axis) with an icosahedral threefold axis coincident with the body diagonal. The origin and the point (1/4, 1/4, 1/4) are equivalent positions and so the search space is restricted to a position along the body diagonal between (0, 0, 0) and (1/8, 1/8, 1/8) and rotation angle about the threefold axis of 120°. Under these constraints, the interparticle distance varies from a maximum of 181.9 Å at the origin to a minimum of 157.5 Å at (1/8, 1/8, 1/8). The minimum interparticle distance in the high-resolution structure of the orthorhombic STMV is 164.4 Å. A virus particle positioned at (0.057, 0.057, 0.057) would have an interparticle distance (165.1 Å) approximately the same as in the orthorhombic STMV. The initial assumption was that the particles in cubic STMV were essentially identical to those observed in the orthorhombic form and, therefore, they must be positioned near (0.057, 0.057, 0.057). However, placing the orthorhombic STMV model in the cubic cell at this position and translating along and rotating about the threefold axis for maxima in the correlation coefficient (CC) produced a number of possible solutions with correlation coefficients in the 0.28–0.30 range for data in the 6–15 Å range. $R$ factors were above 0.50 in all cases. In other words, there were no obvious solutions resulting from these searches.

The AFM work reported herein shed much light on the problem. First, it suggested that the particle diameter was approximately 180 Å, which implies that the particle lies either on the origin or very close to it. Second, the packing of particles appeared to be hexagonally close packed. The only way to obtain such packing in space group $P2_13$ is for particles to be centered on the origin, in which case the AFM observed crystal face would be the (111) face.

In accordance with these facts, the orthorhombic STMV model composed of protein, helical RNA, and sulfate anion was placed at the origin in the orthorhombic orientation and noncrystallographic symmetry operators with a variable skew matrix and skew translation were employed. The model was expanded along the radial vector through the centers of mass of the model components, rotated about, and translated along the body diagonal. The search was monitored by the correlation coefficient based on $F$. Figure 1 illustrates the variation in CC and $R$ with respect to these operations. Clearly the particle is centered at the origin and expanded by about 5 Å. The skew angle (equivalent to the rotation angle) was estimated to be about 10.8°. Continued refinement of these parameters with rigid body refinement of the model gave final values of 11.19° for the skew angle and ~5.7 Å for the expansion. Figure 2 is an illustration of the STMV virion showing the protein coat and the double-helical RNA inside. The change in particle size is also tabulated as are results from the rigid body refinement. It should be noted that the 5.7 Å radial expansion of the particle is a 7.8% change in the mean radius and translates directly to a 7.8% increase in tangential surface distances at the mean radius. Thus, a 2.80 Å hydrogen bond would become a 3.02 Å hydrogen bond, well within reasonable hydrogen bonding distances even without consideration of molecular changes to accommodate the increase. Table 1 shows the progression of the refinement. Included in the table are values corresponding to refinement by conjugate gradient minimization of all protein atoms with the RNA and sulfate fixed and group B factor refinement for the three moieties. We regard the best treatment of this structure at 5 Å resolution to be the rigid body model. However, it is quite clear that a dramatic improvement in $R$ value is obtained while maintaining a reasonable protein model despite individual atom refinement against low-resolution data. As indicated by the rms deviations from ideality, this refined model has better geometry than the high-resolution model of the orthorhombic crystal form. Crystallographic calculations were made with the program XPLOR (Brünger, 1991; Brünger et al., 1987).

In this refinement process we made no attempt to rebuild the model to maps. We desired to restrain the model as closely as possible to the high-resolution orthorhombic structure. A few observations may be made beyond those already noted. First, the broadness of the expansion curve in Fig. 1a may reflect a size variability in the cubic form. For this reason, the expansion search is plotted with a similar expansion search in the orthorhombic structure. Higher $B$ factors may also reflect the presence of particle size variability in the cubic crystal form. Second, the highly elevated $B$ factors of the RNA most probably reflect a degradation of the RNA due to high pH or aging.

Measurement by AFM of the heights of STMV particles adsorbed on cleaved mica surfaces, in water or buffer,
indicates that STMV preparations used for crystallization contain a few percentages of virus particles that are irregular in terms of size. Freshly prepared STMV solutions or mother liquors that produce orthorhombic crystals are most uniform and are almost exclusively populated by 17-nm particles with low polydispersity. Nonetheless, even in these preparations a small number of particles could be observed, as in Fig. 3c, which had sizes significantly less than 17 nm. As the solutions aged, polydispersity of particle size increased. These older solutions, which generally yielded cubic crystals, contained many particles having sizes in the range of 14 to 18 nm, with most particles about 18 nm in size.

