Deoxyribonucleic Acid in Dahlia Mosaic Virus

I. Fujisawa, M. Rubio-Huertos, and C. Matsui

Plant Pathology Laboratory, Faculty of Agriculture, Nagoya University, Nagoya, Japan; Instituto Jaime Ferran de Microbiologia, CSIC, Madrid, Spain.

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ABSTRACT

Dahlia mosaic virus was purified from infected *Verbesina encelioides* by differential centrifugation and sucrose density-gradient centrifugation. The purified preparation revealed a typical ultraviolet absorption spectrum for a nucleoprotein, and consisted of infective particles about 45 nm in diam. Thymidine-^{3}H was incorporated preferentially into the virus and the nucleic acid isolated from the purified virus was digested completely with deoxyribonuclease, but not digested with ribonuclease. The virus therefore contains deoxyribonucleic acid. X-bodies associated with dahlia mosaic virus infection were stained with conjugated anti-cauliflower mosaic virus globulin.

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In plant virus classification (7), the caulimovirus group consists of cauliflower mosaic virus (CaMV), carnation etched ring virus (CERV) and dahlia mosaic virus (DMV). All of them are spherical particles of 40-45 nm in diameter (1, 2, 4, 5). They are restricted in or on the characteristic intracellular X-bodies within host cells (4, 6, 10, 11). Furthermore, there are serological relationships between them (1, 2, 8). CaMV and CERV contained DNA as viral nucleic acid (4, 5, 12, 13). However, little is known about nature of the nucleic acid in DMV.

This paper deals with nature of nucleic acid in DMV and serological properties of DMV.

MATERIALS AND METHODS.—*Virus and plants.* — The virus was increased in dahlia (*Dahlia variabilis* Desf.) and *Myzus persicae* Sulz. was used to transmit DMV from infected dahlia to young seedlings of dahlia, verbesina (*Verbesina encelioides* L.) or zinnia (*Zinnia elegans* Jacq.). The X-bodies associated with infection of DMV isolate used in the present study were larger in number in dahlia plants than in verbesina or zinnia plants.

Purification.— About 3 mo after aphid-inoculation with DMV, infected verbesina leaves were harvested. Five

