Some Properties of the Particles of Three Tobravirus Isolates

By J. I. COOPER AND M. A. MAYO

Scottish Horticultural Research Institute, Invergowrie, Dundee, Scotland

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SUMMARY

When centrifuged in caesium chloride solutions, purified preparations of uncloned cultures of the CAM and PRN strains of tobacco rattle virus contained more than one component but single lesion isolates of each strain contained only one component with buoyant densities of 1.306 and 1.324 g./ml. respectively. The buoyant density of the SP5 strain of pea early-browning virus was 1.310. As determined by electron microscopy the diameters of the particle and the core were respectively 23 and 5 nm. for PRN and about 21.5 and 4 nm. for both CAM and SP5. The mol. wt of the single virus protein, obtained from each freshly purified virus and estimated by polyacrylamide gel electrophoresis, were 28,500 (CAM and PRN) and 24,000 (SP5). Each virus produced nucleoprotein particles of two predominant lengths and these yielded two RNA species whose mol. wt were proportional to those lengths. The mol. wt (× 10^-9) estimated by polyacrylamide gel electrophoresis were 0.7 and 2.5 (CAM), 1.0 and 2.5 (PRN) and 1.3 and 2.5 (SP5).

At pH 8.6 the electrophoretic mobility of the long particle of CAM was 16% greater than that of the short particles. However, the long particles of CAM have few if any antigenic determinants not possessed by short particles.

INTRODUCTION

The tobravirus group (Harrison et al., 1971) consists of plant viruses with split genomes contained in straight tubular particles of two main lengths and with nematode (Trichodorus spp.) vectors. The group includes various isolates of tobacco rattle and pea early-browning viruses; its type member is the PRN isolate of tobacco rattle virus. These viruses contain RNA (Harrison & Nixon, 1959a, b; Gibbs & Harrison, 1964) embedded in a helical array of protein subunits (Nixon & Harrison, 1959; Finch, 1965; Offord, 1966). Estimates of the mol. wts of the proteins of different isolates, based on amino acid analyses, range from 24,000 (Offord & Harris, 1965) to 19,000 (Semancik, 1970). Polyacrylamide gel electrophoresis has given good estimates of mol. wt of both protein (Weber & Osborn, 1966) and RNA (Bishop, Claybrook & Spiegelman, 1967), and we have used this technique to compare the proteins and RNA species of three tobraviruses; the PRN isolate of tobacco rattle virus (from Scotland), the CAM isolate of tobacco rattle virus (from Brazil) and the SP5 isolate of pea early-browning virus. We have also compared features of their morphology and buoyant densities.

Isolates of tobacco rattle virus show great serological differences (Harrison & Woods, 1966) but Harrison & Nixon (1959a) found no difference in serological properties or electrophoretic mobility between the long and short particles of the PRN isolate. We have re-examined these properties using the CAM isolate, whose long and short particles are more readily separated than those of PRN.
METHODOLOGY

Viruses. The CAM and PRN isolates of tobacco rattle virus were subcultures of the isolates described by Harrison & Woods (1966) and could be distinguished from the SP5 isolate of pea early-browning virus (= the type culture of the English form; Gibbs & Harrison, 1964) by the length of their short particles and by the symptoms induced in infected plants. In inoculated leaves of Phaseolus vulgaris L. cv. The Prince, CAM and PRN caused pin-point chocolate-brown local lesions whereas SP5 produced larger, often ring-like, lesions.

All three isolates were propagated in Nicotiana clevelandii Gray plants grown in a glasshouse at about 20°C. The systemically infected leaves were harvested approximately 14 days after the plants were inoculated, minced and the sap expressed through muslin was stored at −15°C for about a month.