If an orthorhombic crystal capable of further growth is placed in a mother liquor containing predominantly 18-nm virions and which spontaneously yields cubic crystals, the orthorhombic seed crystal will not grow. Thus it appears that 18-nm particles can only be incorporated into orthorhombic crystals as impurities.

A concurrent observation in the course of our AFM investigations of STMV crystals, illustrated in Fig. 4, was that orthorhombic crystals exhibit a very high density of point defects and absences in their lattice, but at the same time, they are composed of very uniform particles essentially invariant. As a consequence, however, the orthorhombic crystals would likely be sensitive to polydispersity. The incorporation of an anomalous particle could promote defect formation. The accumulation of stress

<table>
<thead>
<tr>
<th>Procedure using 5–20 Å data with a 5σr cutoff</th>
<th>Skew angle (°)</th>
<th>Radial expansion (Å)</th>
<th>(B) protein (Å²)</th>
<th>(B) RNA (Å²)</th>
<th>(B) sulfate (Å²)</th>
<th>R value</th>
<th>CC value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orthorhombic STMV model (protein, RNA, and SO₄ domains) placed in cubic cell with orthorhombic orientation and B factors</td>
<td>0.00</td>
<td>0.00</td>
<td>15</td>
<td>101</td>
<td>7</td>
<td>0.881</td>
<td>0.015</td>
</tr>
<tr>
<td>Optimized skew angle and expansion</td>
<td>11.11</td>
<td>5.38</td>
<td>15</td>
<td>101</td>
<td>7</td>
<td>0.457</td>
<td>0.382</td>
</tr>
<tr>
<td>Optimized translation along body diagonal</td>
<td>11.11</td>
<td>5.38</td>
<td>15</td>
<td>101</td>
<td>7</td>
<td>0.456</td>
<td>0.381</td>
</tr>
<tr>
<td>Optimized B factors by group B factor refinement for each domain</td>
<td>11.11</td>
<td>5.38</td>
<td>71</td>
<td>349</td>
<td>8</td>
<td>0.451</td>
<td>0.417</td>
</tr>
<tr>
<td>Optimized skew angle followed by rigid body refinement</td>
<td>11.10</td>
<td>5.38</td>
<td>71</td>
<td>349</td>
<td>8</td>
<td>0.405</td>
<td>0.557</td>
</tr>
<tr>
<td>Optimized B factors by group B factor refinement for each domain</td>
<td>11.10</td>
<td>—</td>
<td>68</td>
<td>378</td>
<td>7</td>
<td>0.404</td>
<td>0.557</td>
</tr>
<tr>
<td>Optimized skew angle and B factors with rigid body refinement</td>
<td>11.25</td>
<td>—</td>
<td>53</td>
<td>386</td>
<td>58</td>
<td>0.402</td>
<td>0.554</td>
</tr>
<tr>
<td>Powell minimization with protein backbone, RNA, and SO₄ fixed followed by group B factor refinement for each domain. WA = 150,000</td>
<td>11.25</td>
<td>—</td>
<td>55</td>
<td>617</td>
<td>2</td>
<td>0.358</td>
<td>0.724</td>
</tr>
<tr>
<td>Optimized skew angle and B factors with bulk solvent correction and rigid body refinement</td>
<td>11.19</td>
<td>—</td>
<td>81</td>
<td>523</td>
<td>2</td>
<td>0.402</td>
<td>0.554</td>
</tr>
<tr>
<td>Apply bulk solvent correction before Powell minimization. Still keeping protein backbone, RNA, and SO₄ fixed and following up with group B factor refinement for each domain. WA = 150,000</td>
<td>11.19</td>
<td>—</td>
<td>80</td>
<td>518</td>
<td>2</td>
<td>0.354</td>
<td>0.748</td>
</tr>
<tr>
<td>Apply bulk solvent correction before Powell minimization. Include protein, RNA, and SO₄ in model but fix RNA, and SO₄. Let all protein atoms refine. Follow up with group B factor refinement for all domains. WA = 150,000</td>
<td>11.19</td>
<td>—</td>
<td>85</td>
<td>564</td>
<td>2</td>
<td>0.261</td>
<td>0.878</td>
</tr>
</tbody>
</table>

Geometry of protein subunit

<table>
<thead>
<tr>
<th>Start model</th>
<th>Refined model</th>
</tr>
</thead>
<tbody>
<tr>
<td>RMSD in bonds (Å)</td>
<td>0.007</td>
</tr>
<tr>
<td>RMSD in angles (°)</td>
<td>2.310</td>
</tr>
<tr>
<td>RMSD in dihedral angles (°)</td>
<td>14.75</td>
</tr>
<tr>
<td>RMSD in improper angles (°)</td>
<td>1.144</td>
</tr>
</tbody>
</table>
from the incorporation of several particles consistently displaying minor variability could do the same.