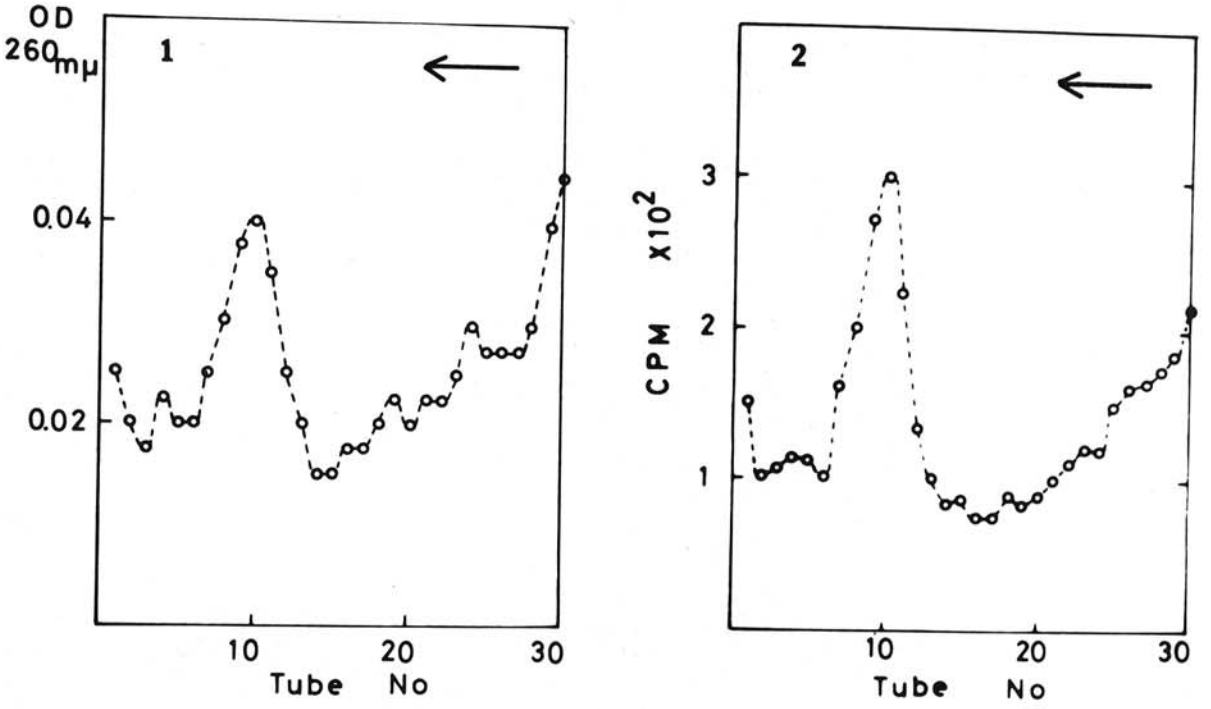


Fig. 1-2. 1) Absorbance (260 nm) of fractions from a sucrose density-gradient centrifugation of a preparation of purified dahlia mosaic virus. Arrow indicates sedimentating direction. 2) Radioactivity profile of a thymidine-^{3}H-labeled dahlia mosaic virus subjected to a sucrose density-gradient centrifugation. Arrow indicates sedimentating direction.

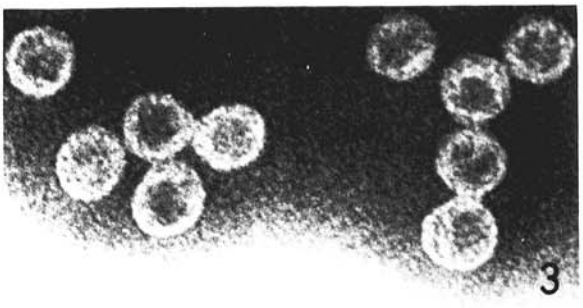


Fig. 3. Electron micrograph of negatively stained (2% potassium phosphotungstate) dahlia mosaic virus particles (× 180,000).

hundred grams of leaves were frozen and then homogenized in 700 ml of cold 0.5 M phosphate buffer, pH 7.5, containing 0.01 M Na_2SO_3. After addition of *n*-butanol to 8.5% (v/v), the homogenate was stirred at 4 C overnight. The virus was purified from the homogenate by the procedures previously described (4).

Isotope uptake.— Infected verbesina leaves were floated for 4 days on an isotope solution containing 1 mCi of uridine-^{3}H (6.0 Ci/mmole) or thymidine-^{3}H (5.0 Ci/mmole) under continuous illumination at 27 C. For preparation of ^{32}P-labeled DMV, infected dahlia leaves were floated on a ^{32}P solution ($H_3P^{32}O_4$; 70-60 Ci/mg) for 4 days under the same conditions as described before. The leaves then were frozen and the radioactive virus was purified from the frozen leaves by the procedures described above.

Isolation and digestion of the nucleic acid.— Procedures for the isolation of the nucleic acid from ^{32}P-labeled DMV, the digestion of the nucleic acid with deoxyribonuclease (DNase) or ribonuclease (RNase), and radioactivity measurements of the nucleic acid digests were described previously (5).

Fluorescent-antibody staining.— CaMV was purified from the sap of infected leaves of *Brassica perviridis* Bailey by differential centrifugation and sucrose density-gradient centrifugation (4). The purified virus was suspended in 0.01 M phosphate buffer, pH 7.0, containing 0.85% NaCl (PBS) for injection. A rabbit received one intravenous injection of 2 mg CaMV per wk for 3 wk. Ten days after the last immunization, the rabbit was bled, and yielded 120 ml of antiserum with a titer of 1/1024.

Isolation of the immunoglobulin G fraction from the antiserum, and conjugation of immunoglobulin G with fluorescein isothiocyanate was carried out by the method of Otsuki and Takebe (9). In order to minimize nonspecific staining, the conjugated globulin was absorbed with acetone extracts of powders of healthy dahlia leaves. After two