Virus purification. Frozen sap containing tobacco rattle virus was thawed overnight and centrifuged at 8000 g for 10 min. The virus was obtained from the supernatant fluid by two cycles of high-speed and low-speed centrifuging. Unless otherwise stated, the pellets obtained by high speed centrifuging were resuspended in 0.02 M-phosphate buffer (sodium and potassium salts, pH 7.3). The same method was used to purify SP5, except that the thawed sap was heated at 50°C for 10 min. before centrifuging at 8000 g. Preparations of separated long and short particles were obtained by centrifuging 2 to 4 times in sucrose density gradients (Brakke, 1960).

Equilibrium sedimentation. Two methods were used. In one, virus in caesium chloride solution was centrifuged using a Beckman SW39L rotor. After centrifuging, the contents of the tubes were discharged by upward displacement with 20% (w/v) sucrose in 40% (w/v) potassium iodide through an u.v. absorptiometer (LKB Uvicord I). The density of two or four drop fractions was determined either by weighing 50 μl. samples (Crawford, 1960) or from refractive index measurements. In the second method virus was centrifuged at 30,000 rev./min. for about 24 hr at 22 to 27°C using the AnD rotor of a Beckman Model E analytical centrifuge.

Buoyant densities were determined by measuring prints of Schlieren diagrams, using a method similar to that described by Szybalski (1968).

Electron microscopy. Preparations containing virus were examined on carbon coated copper grids in a Siemens Elmiskop IA. Virus in caesium chloride solutions was dried on to the grids and irrigated with stain in situ. Virus in phosphate buffer solutions was sedimented and resuspended in water before mixing with stain. Particle length measurements were made and running mean distributions calculated as described by Harrison, Nixon & Woods (1965). When the structure of virus particles was to be examined in detail, preparations were dialysed against 0.1 M-ammonium acetate for periods of up to 7 days before staining (Nixon & Harrison, 1959) and micrographs taken at a magnification of 40,000 x.

Four negative stains were compared using CAM and PRN: (a) phosphotungstic acid (2% w/v) adjusted to pH 6.8 with sodium hydroxide; (b) ammonium molybdate (2% w/v), pH 5.3; (c) uranyl formate (50% saturated), pH 3.8; (d) uranyl formate in sodium hydroxide (Barnett & Murant, 1970).

The dimensions of long and short virus particles of each isolate were compared by measurements of micrographs. When different viruses were compared, micrographs were taken of mixtures of either CAM and PRN or CAM and SP5, the viruses being identified from their particle lengths.

To measure the total width of virus particles and the diameter of the central canal (core width), images of individual particles were scanned using a microdensitometer (Joyce-
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Loebl, Gateshead-on-Tyne) with table magnification 50:1 and slit width 0·2 mm. Particles were scanned at right angles to their long axis, with the density being averaged over the equivalent of about 50 nm. of the particle length. In some instances, particles lying end on were also scanned using a slit width of 0·1 mm; the density was averaged over the apparent diameter of the central canal.

Polyacrylamide gel electrophoresis. Protein samples were prepared by boiling virus for 1 min. in 1 % (w/v) 2-mercaptoethanol in 0·01 M-sodium phosphate (pH 7·0). The use of 8 M-urea and 0·1 % w/v dithiothreitol in place of 2-mercaptoethanol did not alter the result. Samples were alkylated with iodoacetamide (Mayo, Murant & Harrison, 1971). Electrophoresis was in gels containing 10 % w/v acrylamide + 0·25 % w/v methylene bisacrylamide, using 0·1 M-sodium phosphate (pH 7·0) plus 0·2 % w/v sodium dodecyl sulphate (SDS). An apparatus with cells of rectangular cross-section (Narayan, Vogel & Lawrence, 1965) was used and after staining with Coomassie blue, gels were scanned using a microdensitometer. Mol. wt were estimated by comparing the mobilities of proteins of known mol. wt with those of the virus proteins. The following proteins were used for calibration: bovine serum albumin (Calbiochem), ovalbumin, carbonic anhydrase, chymotrypsinogen (Sigma), turnip yellow mosaic virus protein and tobacco mosaic virus protein.

