

Changes Induced by Magnesium Ions in the Morphology of Some Plant Viruses with Filamentous Particles

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(Accepted 4 June 1971)

SUMMARY

Particles of henbane mosaic virus in extracted plant sap were usually straight and 900 nm. long but occasionally flexuous and 800 nm. long. The length and flexuousness of the particles depended on the composition of the extracting medium. When exposed to magnesium ions (0.05 M), the particles were long and straight but when exposed to 0.05 M-EDTA they were shorter and flexuous. Similar morphological differences were found when pepper veinal mottle virus or bean yellow mosaic virus was extracted in the two solutions. With pepper veinal mottle virus, each form of particle could be converted to the other by changing the medium.

INTRODUCTION

Watson, Plumb & Woods (1971) found that negatively stained preparations of purified henbane mosaic virus (HMV), when viewed in the electron microscope, showed rather straight particles with a modal length of about 830 nm. The particle length was very variable, probably because many particles had broken during purification, but none resembled the flexuous 730 nm. particles described by Brandes (1959). Extracts of infected plants gave straight particles about 900 nm. long (R. T. Plumb & D. A. Vince, personal communication). Extending their work, we found that, although most preparations gave these straight 900 nm. particles, occasional preparations gave predominantly flexuous particles with a modal length of about 800 nm. Only rarely were the two types of particle seen in the same preparation. In this paper, we describe our investigation into the relation between the two types of particle.

METHODS

Infected leaf was ground, using a pestle and mortar, in distilled water or buffer solution using 2 ml./g. of leaf. The extracted sap was mixed with twice its volume of 2% sodium phosphotungstate pH 6.8, sprayed on to carbon-coated mounts and examined in the Siemens Elmiskop 1 A electron microscope. Photographs were taken at a magnification of 40,000. The magnification was set for each series of samples by adjusting the objective, intermediate and projector lens' currents to values known, by calibration, to give the required result and it was periodically checked by examining a catalase crystal or by reference to a 70 μ m. hole.

RESULTS

The two types of particle were not confined to infections with specific HMV isolates. Several different cultures, maintained by Dr M. A. Watson at Rothamsted, were examined and each often gave 800 nm. flexuous particles when water extracts were made soon after

the plants developed vein-clearing symptoms, but gave only straight 900 nm. particles when the same plants were extracted some days later. Numerous examinations showed that flexuous 800 nm. particles were found more often in extracts of young leaves or of young and recently infected host plants than in extracts of old leaves or of older plants infected for more than 2 weeks. Also, they were found more often in extracts of *Datura stramonium* L. than in tobacco extracts and more often when source plants were kept above 20° than at lower temperatures.

We concluded that, either we were dealing with two viruses of which the one with 800 nm. particles multiplied faster at first but was eventually largely suppressed by the one with 900 nm. particles, or the length and straightness of the HMV particle depended on the conditions in the sap during extraction. A virus mixture seemed the less probable explana-

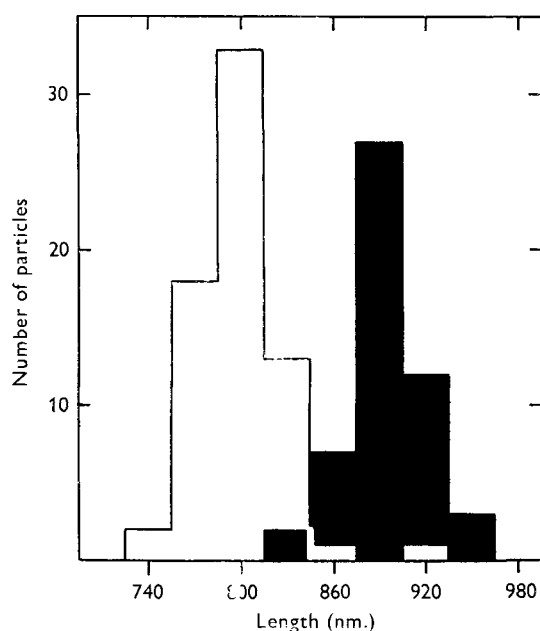


Fig. 1. Particle length distributions of henbane mosaic virus extracted from leaves of *Datura stramonium* L. in 0.05 M-EDTA (□, 68 particles) and 0.05 M-MgCl₂ (■, 51 particles).

tion because extracts of the two halves of the same leaf sometimes gave particles of different types. Also, by extracting sap in 0.07 M-phosphate buffer pH 7.5, we usually, but not always, obtained 800 nm. particles from plants that had previously given only 900 nm. particles when extracted in water.

