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Electron Microscopic Observation on Dolichos Enation Mosaic Virus

By D. McCARTHY* AND R. D. WOODS
Rothamsted Experimental Station, Harpenden, Hertfordshire

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SUMMARY

Sap from plants infected with a strain (NDEMV) of dolichos enation mosaic virus (DEMV) contains many ringlike particles, but few particles of about 3000 Å, the normal length for viruses of the tobacco mosaic virus group. In purified preparations the mean length of normal particles of NDEMV was 3000 Å and that of DEMV, 2860 Å. Staining with uranyl formate clearly revealed the fine structure of these particles and in the ringlike particles it penetrated to the expected position of the ribonucleic acid.

INTRODUCTION

From dolichos enation mosaic virus (DEMV), which is serologically related to tobacco mosaic virus, a strain (NDEMV) was isolated that causes necrotic lesions in beans and has some unusual properties (Kassanis & McCarthy, 1967). Preparations of the strain (NDEMV) isolated from plants kept at 20° contained many broken and ringlike particles with few particles of normal length (3000 Å) whereas preparations from plants kept at 36° contained many more particles of normal lengths, some broken and a few ringlike particles. DEMV did not show such sensitivity to changes in temperature and preparations from plants kept at 20° or 36° were very similar to preparations of NDEMV from plants kept at 36°.

This paper describes the structure, as shown by electron microscopy, of DEMV and NDEMV from plants grown at 20°.

METHODS

The materials used were as reported by Kassanis & McCarthy (1967), but purified preparations were further fractionated on the basis of their particle sizes by passage through a column of 4% (w/v) agar (Steere & Ackers, 1962). For electron microscopy negatively stained specimens were deposited on carbon support films covering seven-hole platinum mounts. Two preparation methods were routinely used: (1) the virus suspension was mixed with an equal volume of 2% (w/v) potassium phosphotungstate pH 6·8 containing 0·2% bovine serum albumin to assist spreading, and then sprayed on to the film; (2) a droplet of the virus suspension was placed on the carbon film, a droplet of 1% uranyl formate (Leberman, 1965) mixed with it and the excess liquid removed after 15 sec. Specimens were examined in a Siemens Elmiskop I at 80,000 v and photographs taken at set magnifications of ×20,000 or ×80,000.

* Present address: Botany Department, Queen Mary College, Mile End Road, London, E. 1.

RESULTS

Examination of infected sap

Preparations of both strains, whether revealed with phosphotungstic acid or uranyl formate, contained rod-shaped particles that did not have the rigid outline usually associated with tobacco mosaic virus. Instead, the particles were slightly curved or bent, sometimes at more than one point. When sap containing particles of NDEMV was stained with phosphotungstic acid the stain penetrated the hollow centre of different particles to two radial distances (Pl. 1, fig. 1). The outer diameter of individual particles was also observed to vary. Some particles of NDEMV in sap stained with uranyl formate had many fractures, and often short particles lay end to end as though formed by a single longer particle breaking during staining. Some of these short particles had flat ends, but many had a stepped appearance possibly indicating an abrupt end to the helical arrangement of protein subunits where the breakage occurred (Pl. 1, fig. 2). Ring-like particles orientated in end view showed a concentric band of stain about midway between the axis and periphery of the particle (Pl. 1, fig. 4), in addition to the central hole. On several occasions crystalline bodies, composed of tightly packed particles, were seen in leaf-dip mounts (Pl. 1, fig. 3). Although some of the repeating dimensions in this lattice are similar to those of the ring-like particles, it is not known whether these bodies are a consequence or a by-product of infection.

The ratio of numbers of short particles to those of normal length in sap from plants infected with NDEMV changed during our work; more broken particles were observed during winter than summer. Several factors may be concerned in this change but differences in average temperature in the glasshouse are probably the most important.