RNA samples were prepared either by cold phenol extraction of purified virus preparations or, more conveniently, by incubating approximately 1 mg./ml. virus with 1·5 mg./ml. pronase (Calbiochem) + 0·5 mg./ml. SDS + 50 mg./ml. sucrose in 0·15 M-sodium chloride solution containing 0·015 M-sodium citrate (Ringborg et al. 1970). The pronase solution was incubated at 37 ° C for 30 min. immediately before use to remove any nucleases. Virus was incubated for 3 to 16 hr at 37 °C; samples were then applied direct to acrylamide gels for electrophoresis or were precipitated with 2·5 vol. ethanol at −18 °C. Electrophoresis was in 2·2 to 2·5 % (w/v) acrylamide gels prepared as described by Loening (1967) and run in 0·02 M-tris-phosphate buffer (pH 7·8), containing 0·2 % (w/v) SDS. After electrophoresis, the gels were examined using an u.v. scanner (Joyce–Loebl, Gateshead-on-Tyne, U.K.). Mol. wt were estimated by comparing the mobilities of the nucleic acid bands with those of ribosomal RNA species of known mol. wt (Loening, 1968). For mol. wt determinations, gels were scanned immediately after electrophoresis to avoid stretching during washing.

Zone electrophoresis. Virus suspensions were electrophoresed in a sucrose gradient at 7 °C in an apparatus similar to that described by Polson & Cramer (1958). Phenol red dye (Gurr) was introduced with the virus. Vertical illumination revealed the light-scattering bands containing virus. Samples of the most mobile components were obtained with a needle inserted from the top of the vertical column, and the less mobile components were drained from the bottom.

Moving boundary electrophoresis. A Spinco Model H apparatus with a 2 ml. quartz Tiselius cell sealed with 1:1 w/v liquid paraffin:petroleum jelly was run at 3 °C. Before the virus was examined in this apparatus it was sedimented and resuspended at a final concentration of 7 to 10 mg./ml. in veronal acetate buffer (pH 8·6, 0·1 ionic strength, Oxoid). Relative mobilities were calculated from Schlieren diagrams. The two components of CAM were separated by removing the more mobile species through the ascending limb of the cell whereas the less mobile species was taken from the descending limb; a needle with an L-shaped end was used to minimize contamination.

Serology. Antisera were prepared in rabbits sensitized by an intravenous injection of 0·5 mg. antigen, followed two or three weeks later by an intramuscular injection of 1·0 mg. antigen emulsified with Freund's incomplete adjuvant (Difco). The rabbits were bled before sensitization and at intervals following the second injection. Precipitin tests were made in
Fig. 1. Isopycnic sedimentation in caesium chloride. The Schlieren diagram was obtained after 18 hr at 30,000 rev./min. in the analytical ultracentrifuge. Schlieren angle 60°. (a) A bulk culture of CAM showing a major and a minor component. (b) A local lesion isolate of CAM showing only one buoyant density component.

glass tubes of 6 mm. internal diameter containing 0.5 ml. of each reactant diluted with 0.85% sodium chloride and partially immersed in a water bath at 37°. Precipitation end points were recorded after 5 hr. Antisera to long or short particles of isolate CAM were prepared using virus fractionated by four cycles of density gradient centrifugation.

**Infectivity assay.** Test plants were inoculated manually using inocula prepared in 0.02 M-phosphate buffer and mixed with Celite (Johns–Manville) to increase the sensitivity of the assay. On the test plant *Chenopodium amaranticolor* Coste & Reyn., treatments were distributed so that each inoculum occurred equally frequently at each leaf position. The lesions were counted 5 days after inoculation. SP5 could be distinguished from the two isolates of tobacco rattle virus by the larger lesions it produced in French bean leaves.