Attempts to separate the two particles by infecting a range of host plants failed until we found that extracts of infected *Petunia hybrida* Vilm. in water invariably contained flexuous 800 nm. particles, even when the plants had been infected for long periods. However, extracts of infected *P. hybrida* in 0.2 M-ammonium acetate buffer pH 5.2, contained only straight 900 nm. particles.

Although it was evident that the type of particle obtained could be controlled to a large extent by extracting in buffers of different pH, it seemed probable that this control depended on the different ions in the buffer solutions, rather than the difference in pH. Phosphate buffer as extractant would precipitate most of the Mg and Ca ions in sap, whereas ammonium

acetate buffer would not, so we tested the effect of adding Mg ions during extraction and of removing them by extraction in solutions containing EDTA. Infected leaf was ground in 0.05 M-ammonium acetate buffer containing either 0.05 M-MgCl₂ (pH of mixture 8.65) or 0.05 M-EDTA (pH of mixture 8.5).

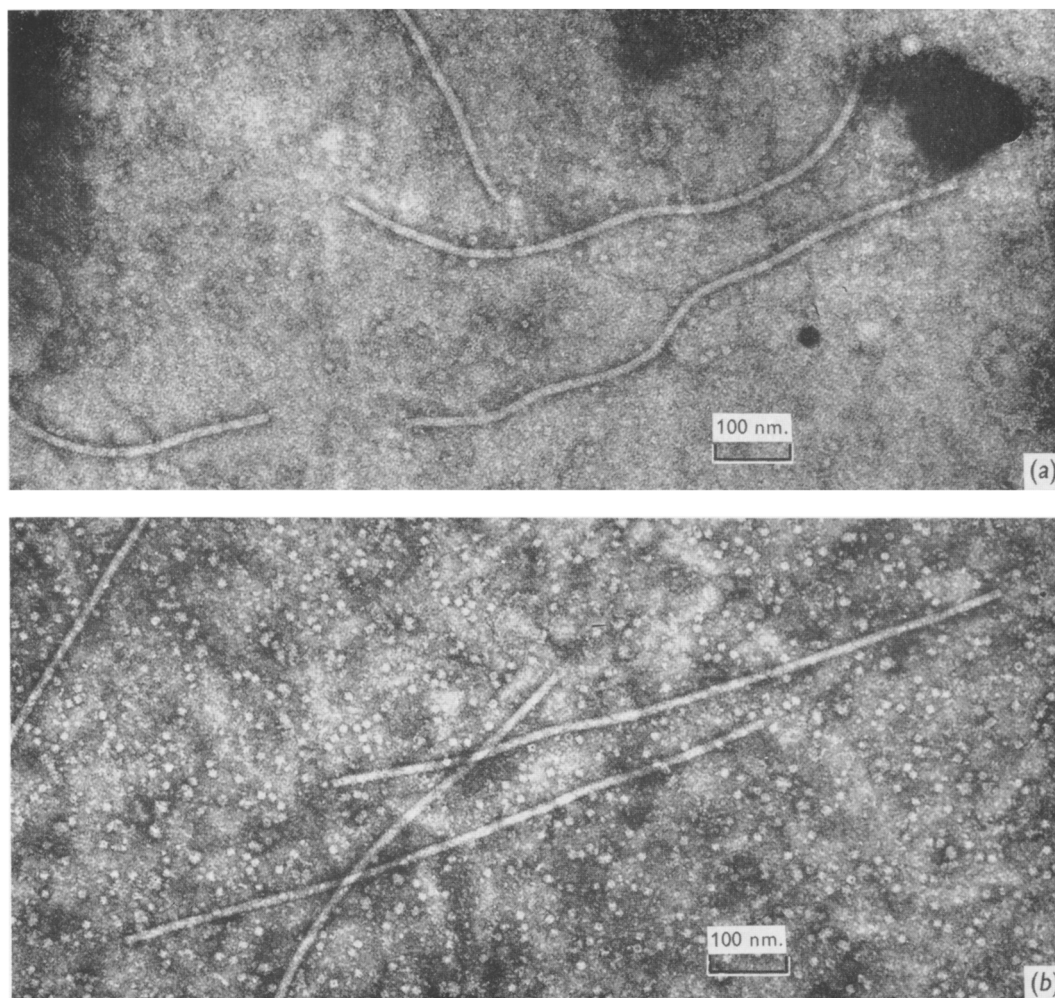


Fig. 2. Particles of henbane mosaic virus extracted from leaves of *Datura stramonium* in (a) 0.05 M-EDTA, (b) 0.05 M-MgCl₂.

Extraction in the EDTA solution gave only flexuous 800 nm. particles, regardless of the identity of the host species, of how long it had shown symptoms, or whether it had been kept above or below 20° (Fig. 1, 2a). By contrast, extractions in the MgCl₂ solution gave only straight 900 nm. particles (Fig. 1, 2b). Extraction in 0.05 M-CaCl₂ also gave straight 900 nm. particles.