Purified preparations

Staining. As the shorter particles observed in preparations of the two strains may have been produced by longer particles breaking during mounting for electron microscopy, the effects of potassium phosphotungstate and uranyl formate on the particles were compared. A preparation of NDEMV containing particles whose lengths ranged from 500 to 3250 Å was obtained by agar-column fractionation. One sample was sprayed directly on to a carbon film and shadowed with platinum-iridium and others were mounted in potassium phosphotungstate or uranyl formate. Little difference was observed in the particle-length distributions when the three samples were measured, except that potassium phosphotungstate induced some particles with lengths 500 to 1000 Å to aggregate end to end. The proportion of the particle population with lengths between 2750 to 3250 Å was similar irrespective of the method of preparation.

Length distribution and fine structure. During routine investigations of particle-size distributions of the two strains, some evidence was obtained that particles of NDEMV had a slightly longer normal length that those of DEMV. To check this, samples consisting largely of normal-length particles were obtained from agar-column fractionations and their length distribution measured after staining with potassium phosphotungstate. Both samples had nearly normal distributions with means at 2860 Å and 3000 Å for DEMV and NDEMV respectively.

When observed in uranyl formate, normal-length particles of both strains had ends that were either slightly rounded or stepped, but rarely exactly square. In some places the surface of particles showed an irregular pattern, but in others, a regular one,

corresponding to the primary and secondary helices. Uranyl formate also penetrated further between the turns of the primary helix in particles of NDEMV than of DEMV (Pl. 1, fig. 5, 6).

Ring-like particles. Ring-like particles devoid of RNA were obtained from purified preparations of NDEMV by fractionation through an agar column (Kassanis & McCarthy, 1967), and examined using uranyl formate. The circle of stain previously seen in the end view of particles from leaf-dip mounts could now be resolved more clearly. Often it was evidently not a continuous circle of stain, but made up of eight small areas of stain spaced roughly symmetrically, and at a radial distance of about 40 Å from the centre of the particle (Pl. 1, fig. 7). In side view these ring-like particles have never been seen to form the stacked-disc structures characteristic of aggregated tobacco mosaic virus protein. However, end-to-end aggregates form with each section composed of 3 to 6 turns of the helix. The staggered fractures which occur between each section suggest that the helical arrangement of subunits ends abruptly, unlike the stable two-turn disc aggregates of tobacco mosaic virus protein.

Aggregation of NDEMV 'A' protein. The frequency in purified preparations of NDEMV in 0·06 M-phosphate buffer pH 8 of particles shorter than 3000 Å, possibly indicates that the coat protein of this virus cannot form a structure as rigid and stable as that formed by tobacco mosaic virus at the same pH. To determine whether this was an inherent characteristic of the protein, the 'A' protein of NDEMV was obtained from a purified preparation by the cold alkali method and allowed to reaggregate in 0·06 M-phosphate buffer pH 5·2 for 18 hr. The 'A' protein was also similarly obtained from a purified preparation of tobacco mosaic virus and allowed to reaggregate under identical conditions. Electron microscopy showed that whereas the 'A' protein of tobacco mosaic virus had aggregated into rod-like particles with lengths greater than 3000 Å, the 'A' protein of NDEMV had aggregated to give the typical ring-like particles and some short rods whose lengths rarely exceeded 1000 Å. The stacked disc structure of reaggregated tobacco mosaic virus protein, reported by Nixon & Woods (1960), was never observed in samples of reaggregated NDEMV protein, but a fine cross-banding was seen in preparations stained with potassium phosphotungstate or uranyl formate. This is unusual, as fine cross-banding is usually seen only in intact particles stained with uranyl formate.

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EXPLANATION OF PLATE

Fig. 1. Electron micrograph of particles of NDEMV from cut leaf, mounted in sodium phosphotungstate.

Fig. 2. As fig. 1, but mounted in uranyl formate.