**RESULTS**

**Equilibrium sedimentation**

To resolve small differences which might exist between long and short particles, isopycnic sedimentation in caesium chloride was done in an analytical centrifuge. Two components with buoyant densities 1.310 and 1.315 g./ml. respectively were resolved when CAM (0.95 mg./ml.) was centrifuged for 18 hr at 30,000 rev./min. (Fig. 1 a). However, when a new isolate, obtained by propagating virus from well separated local lesions on two successive occasions, was purified and centrifuged to equilibrium in caesium chloride it gave a single Schlieren peak (Fig. 1 b) at a buoyant density of 1.306 g./ml. This indicates that long and short particles have the same buoyant density and that the bulk culture of CAM used in the first experiment was a mixture of two variants differing in buoyant density.
Fig. 2. Analytical sedimentation at 30,000 rev./min. (a) Isopycnic sedimentation of a bulk culture of PRN in caesium chloride after 18 hr. Schlieren angle 60°. Four buoyant density components are visible. (b) Isopycnic sedimentation of a local lesion isolate of PRN after 18 hr. Schlieren angle 70°. Only one buoyant density component was found. (c) Isopycnic sedimentation of a single lesion isolate of PRN in caesium chloride initial density 1.324 g./ml. for 3 hr. Schlieren angle 70°. Four components, one pair moving from left to right and one pair from right to left corresponding to short particles (outer components) and long particles (inner components).

Preparations of a bulk culture of PRN contained four components with buoyant densities of 1.319, 1.324, 1.330 and 1.354 g./ml. (Fig. 2a). However, an isolate obtained from the PRN culture by two successive passages through single local lesions contained only one component (Fig. 2b). Here again, long and short particles have the same buoyant density and the bulk culture contained a mixture of virus variants. That particles with two distinct sedimentation coefficients were present together in preparations of the local lesion isolate was apparent on several occasions because, after 3 to 5 hr centrifugation, four boundaries were seen in Schlieren diagrams (Fig. 2c). The two outermost peaks were broader than the inner pair, probably because the material responsible for their formation had a greater rate...
Table 1. Some properties of CAM, PRN and SP5

<table>
<thead>
<tr>
<th>Virus</th>
<th>Buoyant density in CsCl* (g./ml.)</th>
<th>Particle diameter† (nm.)</th>
<th>Core diameter† (nm.)</th>
<th>Protein mol. wt</th>
<th>RNA mol. wt (× 10⁻⁶)‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAM</td>
<td>1.306</td>
<td>21.7</td>
<td>3.7</td>
<td>28,500</td>
<td>2.5</td>
</tr>
<tr>
<td>PRN</td>
<td>1.324</td>
<td>23.1</td>
<td>5.1</td>
<td>28,500</td>
<td>2.5</td>
</tr>
<tr>
<td>SP5</td>
<td>1.310</td>
<td>21.3</td>
<td>4.1</td>
<td>24,000</td>
<td>2.5</td>
</tr>
</tbody>
</table>

* Data from isopynic sedimentation in analytical ultracentrifuge.
† Mean values from data in Table 2 (uranyl formate + NaOH).
‡ Data from 2.5% acrylamide gels.

Fig. 4. Polyacrylamide gel electrophoresis of virus RNA. The traces are of extinction at approximately 260 nm. Electrophoresis was from left to right. Experiment 1: (a) CAM, (b) PRN and (c) 1 part CAM plus 1 part PRN. Experiment 2: (d) CAM, (e) SP5 and (f) 1 part CAM plus 1 part SP5.