Images of the cubic crystal lattice are significantly different from those of the orthorhombic lattice. There is a continuous height variation, visibly present, from particle to particle. This implies that the virions in the cubic crystals are characterized by a more extensive size heterogeneity. Virus particles in the cubic crystal lattice, which contains 25% more solvent per virion, apparently have less rigorously defined, more flexible interactions with neighbors. The cubic lattice, therefore, would be less sensitive to perturbation by incorporation of particles of polydisperse character. This would allow particles significantly different in size to incorporate, while the tolerant nature of the lattice would allow it to remain unstressed and defect free.

A second transition of structural consequence was also studied, but using AFM alone. If particles from either orthorhombic crystals (17-nm particles) or cubic crystals (18-nm particles) are spread and adsorbed to freshly

![Image](https://example.com/image.png)

**FIG. 3.** Two isolated STMV particles in water adsorbed onto freshly cleaved mica in (a). Height and width measurements are shown in (b). The height of 17 nm corresponds exactly with the center-to-center distance of STMV particles in the orthorhombic crystals and is an accurate measure of the true diameter of the icosahedral particle as confirmed by X-ray structure determination. The apparent width of the particle, however, is 45 nm, which is larger than the correct diameter. The scan area in (a) is 250 × 500 nm². Many freshly prepared, individual STMV particles are seen adsorbed onto freshly cleaved mica directly from solution in (c). AFM imaging is in water in a fluid cell and the scan area is 500 nm². The vast majority of the particles have diameters, from height measurement, of 17 nm, in agreement with the X-ray structure. Occasional aberrant particles of other sizes, both larger and smaller, are also observed. Overall, however, the distribution is very narrow.
cleaved mica, then, when imaged in buffer, under physiological conditions of pH, temperature, and ionic strength, they yield average heights in the AFM of 17 and 18 nm, respectively, though, because of the barely perceptible difference, there is extensive overlap of the two distributions.

If STMV preparations used for crystallization are dried in air on the surface of cleaved mica before imaging, the sizes of the STMV particles are seen to decrease substantially. Particles from freshly prepared solutions, those which yield orthorhombic crystals, or from redissolved orthorhombic crystals, decrease in size to 12 nm, and the size distribution of the dried particles is very uniform. These are seen in Fig. 5a. STMV particles from redissolved cubic crystals also decrease in size, but exhibit a broad distribution of sizes when adsorbed to the mica surface over the range 10 to 12.5 nm. These are seen in Fig. 5b. The heights measured for the dried particles were insensitive to AFM tip pressure, which was varied over a broad range. This indicates that the particles indeed have reduced diameters and are not yielding reduced heights as a transient deformation in response to tip pressure.

We assume that STMV particles decrease in size as a consequence of drying due to the adhesion force which binds the particles to the mica surface. Because STMV particles are not completely rigid in structure, their vertical dimension, which is in the direction of the adhesion force, is reduced. No significant change in lateral dimension was recorded. This was determined by measuring the center-to-center distances within dried masses of particles exhibiting reduced heights. Virus particles of uniform size from orthorhombic crystals also show uniform mechanical properties and deform to the same final size. More aged STMV particles, those from cubic crystals, which have a broader size distribution, also exhibit a greater range of mechanical properties and are seen to deform in a much less consistent manner. This is illustrated in Figs. 5c and 5d.

To show that the deformation of the virus particles is indeed due to their unique mechanical properties and is not a general feature of hydrated biological specimens, we simultaneously imaged, by AFM, both STMV particles and their rod-shaped helper virus tobacco music virus (TMV). TMV, unlike STMV, exhibits a diameter of 18 nm when fully hydrated, and this remains unchanged by drying. We also imaged 0.4-μm polystyrene spheres as a control. Polystyrene particles also did not change diameter significantly upon drying, did not deform, and in polycrystalline masses showed center-to-center distances equal to their heights.