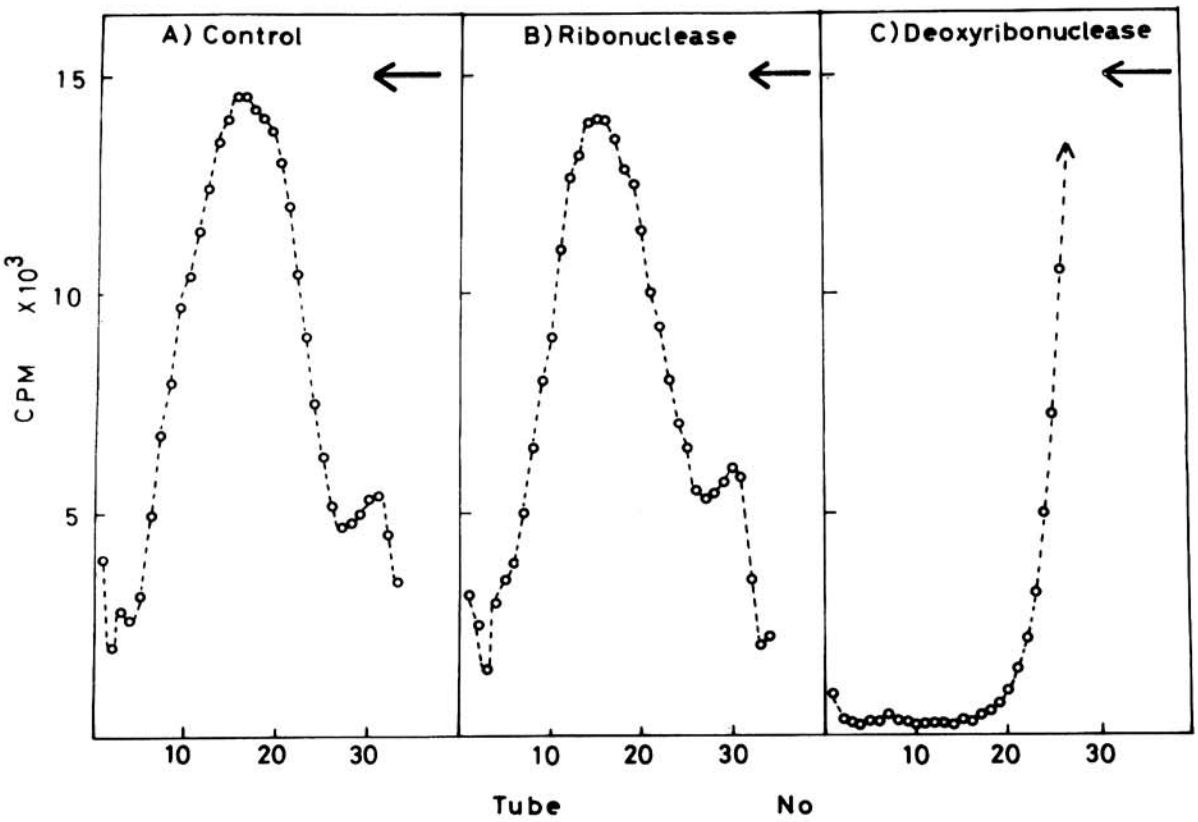


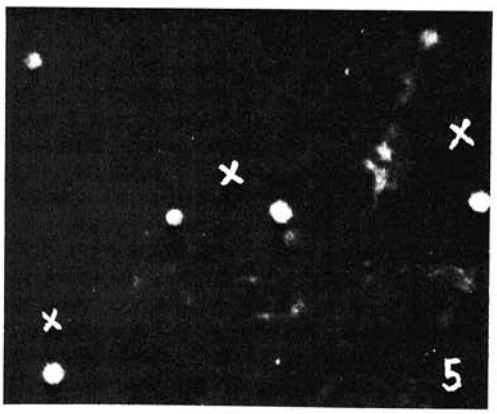
Fig. 4-A, B, C. Radioactivity profile of centrifuged sucrose density gradient containing nucleic acid isolated from ^{32}P-labeled dahlia mosaic virus. **A)** Control. **B)** Treated with ribonuclease. **C)** Treated with deoxyribonuclease. Arrow indicates sedimentating direction.

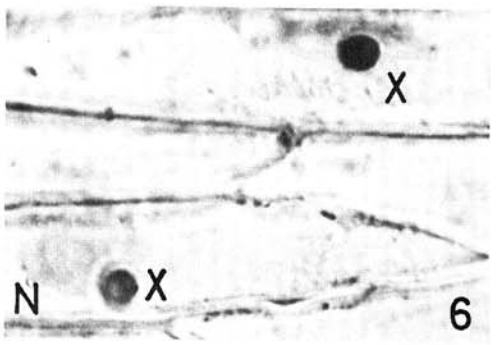
adsorptions with acetone powder preparations, the mixture was centrifuged at 10,000 *g* for 20 min to remove insoluble material. The supernatant solution was used for staining. The epidermal layer was stripped from infected dahlia leaves and fixed with acetone for 2 hr at room temp. After fixation, the specimen was washed through four changes of PBS and then stained by immersing the specimen in conjugated globulin solution at 36 C. After 24 hr in the conjugated globulin solution, the specimen was washed in PBS as described above and immersed in PBS containing 10% glycerin. The stained specimens were examined under a fluorescence microscope (9).

RESULTS AND DISCUSSION.—*Purified virus particles.*– The absorbance at 260 nm of each fraction after sucrose density-gradient centrifugation of DMV from infected verbesina leaves is shown in Fig. 1. The absorbance peak occurred in tube No. 10. The ultraviolet absorption spectrum of this fraction was typical of nucleoprotein with maximum and minimum absorption at about 260 and 240 nm, respectively. The ratio of the absorbancies, 260/280, was about 1.35. This value is close to those reported for CaMV (1.40) and for CERV (1.38). Electron microscopy showed that this fraction consisted of spherical particles about 45 nm in diam (Fig. 3). Since these spherical particles were infective to verbesina and zinnia plants, and characteristic X-bodies were produced in the infected leaf cells, these spherical particles were considered to be DMV.

