Morphological Studies of Maize Mosaic Virus I*

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SUMMARY

Particles of maize mosaic virus I are bullet-shaped, or hemispherical at both ends. Bullet-shaped particles in phosphotungstic acid measure 2550 × 900 Å, those in uranyl acetate 2410 × 730 Å. The particles appear to consist of an envelope with thread-like or knob-like protrusions, a helical structure composed of beaded units, a hollow cylinder and an inner core. The last has no visible structure but disintegrates often in somewhat spherical masses.

INTRODUCTION

Particles believed to be the viral agent of maize mosaic virus I (Smith) have been studied in thin sections of corn leaves, osmium fixed droplets of plant sap preparations (Herold, Bergold & Weibel, 1960) and sections of organs of the insect vector (Herold & Munz, 1965). The shape and structure of the particles are very different from those of other plant viruses. A detailed investigation of this virus was therefore made on particles in suspension by negative staining methods and on embedded particles by double staining.

METHODS

Corn plants (Zea mays L.) infected in the greenhouse with maize mosaic virus I by viruliferous Peregrinus maidis (Ashm.) were used. Most preparations were made according to Brandes’s (1961) dipping method slightly modified by us, on Formvar coated grids reinforced with carbon. The dippings were mostly made in water. Droplets of 2% (w/v) aqueous phosphotungstic acid (PTA) adjusted with NaOH to pH 6, 1% aqueous uranyl acetate, or the complex of uranyl ions and ethylenediaminetetra-acetate (EDTA) at pH 7, pH 6 or pH 4 (van Bruggen, Wienberga & Gruber, 1962) were added to the water droplets immediately after the dipping or at intervals from 10 min. to 24 hr. Several dippings were made directly in PTA. The excess liquid was sucked off with a thin cotton thread. A few preparations were made with purified virus suspensions. Leaves were macerated in a mortar with 0.01 M-phosphate buffer pH 8 to make a 20% (w/v) suspension. The sap was filtered through nylon cloth, clarified by centrifugation at 3,000g for 15 min. stored for 24 hr at 4°, clarified once more and centrifuged in a Spinco Model L ultracentrifuge at 11,000 rev./min. for 1 hr. The sediment was suspended in phosphate buffer (half the original weight of the leaves) mixed 1:1 with PTA and sprayed on to the grids with a Vaponefrine nebulizer.

Pieces of leaves were fixed in 1% Veronal-buffered osmic acid, treated with 1%

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aqueous uranyl acetate, embedded in methacrylate (Herold et al. 1960) and cut with a diamond knife in a Porter–Blum microtome. The sections were stained for 10 min. in 4% aqueous uranyl acetate at room temperature and subsequently in lead citrate for 2 min. at 60° (Reynolds, 1963). Suspensions and sections were examined in a Siemens Elmiskop I electron microscope.

**RESULTS**

The rod-shaped particles of maize mosaic virus I were either bullet-shaped or hemispherical at both ends. The bullet-shaped particles from PTA preparations measured 2550 × 900 Å (Fig. 1, Table 1), those from uranyl acetate 2410 × 730 Å (Table 1). Very few particles with both ends hemispherical were found in PTA (Pl. 1, fig. 3). Their dimensions were similar to those of the bullet-shaped ones. In uranyl acetate preparations there were two types of particles with both ends hemispherical: highly contrasted ones with visible inner structures and weakly contrasted ones without visible inner structures (Pl. 2, figs 8, 11; Pl. 3, fig. 12). The highly contrasted particles had almost the same dimensions as the bullet-shaped ones. The weakly contrasted particles measured 2770 × 900 Å (Table 1).