Mol. wt of protein and RNA

The mol. wt of protein from CAM was the same as of that from PRN, when virus first centrifuged from thawed sap four days before was examined (Table 1): CAM and PRN proteins were not resolved when co-electrophoresed. However, the mol. wt of protein from SP5 was consistently smaller than that from either of the tobacco rattle virus isolates. Proteins...
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Table 2. Effect of staining method and particle orientation on estimates of total diameter and core diameter of virus particles

<table>
<thead>
<tr>
<th>Virus isolate</th>
<th>Particle type</th>
<th>Stain</th>
<th>Total particle diameter</th>
<th>Core diameter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Uranyl formate/NaOH</td>
<td>Phosphotungstic acid</td>
<td>Ammonium molybdate</td>
</tr>
<tr>
<td>CAM</td>
<td>End</td>
<td>21.9 ± 2.9</td>
<td>5.9 ± 1.6</td>
<td>20.1 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>Long</td>
<td>21.7 ± 2.6</td>
<td>3.6 ± 0.9</td>
<td>23±1±2±7</td>
</tr>
<tr>
<td></td>
<td>Short</td>
<td>21.4 ± 2.7</td>
<td>3.7 ± 0.8</td>
<td>23±2±2±4</td>
</tr>
<tr>
<td>PRN</td>
<td>End</td>
<td>23.0 ± 2.4</td>
<td>7.2 ± 1.3</td>
<td>21±6±2±0</td>
</tr>
<tr>
<td></td>
<td>Long</td>
<td>23.2 ± 2.1</td>
<td>4.9 ± 0.7</td>
<td>23±2±2±4</td>
</tr>
<tr>
<td></td>
<td>Short</td>
<td>23.2 ± 2.4</td>
<td>5.2 ± 0.8</td>
<td>23±2±2±4</td>
</tr>
<tr>
<td>SP5</td>
<td>Long</td>
<td>21.5 ± 1.7</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Short</td>
<td>22.2 ± 2.7</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Long+ short</td>
<td>20.3 ± 2.3</td>
<td>4.1 ± 1.0</td>
<td>—</td>
</tr>
</tbody>
</table>

* Dimensions are expressed in nm ± 2 x standard error.

prepared from separated long and short particles of CAM were indistinguishable. Alkylation of CAM protein before electrophoresis did not affect the estimate of mol. wt.

As expected, the mol. wt of the nucleic acids from each virus reflected the predominantly bimodal particle length distribution characteristic of tobraviruses (Table 1). The ratio of mol. wt to particle length was constant for the short particles of all three strains but, when co-electrophoresed neither the larger RNA of PRN nor SP5 were resolved from that of CAM (Fig. 4) although the long particles of SP5 are about 6% longer than those of CAM.

Morphology of the three viruses

Preparations stained with ammonium molybdate were of low contrast but the axial periodicity and the central canals of the virus particles were distinct. When phosphotungstic acid was used, the central canals of particles were often indistinct and the periodicity only visible for short distances. Aqueous uranyl formate gave obvious cross banding but the cores of particles were less apparent; also, the particles appeared curved, non-uniform in diameter and broken into short pieces. No measurements were made of virus in this negative stain. However, uranyl formate in sodium hydroxide penetrated the central canal without distorting particles or affecting their length.

The effects of the different staining methods on the mean total diameters and mean core diameters of particles of PRN, CAM and SP5 (bulk cultures) are shown in Table 2. Particles stained with ammonium molybdate were 6 to 8% narrower than particles stained in either PTA or in uranyl formate + sodium hydroxide. Mean core diameters also differed and were smallest (79% of value in other stains) when uranyl formate + sodium hydroxide was used. In all three stains, particles of PRN were consistently wider and had wider central canals than particles of CAM, the ratios being consistent whichever stain was used. Particles of SP5 were about the same diameter as those of CAM. There was no significant difference in mean total diameter or mean core diameter between the long and short particles of either CAM or PRN. When particles of CAM or PRN lying at right angles to the electron mean, or parallel with it, were measured, small but statistically non-significant differences in mean total diameter were observed when uranyl formate plus sodium hydroxide or ammonium molybdate was
Table 3. Dimensions of three viruses examined in two mixed preparations

<table>
<thead>
<tr>
<th>Mixture 1</th>
<th>Mixture 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>SP5</td>
<td>CAM</td>
</tr>
<tr>
<td>CAM</td>
<td>PRN</td>
</tr>
<tr>
<td>Mean total diameter</td>
<td>20.6 ± 2.5*</td>
</tr>
<tr>
<td>Mean core diameter</td>
<td>4.4 ± 0.8</td>
</tr>
</tbody>
</table>

* Dimensions are expressed in nm ± 2 × standard error.