STMV particles are not restored to their original sizes if they are rehydrated by filling the fluid cell with water or buffer. After drying, they consistently show the same sizes and size distribution whether they are subsequently imaged in air, water, buffer, propanol, or ethanol. Thus the deformation produced by drying appears to be irreversible.

**DISCUSSION**

Because the maximum resolution of the X-ray data from cubic crystals is no more than 5 Å, a well-refined
model of the expanded 18-nm STMV particle cannot be obtained. Nonetheless, our results lead us to conclude that the striking decline in diffraction resolution is probably due to variability in the size and detailed structure of the 18-nm particles. Inspection of the AFM images of orthorhombic crystals show that they have a very high incidence of point defects and other lattice imperfections, yet they diffract to 1.8 Å resolution. On the other hand, cubic STMV crystals exhibit lattices that are almost flawless. There is a near complete absence of vacancies and point defects. Thus, the difference in diffraction resolution seems not due to crystal defect structure or density, but to particle properties.

Freshly prepared STMV solutions are relatively uniform in terms of the particle size distribution, and orthorhombic crystals contain, essentially, only these 17-nm particles. The populations, however, contain some anomalous particles; probably some swollen 18-nm particles, but smaller particles as well. The orthorhombic crystals, which diffract to 1.8 Å resolution, are very demanding in the order they require and the size of the particles they select. If, however, aberrant particles are occasionally but inevitably incorporated, because of the rigor of the lattice, strain would result and defects would be produced. Thus the orthorhombic crystals have a rigid and precisely defined lattice that diffracts to high resolution, but it may not be able to tolerate interlopers. Aberrant particles may be the sources of the myriad patterns of defects that occur in the orthorhombic crystals.

Another possibility is that the populations of native STMV virions which usually form orthorhombic crystals are in fact imperceptibly heterogeneous in size or structure. This would not be evident from X-ray diffraction,
which reveals only the time- and space-averaged structure. If this were the case, then because of the rigor of the lattice, strain could accumulate over long distances due to the microheterogeneity of the particles. Defect formation would serve to relieve this collective stress in the lattice. This mechanism is consistent with the findings of Rimai et al. (2000), who studied the adhesion of particles in the nanometer to micrometer size range. They show that for small particles of the sizes of STMV, accumulated strain can become quite large, resulting in plastic deformations.

Two observations seem to argue in favor of the latter hypothesis. First, we cannot identify any particles in the immediate neighborhood of the defects or vacancies that are definitely aberrant or of abnormal size. The second is that as the number of 18-nm particles increases with age, the number of absences and defects in the lattice do not increase. That is, we see no significant correlation between the number of defects and the number of abnormal particles.

The cubic crystals of STMV, on the other hand, have a very forgiving lattice that accepts a broad range of particle sizes and structures. The consequences of this lack of discrimination are twofold. First it accommodates diversity without stress and is virtually defect free. Second, because of its diversity and lack of precision, it only diffracts to low resolution.

We cannot detail alterations in the bonding interactions between protein subunits in the capsid upon swelling to 18-nm diameters except to say that the protein appears to move radially as dimer units. This is to be expected given the strong interactions between dyad-related monomers. This implies, however, that there must be significant change in the interactions between threefold and fivefold related subunits. It was clear, even from the 5 Å refinement of the cubic crystals, that RNA helical segments, strongly bound by the capsid in the native structure, move radially with the protein dimers upon expansion. The protein–nucleic acid interactions are, therefore, largely or entirely preserved.

In the native STMV structure, 10,080 structural water molecules were identified. This was in addition to the roughly 30% of the particle volume at the center of the virion which must be occupied by bulk solvent. Clearly, there must be an extensive rearrangement of the water structure and water-bonding networks that otherwise contribute to maintaining the integrity of the particles. The rearrangement of water molecules must, in fact, be a major feature of the transition. An 11 Å increase in the diameter of the virion represents a 22% increase in the volume of the particle. Thus, in addition to a major rearrangement of structural water molecules, there must also be a substantial increase in the amount of bulk solvent inside the particle. The additional water may in turn contribute to the general variability or structural instability of the 18-nm virions.

The transition, observed by AFM as a consequence of drying, shows that the mechanical structure of the virions is weakened upon swelling. As a consequence, when swollen particles dry and collapse, the particles display a variety of sizes and shapes. Native particles, on the other hand, even when dried and made to collapse, do so in a uniform manner, reflecting their greater mechanical stability. This may be a result of more intact and structurally rigid RNA, maintenance of rigorous protein–nucleic acid structure, or simply the integrity of the water structure and inter-subunit interfaces in the capsid.