![Graphs showing length and diameter distribution of maize mosaic virus.](image)

Table 1. *Dimensions of differently stained and shaped virus particles*

<table>
<thead>
<tr>
<th>Particles</th>
<th>Staining</th>
<th>Contrast</th>
<th>Inner structure</th>
<th>Length Ŕ ±200</th>
<th>Width Å ±100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bullet-shaped</td>
<td>PTA*</td>
<td>High</td>
<td>Visible</td>
<td>2550 (127)†</td>
<td>900 (130)</td>
</tr>
<tr>
<td>Bullet-shaped</td>
<td>UrA†</td>
<td>High</td>
<td>Visible</td>
<td>2410 (53)</td>
<td>730 (53)</td>
</tr>
<tr>
<td>Both ends hemispherical</td>
<td>UrA</td>
<td>Low</td>
<td>Not visible</td>
<td>2770 (24)</td>
<td>900 (24)</td>
</tr>
</tbody>
</table>

* PTA: phosphotungstic acid.
† Numbers in parentheses indicate the number of particles measured.
‡ UrA: uranyl acetate

Three or more bullet-shaped particles often lay with their flat ends together forming star-like figures (Pl. 1, fig. 1). Sometimes broken particles (Pl. 3, fig. 15) or disc-like fragments were seen (Pl. 3, fig. 17). An occasional particle had an appendage apparently adhering to the flat end (Pl. 1, fig. 7).
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Ultrastructure

From studies of electron micrographs of differently treated preparations the following architecture of the particles is suggested. On the outside the particles have an envelope. This has thread-like protrusions which in PTA mountings are upright and approximately 70 Å high (Pl. 1, figs 1-7; Fig. 2). In uranyl acetate + EDTA preparations (pH 7, pH 6 and pH 4) the protrusions are contracted showing somewhat thicker knob-like forms (Pl. 3, fig. 12). In uranyl acetate without buffer the protrusions are coarse and knob-like (Pl. 2, figs 8, arrows, 9) or they are not recognizable (Pl. 2, figs 8, 11). In the weakly contrasted particles the whole surface of the envelope appears coarsely granulated in uranyl acetate and in uranyl acetate + EDTA slightly
granulated (Pl. 2, figs 9, 11; Pl. 3, fig. 12). Sometimes the envelope is seen as an irregular structure lying beside a particle without envelope (Pl. 3, fig. 16).

Adjacent to the envelope is a helical structure running across the particle (Pl. 1, figs 1–7; Pl. 2, figs 8–10; Pl. 3, figs 12, 14–16, 18; Fig. 2). This structure seems to consist of beaded units of approximately 45 Å in diameter and approximately 34 units in one turn. The distance from turn to turn is approximately 52 Å. There are about 40 turns and consequently 1360 units in one particle. The units mostly show a hexagonal arrangement (Pl. 1, figs 5, 7; Pl. 2, fig. 8; Pl. 3, fig. 15); however, sometimes a square arrangement is observed (Pl. 1, fig. 4). In disintegrating particles the union between the turns becomes loosened and the helix uncoils (Pl. 2, figs 8, 10; Pl. 3, fig. 14) or groups of units break out (Pl. 1, fig. 6).

Often an electron-clear zone is seen between the envelope and the helix (Pl. 1, figs 1, 2, 6, 7). This is due to shrinkage of the helix, while the envelope sticks in the embedding material.

Inside the helix there seems to be a hollow cylinder filled (Pl. 2, figs 8, 9; Pl. 3, figs 14, 15, 17, 18; Fig. 2) with undifferentiated material. However this material often disintegrates in spherical masses so that 'empty spaces' appear which are filled with staining substance (Pl. 1, figs 1, 2; Pl. 2, figs 8, 9; Fig. 2). Sometimes the inner material seems to have been lost, leaving only a small zone outlining the empty channel (Pl. 3, fig. 13).

Both highly and weakly contrasted intact particles and disintegrated particles are found in the same ratio in dippings prepared in PTA and in those made by first dipping in water and then adding PTA or uranyl acetate 10 min. to 24 hr later. Many more disintegrated particles are found in purified sap preparations than in dippings.

**DISCUSSION**

Recently several viruses have been described with shape, dimensions and ultrastructure similar to those of maize mosaic virus. All have three or four structural units. All particles have an outer coat with fine surface projections (Ditchfield & Almeida, 1964), which were described as bead-like projections (Harrison & Crowley, 1965; Kitajima & Costa, 1966), a series of loops or small knobs (Hitchborn, Hills & Hull, 1966) or loops (Bergold & Munz, 1967). Our findings of hair-like protrusions in PTA and knob-like ones in uranyl acetate preparations are difficult to interpret. Both substances are negative stains (van Bruggen et al. 1962); however, PTA is considered to preserve fine details better than uranyl acetate (Bradley, 1962; Home & Wildy, 1963). We therefore assume that the protrusions are indeed hair-like. In uranyl acetate preparations the protrusions seem to be contracted and less well preserved resulting in a knob-like appearance. Addition of EDTA to uranyl acetate somewhat improves the preservation of the protrusions. Berkaloff & Thiéry (1963) also observed constriction and noticed that the density of the outer parts of the envelope of influenza virus appeared greater in uranyl acetate staining than in PTA staining.