Fig. 5. Zone electrophoresis of CAM in a sucrose gradient. Left, photograph showing two light scattering bands obtained by electrophoresis at 1.5 v/cm. for 22 hr. Movement is from bottom to top. Centre, microdensitometer tracing of negative of photograph at left, showing one peak with a pronounced shoulder. Right, particle length distributions of: top, the more mobile component showing similar amounts of long and short particles; centre, the less mobile component showing few long particles; bottom, the virus preparation before electrophoresis.

used. However, the mean diameters of the cores of CAM or PRN particles in end view were significantly ($P < 0.001$) greater than when particles of these viruses lying at right-angles to the electron beam were measured. Perhaps the flared end (Harrison & Woods, 1966) of the virus particles held a small pool of stain. Alternatively, the phenomenon may be caused by penetration of stain between virus subunits, which would be most evident in particles viewed end on because of the optical reinforcement of stain held throughout the length of the particles.

When particles of CAM and SP5 in the same electron micrographs were measured, the total diameter of CAM was significantly ($P < 0.001$) greater, and its core diameter was significantly smaller ($P < 0.001$) than that of SP5 (Table 3). CAM particles were significantly ($P < 0.001$) narrower and had a significantly ($P < 0.001$) smaller core than particles of PRN.
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Table 4. Distribution of infectivity after moving boundary electrophoresis

<table>
<thead>
<tr>
<th>E260</th>
<th>Ascending limb, leading component</th>
<th>Descending limb, trailing component</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>4005</td>
<td>192</td>
</tr>
<tr>
<td>0.01</td>
<td>435</td>
<td>17</td>
</tr>
</tbody>
</table>

Infectivity expressed as the total number of lesions in 8 leaves of Chenopodium amaranticolor.

Zone electrophoresis of CAM

CAM separated into two components differing in mobility when 1 ml. virus (bulk culture) preparation (0.6 mg.) was exposed to a potential gradient of 1.5 V/cm for 22 hr. Microdensitometer scans of the photograph of the tube at the end of the run showed one peak with a pronounced shoulder (Fig. 5). The more mobile component contained proportionately more long virus particles than the preparation before electrophoresis whereas the less mobile component contained proportionately fewer (Fig. 5). Phenol red moved further from the origin than zones containing virus and values for RO (van Regenmortel, 1968) were 0.43 and 0.39 respectively.

Moving boundary electrophoresis of CAM

During electrophoresis of a cloned culture, two Schlieren peaks appeared and migrated at different rates towards the anode. The ratio of the peak area of the less mobile component to that of the more mobile was 3.5:1. When virus from the two boundaries was removed from the electrophoresis cell at the end of the experiment and assayed for infectivity, samples from the more mobile boundary from the ascending limb were at least twenty times more infective than those from the less mobile boundary from the descending limb (Table 4). The more infective samples also contained a greater proportion of long particles than the samples from the slower moving boundary but the separation was incomplete (Fig. 6, bottom). Attempts to improve separation by counter current compensation were unsuccessful.

Electrophoresis of preparations containing either predominantly short particles or predominantly long particles of CAM, obtained by two cycles of rate density gradient sedimentation showed that the long particles migrated faster than the short ones (Fig. 6, top and middle) confirming the indication obtained from the zone electrophoresis experiments.

Estimates were made of the electrophoretic mobility of long and short particles of CAM. Although precise temperature control was not possible the mean ratio of the mobility of long particles to that of short particles was 1.16:1 (Table 5).