The deformation that is observed upon drying, both for 17- and 18-nm swollen particles, represents a decrease in the particle volume of 30 to 40%. In terms of mass, this decrease could be accommodated by loss of the water at the center of the particle and other associated water molecules. It does not, however, seem conceivable that this degree of deformation could take place without radical structural changes in both the protein capsid and its interacting nucleic acid. Whether the icosahedral particles are ruptured by the shrinkage we cannot say, but that remains a possibility.

The two transitions observed here suggest that the icosahedral architecture of the STMV virion is robust, but nonetheless subject to alteration. It is clearly dependent on protein–protein, protein–nucleic acid, and interactions involving the extensive water networks. When RNA is degraded, pH-inspired transition is promoted. When water structure is destroyed, deformation becomes possible. In some cases, e.g., the pH transition from 17 to 18

![FIG. 6. A cubic crystal of satellite tobacco mosaic virus grown in microgravity aboard the U.S. space shuttle. The crystal has edges of approximately 1 mm and is growing from a glass substrate.](image-url)
nm diameter, the process is reversible, in other cases, e.g., the drying response, it is not.

The transition of native $T = 3$ icosahedral plant virus to swollen states is not uncommon, and its causes have been discussed by Kaper (1975). Swelling occurs as a function of pH and ionic strength, and due to the presence or absence of metal ions such as Ca$^{2+}$ and Mg$^{2+}$.

Virion swelling has been extensively studied for Southern bean mosaic virus (Wells and Sisler, 1969; Kruse et al., 1982), Tomato bushy stunt virus (Golden and Harrison, 1982), Cowpea chlorotic mottle virus (Kruse et al., 1980), and Brome mosaic virus (Bancroft et al., 1968; Incardona and Kaesberg, 1964). STMV is to our knowledge, however, the first $T = 1$ icosahedral virus observed to undergo such a transition.

**MATERIALS AND METHODS**

Crystallization and data collection

STMV was purified from leaves of infected tobacco plants (*nicotiana tabacum*) as previously described (Koszelak et al., 1989; Larson et al., 1993a,b) and was recrystallized from bulk solution by the addition of ammonium sulfate to 15% saturation. STMV particles were completely dissociated in SDS running buffer and were subjected to SDS–PAGE using the procedure of Laemmli (1970). A 10% acrylamide gel was used and stained with Coomassie brilliant blue. Pure STMV characteristically gave only a single-coat protein band at 232 KUZNETSOV ET AL.  202.5 Å), seen in Fig. 6, which diffracts to no better than 5 Å resolution, and the cubic form (P213, $a = 175.8$ Å, $b = 169.9$ Å, $c = 244.6$ Å, $\beta = 92.7^\circ$) which grows at temperatures below about 10°C, and which diffracts to 5 Å resolution, and the cubic form (P213, $a = 175.8$ Å, $b = 169.9$ Å, $c = 244.6$ Å, $\beta = 92.7^\circ$) which grows at temperatures below about 10°C, and which diffracts to 5 Å resolution, and the cubic form of STMV can be obtained in at least three different crystal forms, all of which have been characterized by X-ray diffraction, and probably a fourth form which remains undefined. All of the crystal forms can be grown to dimensions exceeding 1 mm. Those which have been studied are an orthorhombic form (I222, $a = 174.3$ Å, $b = 191.8$ Å, $c = 202.5$ Å) from which the structure of the virus has been determined to 1.8 Å resolution (Larson et al., 1998), a closely related monoclinic form (I2, $a = 175.8$ Å, $b = 169.9$ Å, $c = 244.6$ Å, $\beta = 92.7^\circ$) which grows at temperatures below about 10°C, and which diffracts to about 2.7 Å resolution, and the cubic form (P213, $a = b = c = 257.2$ Å), seen in Fig. 6, which diffracts to no better than about 5 Å resolution (Koszelak et al., 1995). The volume per viral particle in the orthorhombic and monoclinic unit cells is about $3.4 \times 10^6$ Å$^3$/virion. In the cubic unit cell there is a 25% increase to $4.25 \times 10^6$ Å$^3$/virion.