Similar tests were made on plants infected with several other viruses. Extracts of plants infected with potato virus S, potato virus X, potato virus Y or tobacco severe etch virus each contained particles of one characteristic appearance and modal length whichever

extracting solution was used. Extracts of plants infected with pepper veinal mottle virus (Brunt & Kenten, 1971) contained flexuous particles 750 nm. long when extracted in the EDTA solution and straight particles 850 nm. long when extracted in the $MgCl_2$ solution. Extraction in the $MgCl_2$ solution of broad bean leaves infected with bean yellow mosaic

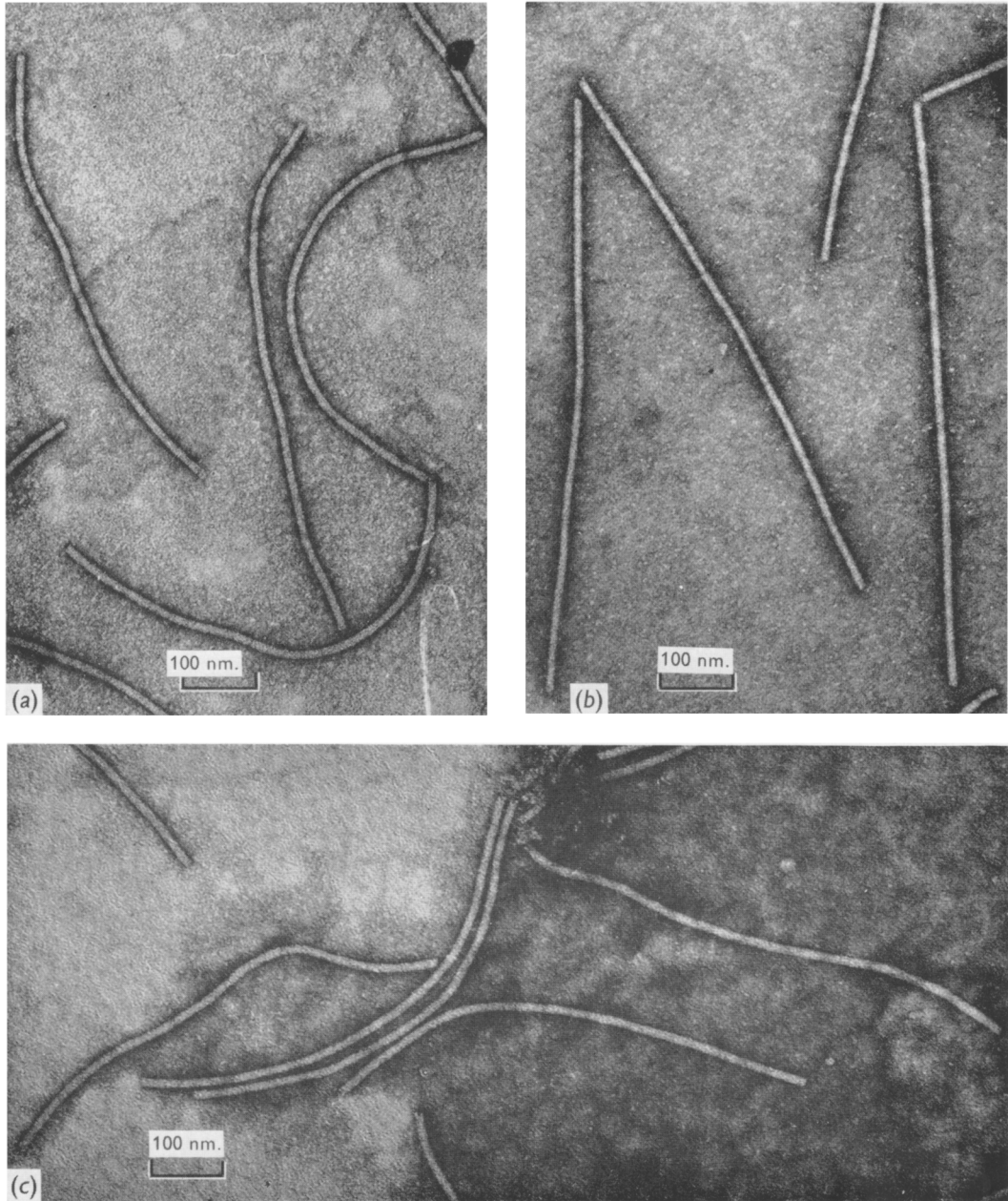


Fig. 3. Particles in a purified preparation of pepper veinal mottle virus (a) in 0.05 M-borate buffer, (b) after adding an equal volume of 0.1 M- $MgCl_2$, (c) treated as (b) and then dialysed against 0.02 M-EDTA.

virus gave straight particles about 800 nm. long, whereas extraction in the EDTA solution gave particles of various types, some straight and about 800 nm. long, others shorter and flexuous; but when this extract was dialysed for 24 hr against 0.02 M-EDTA pH 7.7 most of the particles were flexuous and about 750 nm. long.