Serological tests

Antiserum prepared against unfractionated CAM (bulk culture) reacted to a similar endpoint against all three antigens, with the titre to unfractionated particles being twice that to either long or short particles (Table 6). When antisera against either short or long particles (bulk cultures) were used the results were similar.

After cross absorption with long particles the antiserum against short particles of CAM failed to react at a dilution of ½ with short particles. Although there were few short virus particles in the preparation composed predominantly of long particles they may have affected this result. Indeed, after cross absorption with short particles, the antiserum against long particles still reacted at ½ with long particles. Hence, although these results require confirmation, long particles of CAM possibly have a few antigens not possessed by short particles.
Before Ascending Descending

Fig. 6. Moving boundary electrophoresis of CAM. Left, tracings of Schlieren diagrams: top, virus preparation containing mostly short particles; centre, virus preparation containing predominantly long particles; bottom, unfractonated virus preparation. Right, particle length distributions of preparation before electrophoresis and of samples from the ascending and descending boundaries.

Table 5. Electrophoretic mobilities of two components of CAM in veronal acetate buffer at pH 8.6 and ionic strength 0.1

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Mobility x 10^5 (cm^2/v sec.) of long particle rich component</th>
<th>Mobility x 10^5 (cm^2/v sec.) of short particle rich component</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.17</td>
<td>5.50</td>
<td>1.17</td>
</tr>
<tr>
<td>2</td>
<td>6.24</td>
<td>5.45</td>
<td>1.15</td>
</tr>
<tr>
<td>3</td>
<td>5.65</td>
<td>4.80</td>
<td>1.18</td>
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<td>4</td>
<td>5.59</td>
<td>5.00</td>
<td>1.12</td>
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<td>6.35</td>
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<td>—</td>
</tr>
<tr>
<td>7</td>
<td>—</td>
<td>5.45</td>
<td>—</td>
</tr>
<tr>
<td>Mean</td>
<td>6.07</td>
<td>5.27</td>
<td>1.16</td>
</tr>
</tbody>
</table>

Data derived from unfractonated virus (experiments 1 to 4) from a preparation of long particles produced by two cycles of density gradient sedimentation (experiment 5), from a preparation of long particles produced by three cycles of density gradient sedimentation (experiment 6) and from a preparation of short virus particles produced by two cycles of density gradient sedimentation (experiment 7).

Table 6. Serological relationship between long and short particles of CAM

<table>
<thead>
<tr>
<th>Antiserum</th>
<th>Short particles</th>
<th>Unfractionated preparation</th>
<th>Long particles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Short particles</td>
<td>64</td>
<td>128</td>
<td>64</td>
</tr>
<tr>
<td>Unfractionated preparation</td>
<td>128</td>
<td>256</td>
<td>128</td>
</tr>
<tr>
<td>Long particles</td>
<td>128</td>
<td>256</td>
<td>128</td>
</tr>
</tbody>
</table>

Figures are reciprocals of precipitation end-points of antiserum, using antigen at 0.05 mg./ml., after 5 hr incubation at 37°C.
Harrison & Woods (1966) showed that CAM was only distantly related to PRN and no serological relationship was detected between sP5 and PRN (Gibbs & Harrison, 1964). However, an isolate of pea early-browning virus and one of tobacco rattle virus from the Netherlands have antigenic determinants in common (Maat, 1963) and, because the buoyant density and particle morphology of sP5 and CAM are similar, these viruses were tested serologically against the heterologous antisera. Using undiluted and diluted antisera with homologous titres of 1/512 and antigens at four times the concentration at their precipitation end point, no heterologous reactions were observed.