Another question arises on the nature of the weakly contrasted particles without visible inner structures in uranyl acetate preparations. They could be complete particles into which for some reason no stain has entered. However, uranyl acetate is also a positive stain for nucleic acids. Because of this property the weakly contrasted particles could be empty envelopes. The transparency of the particles as observed in
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some micrographs (Pl. 2, fig. 8) cannot be used to determine their nature because particles with visible inner structures are also transparent (Pl. 2, fig. 9). Harrison & Crowley (1965) considered the weakly contrasted particles of lettuce necrotic yellows virus to be empty bags. But in Harrison and Crowley's, as in our case, the fact that these supposed envelopes appear to be entire and do not show any opening which might be expected in empty bags remains without explanation. Shadow casting experiments are under investigation to decide this question.

A beaded helix adjacent to the envelope was first reported in vesicular stomatitis virus (Hacket, 1964; Ditchfield & Almeida, 1964; Simpson & Hauser, 1966; Bergold & Munz, 1967). It was also suggested for lettuce necrotic yellows virus (Harrison & Crowley, 1965). In a virus of Gomphrena the core was supposed to represent the nucleocapsid. It appeared to be formed by a stack of rings or a helix built up of smaller units (Kitajima & Costa, 1966). The helical structure described by us in maize mosaic virus is also considered to be the nucleocapsid with the capsomeres. There are more beads in one turn and more turns in one particle than in the other viruses. The distance from turn to turn is 52 Å; that is, a little more than that reported for the other viruses. The union between the turns seems to loosen much more easily in uranyl acetate than in PTA, because the uncoiled helices are mostly observed in uranyl acetate. On the other hand in PTA the union from bead to bead appears to loosen more easily. The hexagonal order of the beads corresponds to that of the capsid of T-even phages (Finch, Klug & Stretton, 1964; Favre et al. 1965; Kellenberger & Boy de la Tour, 1965) and to the nucleocapsid of vesicular stomatitis virus (Bergold & Munz, 1967). The square order observed in a few particles seems to be accidental.

The existence of the hollow cylinder is assumed from the homogeneous zone inside the helix observed in disc-like fragments or whole particles in suspensions and from cross sections. The inner material seems to be very unstable because it has often separated in spherical masses or flows out.

A comparison of the present results with earlier ones from particles not specially stained (Herold et al. 1960) is depicted in Fig. 2. The envelope E corresponds to the outer membrane M₁, the helical structure H to the inner membrane M₂, the cylinder Cy to the outer Zone Z₁ and the inner material Im to the core C. The disintegrated inner material yields the spherical masses Sm and the 'empty spaces' Es. This last corresponds to the inner Zone Z₂, which is only a shrinkage space. The differences in the dimensions of the whole particles as well as their components are explained by shrinkage during the embedding process and flattening while drying on the grids. Values of diameters for sprayed particles about 25 to 75% higher than those for embedded particles have been demonstrated with other viruses (Chambers, Crowley & Francki, 1965). In maize mosaic virus we found extremely large differences for width: 480 Å for embedded and 900 Å for sprayed particles. We believe that this large difference depends on failure of staining of the protrusions in the osmium-fixed embedded particles or that the protrusions contract and adhere to the envelope. In both cases the electron-clear spaces between the particles in crystalline array would be the position of the interlocked protrusions. Measurements of particles in PTA without their protrusions or of particles fixed only in osmium vapour, where the protrusions are not visible, result in diameters of approximately 760 Å. This is 50% more than that of embedded particles, which corresponds well with the differences found in other viruses. The smaller dimensions of the highly contrasted particles in uranyl acetate could be
explained by the above-mentioned contraction of the protrusions. The somewhat higher values for the weakly contrasted particles without recognizable protrusions in uranyl acetate could be explained by extreme flattening, and would support the theory that these particles are empty envelopes.