It was possible that each of the two extracting solutions released one type of particle and destroyed the other, although it seemed improbable that all our cultures were mixtures. However, to eliminate this possibility, further tests were made with a purified preparation of pepper veinal mottle virus kindly provided by Mr R. H. Kenten. The purified virus was in 0.05 M-borate buffer and electron microscopy showed flexuous particles with a modal length of about 700 nm. (Fig. 3*a*). The particles were shorter and their length more variable than those seen in sap, probably because some had broken during storage. After adding an equal volume of 0.1 M-MgCl₂, all the particles were straight and their modal length was about 800 nm. (Fig. 3*b*). After the MgCl₂-treated preparation had been dialysed for 24 hr against 0.02 M-EDTA pH 7.7, it again contained only the short flexuous particles (Fig. 3*c*). Re-treatment of the dialysed sample with MgCl₂ restored the long straight particles.

We think that, in the three different viruses for which we have described morphological changes, Mg ions may form bridges between carboxyl groups of the virus protein and that these bridges may alter the conformation of the protein subunit, so changing the length and rigidity of the virus particle. However, some experiments were done with HMV in which Mg-treated sap, with or without added PTA, was sprayed on to mounts that were then either vacuum-dried or air-dried before shadowing with platinum-iridium. Unless preparations were vacuum-dried (put in the microscope vacuum soon after spraying), most particles were about 850 nm. long and few were as long as 900 nm. Particles were straighter when the sap had been treated with PTA than when it had not. So it seems that vacuum-drying and the presence of PTA help produce the straight 900 nm. particles or, more probably, preserve them by preventing contraction and flexing.

DISCUSSION

That the particle lengths of at least three different viruses depend on the extraction medium and that, unless the extraction medium is controlled, extracts of the same leaves will give different and unpredictable results, has obvious implications for diagnosis by electron microscopy. The possibilities of misdiagnosis are well illustrated by recent reports by Bode, Brandes & Paul (1969) and Harrison & Roberts (1971) of slightly flexuous particles with a modal length of 900 to 925 nm. in plants infected with *Atropa* mild mosaic virus. Dr B. D. Harrison kindly supplied a tobacco plant infected with this virus and, by extracting its leaves in the EDTA and MgCl₂ solutions we obtained, respectively, flexuous 800 nm. and straight 900 nm. particles. The particles were indistinguishable from those of HMV extracted similarly and sap from the tobacco plant reacted specifically with an antiserum to HMV. The reports by Taylor & Smith (1968) and Bos (1970) of a host-dependent variation in the length of the particles of some bean yellow mosaic virus isolates may also be explained by different conditions in the saps of the different hosts.

If Mg ions can have such large effects on the morphology of some viruses, particles differing only slightly in structure from one another may react differently during extraction, and these reactions may not be easily controlled by standardizing the extracting solution. Then, related virus strains may yield different types of particle, whatever the extraction method used. If so, a small difference in particle morphology is not a reliable criterion for separating viruses into different groups. For example, the only property that clearly

separates potato virus Y (730 nm.), bean yellow mosaic virus (750 nm.) and HMV (800 nm.) from one another is particle length.

Lovisolo & Bartels (1970) recently reported a particle length of 850 nm. for HMV. None of our isolates of HMV showed the flexuous 730 nm. particles described by Brandes (1959), although they produced symptoms indistinguishable from those described for HMV (Hamilton, 1932). However, by passage through *Solanum demissum* 'SdA' (Cockerham, 1958) and by early subcultures from infected *Petunia hybrida*, we separated a strain of potato virus Y from one of our HMV cultures, although this contamination was not detected serologically or by electron microscopy. Bawden & Kassanis (1941) failed to detect potato virus Y serologically in plants also infected with HMV and showed that HMV suppresses the multiplication of potato virus Y. When originally described, HMV was associated with a strain of potato virus Y (Watson, 1937), and a culture of HMV that had been freeze-dried and stored for several years before being sent to us by Dr M. Hollings, contained only potato virus Y. Perhaps the isolate examined by Brandes (1959) originated from a mixture of HMV and potato virus Y from which HMV was lost during preservation or subculture. In view of this possibility, the reported serological relationship between HMV and members of the potato virus Y group (Bartels, 1963/64) should be re-examined.

We gratefully acknowledge the helpful suggestions and cooperation of many of our colleagues.

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(Received 19 April 1971)