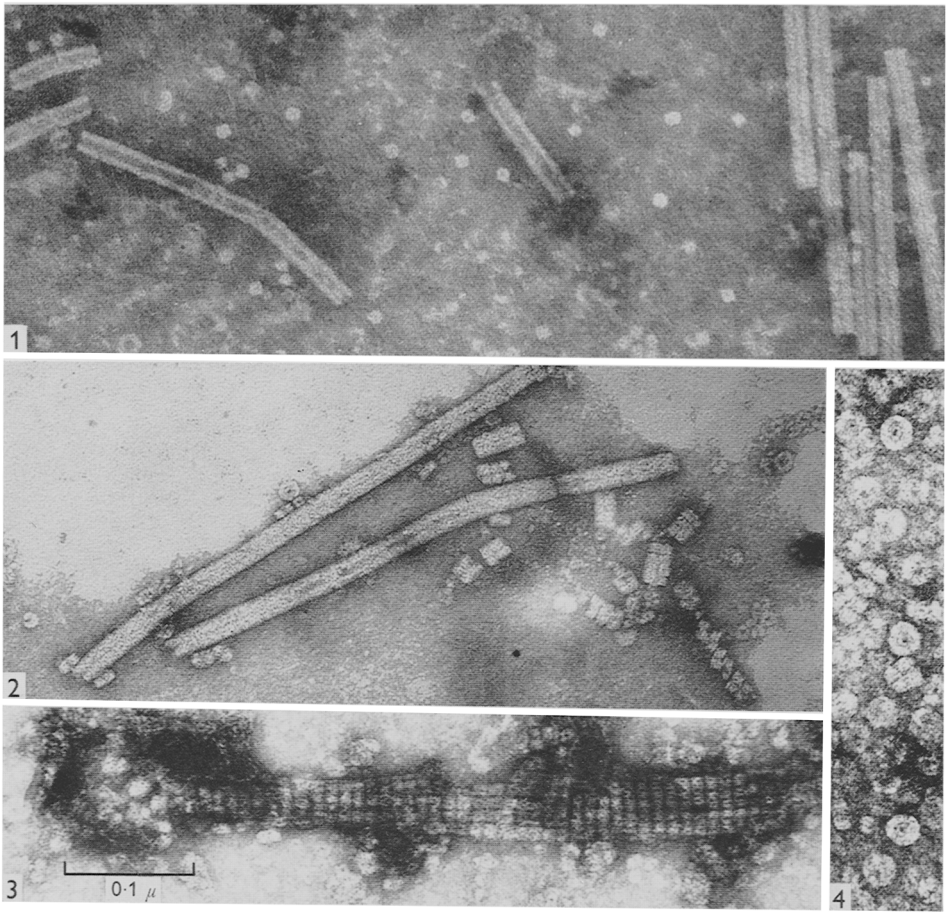
Fig. 3. Electron micrograph of crystalline body seen in cut leaf mounts, mounted in sodium phosphotungstate.

Fig. 4. Electron micrograph of 'rings' from cut leaf mounted in sodium phosphotungstate showing a concentric band of stain.

Fig. 5. Electron micrograph of a particle of NDEMV stained with uranyl formate.

Fig. 6. Electron micrograph of a particle of DEMV stained with uranyl formate.

Fig. 7. Electron micrograph of 'rings' from purified preparation of NDEMV stained with uranyl formate showing eight areas of stain, about 40 Å from the centre of the particle.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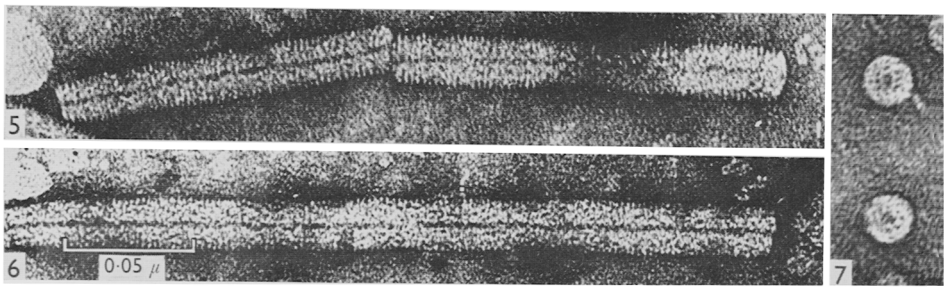
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