More or less contrasted and disintegrated particles occur in the same ratio in different preparations. This may indicate that the penetration of stain and the disintegration of the particles are not a function of the time from the release from the cell until their fixation. More probably, the particles have varying stabilities when they come out from the cell. The finding of more disintegrated particles in purified sap preparations than in dippings indicates damage to the particles during the purifying process.

Viruses with shape and ultrastructure similar to maize mosaic virus have been found in other plants (Lee, 1964; Harrison & Crowley, 1965; Chambers et al. 1965; Kitajima, 1965; Kitajima & Costa, 1966; Hitchborn et al. 1966; MacLeod, Black & Moyer, 1966), in mammals (Howatson & Whitmore, 1962; Davies et al. 1963; Hacket, 1964; Ditchfield & Almeida, 1964; Simpson & Hauser, 1966; Bergold & Munz, 1967), in fish (Zwillenberg, Jensen & Zwillenberg, 1965), and in insects (Berkaloff, Bregliano & Ohanessian, 1965). Of the five above-mentioned plant viruses, three are transmitted by leafhoppers (Herold, 1963; Lee, 1964; MacLeod et al. 1966) and one by aphids in a circulative manner (Stubbs & Grogan, 1963). Another of the plant viruses is transmitted mechanically but the insect transmission has not been tested (Kitajima & Costa, 1966) and it is not known how the last one is transmitted (Hitchborn et al. 1966). The other viruses are transmitted by arthropods with the exception of rabies virus (Davies et al. 1963), and the fish virus (Zwillenberg et al. 1965). It is suggested, therefore, that the majority of this group of viruses has a common tropism to insects.

There is even some evidence that the intimate membrane of a granulosis virus (Smith & Hills, 1962) and the inner membrane of a nuclear polyhedrosis virus have a coiled component (Harrap & Juniper, 1966). This could indicate a relationship between the morphological group discussed here and certain insect pathogenic viruses.

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REFERENCES


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EXPLANATION OF PLATES

PLATE 1

Figs 1–7. Electron micrographs of virus particles stained with PTA.
The bar represents 1000 Å.

Fig. 1. Star-like grouping of bullet-shaped particles.
Fig. 2. Two bullet-shaped particles with broad shrinkage zones on different sides.
Fig. 3. Particles with both ends hemispherical.
Fig. 4. Particle with hexagonal and square arrangement of the beads.
Fig. 5. Particle at higher magnification showing the beads.
Fig. 6. Disintegrating particle from which groups of the beads are broken out.
Fig. 7. Particle with a tail-like appendix at the flat end.

PLATE 2

Figs 8–11. Electron micrographs of virus particles stained with uranyl acetate.
The bar represents 1000 Å.

Fig. 8. (a) Highly contrasted bullet-shaped particles with visible inner structure. (b) Weakly contrasted particle without visible inner structures lying partly over a particle of type (a). (c) Partly disintegrated particles of type (a) with somewhat uncoiled helices. Arrows mark particles with coarse knob-like protrusions.
Fig. 9. Highly contrasted particles lying partly over weakly contrasted ones.
Fig. 10. Disintegrated particle with loosened and uncoiled helix.
Fig. 11. Weakly contrasted particle without visible inner structure.

PLATE 3

Figs 12–17. Electron micrographs of virus particles stained in the following manner:
fig. 12, uranyl acetate + EDTA pH 7; fig. 13, PTA; figs 14 to 17, uranyl acetate. The bar represents 1000 Å.

Fig. 12. Weakly contrasted particles without visible inner structures and highly contrasted particles with visible inner structures.
Fig. 13. Bullet-shaped particle with part of the inner material missing.
Fig. 14. Particle with parts of the envelope missing and the helix loosened.
Fig. 15. Broken parts of a particle.
Fig. 16. Particle with the envelope lying beside it.
Fig. 17. End or disc-like fragment of a particle.
Fig. 18. Section of embedded particles stained with uranyl acetate and lead citrate showing the envelope, the cross striations of the helical capsid and the inner material, according to the cutting plane of the particles. The bar represents 1000 Å.
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(Facing p. 234)