From solutions of freshly prepared STMV the orthorhombic form and the monoclinic form can be predictably and reproducibly grown by fixing the temperature at $>15^\circ$C (orthorhombic) or $<10^\circ$C (monoclinic). The two crystal forms will repeatedly transform, one into the other, if the temperature is manipulated up and down over several days. The cubic crystal form is, however, virtually never observed, though it grows under identical conditions, unless the pH is increased to about pH 8.5, or preparations that are months to years old are used. Aged preparations of virus reproducibly yield cubic crystals, even at neutral pH, and at both room and cold temperatures.

Two cubic crystals were used for data collection, both of unusual size, having volumes of $\sim 1.5$ mm$^3$. These were grown by liquid–liquid diffusion in the Advanced Protein Crystallization Facility (APCF) during a 12 day mission in microgravity (International Microgravity Laboratory II) aboard the U.S. space shuttle as described previously (Koszelak et al., 1995). Crystals were mounted in quartz capillaries by conventional methods. Data were collected at 17°C on a San Diego Multiwire Systems area detector system utilizing two detectors at crystal-to-detector distances of 940 and 1000 mm. A Rigaku RU-200 rotating anode generator fitted with a Supper graphite monochromator operating at 45 kV and 175 mA supplied the CuK$_\alpha$ radiation. Crystals were rotated through 50° sectors about the $\alpha$-axis in 0.10° increments at appropriate $\chi$ and $\varphi$ settings. Both data sets were complete to 5 Å resolution but were averaged to achieve high redundancy ($\sim 3.4$ obs/refl). Table 2 illustrates the poor quality of the data. Although the data are complete to 5 Å, the falloff in $I/\sigma$ is dramatic. For this reason, only data in the 5–20 Å range with $F \geq 5\sigma_F$ were used in the structure solution efforts described below.

AFM

Virus particles were adsorbed onto freshly cleaved mica and examined both in air and in their mother liquor in sealed fluid cells. Virus crystals, grown ex situ, were transferred to sealed fluid cells of about 60 μl volume; the crystals were fixed to the glass substrate by pinning them beneath fine glass fibers and examined under solutions identical to their mother liquors. All operations were carried out at 27°C. The AFM instrument was a Digital Nanoscope III (Digital Instruments, Santa Barbara, CA) and images were collected in tapping mode using silicon nitride, oxide-sharpened tips. Most procedures were those described in detail in earlier work on the crystallization of macromolecules as studied by AFM (Kuznetsov et al., 1997, 1999; Malkin et al., 1999).

The measurement of particle size and the dimensions of features of individual particles were treated with some care. Sizes of individual particles adsorbed to the mica, as in Fig. 3, appear considerably larger because the
image obtained is the convolution of the AFM tip shape with that of the particle. That is, the tip is not infinitely sharp and its curved surface immediately adjacent to the absolute tip causes vertical displacement of the cantilever and, therefore, gives rise to edges in the image before as well as after the absolute tip encounters the object. This does not, however, affect the total vertical displacement of the cantilever. As a consequence, single objects visualized by AFM appear broader than their true dimensions, but yield an accurate and precise vertical dimension. For roughly spherical particles, such as icosahedral viruses, although their diameter appears greater than is in fact the case, the vertical “height” of the particles gives a remarkably accurate value for their true diameter.

In the course of our studies on viruses, we have examined eight different varieties whose capsid sizes are accurately known from electron microscopy, light scattering, or X-ray crystallography. These have included STMV, Cucumber Mosaic Virus (CMV), Turnip Yellow Mosaic Virus (TYMV), Brome Mosaic Virus (BMV), herpes virus, iridovirus, TMV, and Cauliflower Mosaic Virus (CaMV). Our images of these viral particles consistently showed them to have lateral dimensions of about 2.5 times their actual diameter. In all cases, however, their heights, as measured by AFM, were accurate to within 1 nm. Therefore, particle diameters cited here are based on the vertical measures of their diameters obtained from AFM.

In the case of crystals of viral particles, the situation is quite different. With viral crystals each particle is embedded in a lattice composed of similar particles. As the AFM tip passes over the surface, the tip never approaches the “bottom” of a single virion (i.e., a surface equivalent to the flat mica substrate described above), before it encounters a neighboring virion. Thus the heights of particles in crystals does not give a true measure of their diameter. However, it is a simple matter to measure the center-to-center distances of the virus particles in the lattices, and because these are generally close packed, these distances do yield an accurate and precise diameter for the particles of better than 1 nm. Fourier transform of the lattice arrays in fact improve these values to considerably better than 1 nm.

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REFERENCES