**DISCUSSION**

Our estimates of the dimensions of virus particles agree reasonably well with those obtained by others. By electron microscopy of PRN in different stains, Nixon & Harrison (1959) reported that the mean total diameter of particles ranged from 17 to 25 nm, according to the stain, and Offord (1966) quotes a value of 25.6 ± 0.3 nm. With somewhat different stains we obtained diameters of 21.6 to 23.2 nm. Similarly Nixon & Harrison (1959) quote a core diameter of 4.0 to 7.0 nm, and Offord (1966) gives a value of 5.4 nm. We obtained core diameters of 4.9 to 5.3 nm. Again by means of electron microscopy, Harrison & Woods (1966) found that sP5 was 7% narrower than a Dutch isolate of tobacco rattle virus serologically similar to PRN; we found sP5 was 8% narrower than PRN. By X-ray diffraction, the cylindrically averaged diameters of PRN and CAM are reported to be 22.5 nm (Finch, 1965) and 20.5 ± 0.5 nm. (Tollin & Wilson, 1971) respectively, whereas our values in uranyl formate + NaOH stain were 23.1 and 21.7 nm respectively. Our estimate of 3.7 nm for the core diameter of CAM compares with that of 4.1 nm obtained by X-ray diffraction (Tollin & Wilson, 1971). Lwoff, Horne & Tournier (1962) suggested that nucleocapsid diameter should be used in the classification of viruses with helical symmetry. However, our measurements of the particles of tobraviruses show that not only does their apparent diameter depend on the methods used but also that serologically related viruses can have diameters differing by up to 8%. Thus, both in theory and in practice, nucleocapsid diameter is a character of limited value for classifying viruses with helical symmetry.

By amino acid analyses, two strains of tobacco rattle virus from the U.S.A., two from Japan, PRN and CAM were all estimated to have proteins containing 174 to 178 amino acids (Semancik, 1966; 1970; Miki & Okada, 1970). However, Gibbs & McIntyre (1970) using a statistical technique to re-examine published amino acid compositions suggested that the number of amino acids in the two North American tobacco rattle isolates (Semancik, 1966) is 190 to 195. Also from amino acid analyses, Offord & Harris (1965) reported that there were 218 amino acids in the protein subunits of an isolate of tobacco rattle virus from the Netherlands. These numbers of amino acids are equivalent to mol. wt in the range 19,000 to 24,000. However, the values of 28,500 which we obtained for CAM and PRN protein using polyacrylamide gel electrophoresis compare with that of 29,000 for an isolate from the U.S.A. (Lesnaw & Reichmann, 1970) and 27,000 for the isolate used by Offord & Harris (Carpenter, Cook & Gibbs, 1971); all four estimates being obtained by similar methods. Thus, although the mol. wt from amino acid composition and those obtained using polyacrylamide gel electrophoresis differ, similar discrepancies are reported with other viruses, including tobacco necrosis and potato X (Miki & Knight, 1968; Lesnaw & Reichmann, 1970). There were no previous estimates of the mol. wt of pea early-browning virus protein.

No correlation was found between particle diameter or nucleoprotein volume of CAM, PRN and sP5, and protein molecular weight.
Different strains of tobacco mosaic virus differ in buoyant density (Ginoza & Atkinson, 1955; Siegel & Hudson, 1959) and it is not surprising that different strains of tobacco rattle virus should differ similarly. However, it is interesting that, before they were passed through single lesions, the bulk cultures of both CAM and PRN contained multiple buoyant density components. The long and short particles of an isolate have the same buoyant density in caesium chloride, but those of CAM apparently differ in electrophoretic mobility. Perhaps this difference in surface charge explains the association of long but rarely of short particles of CAM with mitochondria (Harrison & Roberts, 1968; Kitajima & Costa, 1969). The long particles become attached to the mitochondria at one end but rarely bridge between adjacent mitochondria, and the protein surface at one end of the particle might well differ in charge from that at the other. However, the protein surfaces at the ends of long and short particles are probably similar; it is ratio of the surface areas of end and side that differs between long and short particles and this difference seems the most plausible explanation of the difference in mean surface charge.

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REFERENCES


Properties of three tobravirus isolates


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