Thymidine-^3H uptake of DMV particles.— The radioactivity associated with each fraction after sucrose density gradient centrifugation of the extract from infected verbesina leaves labeled with thymidine-^3H is shown in Fig. 2.The radioactive peak appeared at tube No. 10 and corresponded to the purified DMV fraction (Fig. 1). On the other hand, no radioactivity was detected in tube No. 10 of virus preparations from leaves administered uridine-^3H. Therefore, it appeared that thymidine-^3H was preferentially incorporated into the viral nucleic acid. These results agree with electron microscope autoradiography of X-bodies in DMV-infected leaf cells labeled with thymidine-^3H (3). Furthermore, in the extracts from healthy verbesina leaves administered thymidine-^3H or uridine-^3H, no radioactivity was detected in tube No. 10 from sucrose-density gradients performed under identical conditions.

Enzymatic digestion of the viral nucleic acid.— Figure 4 shows the radioactivity profiles of untreated, RNase-treated, and DNase-treated DMV nucleic acid preparations that were subjected to sucrose density-gradient centrifugation. The viral nucleic acid preparation yielded a single radioactive peak from tubes 14 to 17 (Fig. 4A). The viral nucleic acid treated with RNase (Fig. 4B) showed a radioactivity profile similar to that of the untreated one (Fig. 4A). On the other hand, the DNase-treated viral nucleic acid preparation yielded no radioactive material in tubes 14 to 17, but did show radioactivity that remained near the meniscus (Fig. 4C). Thus, it is concluded that the nucleic acid isolated from DMV particles was susceptible to





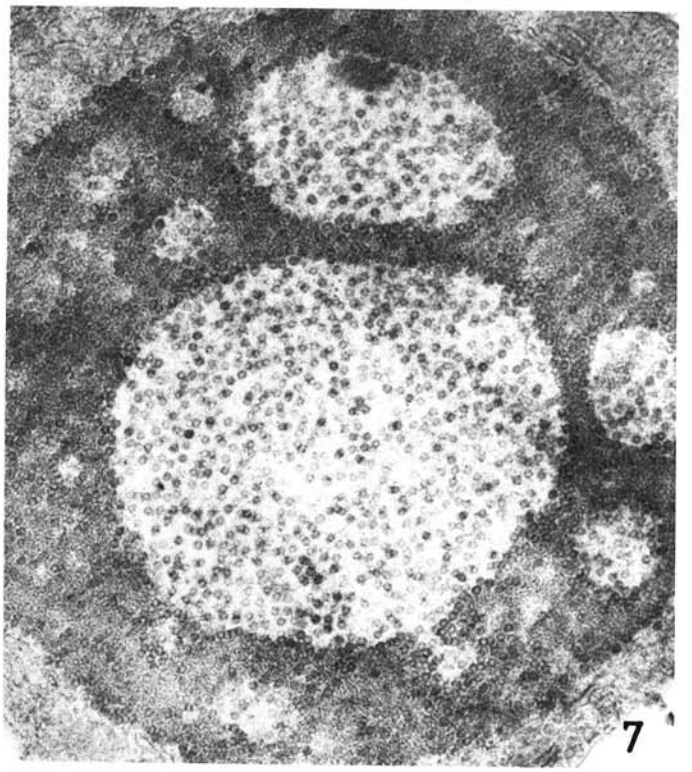


Fig. 5-7. 5) Fluorescence micrograph of conjugated anti-cauliflower mosaic virus globulin-stained epidermis from dahlia mosaic virus-infected leaf (×475). X indicates X-body. **6)** Light micrograph of phloxine-stained epidermis from infected dahlia leaf (×1,200). X indicates X-body. N indicates nucleus. **7)** Electron micrograph of X-body within infected dahlia leaf cell (×27,000).

DNase but resistant to RNase. The specific incorporation of thymidine-^{3}H into DMV and DNase hydrolysis of the nucleic acid isolated from DMV suggest strongly that the nucleic acid in DMV is DNA.

Immunocytological observations.— In the epidermal strips of DMV-infected dahlia leaves stained with conjugated anti-CaMV globulin solution, intense yellow green fluorescence was detected only on round bodies but not on the other cell organelles (Fig. 5). Comparison of light micrographs of the epidermal strips of infected leaves stained with phloxine (Fig. 6) (4) and electron micrographs of infected leaf tissues (Fig. 7) (4), it is clear that these round bodies stained specifically with conjugated anti-CaMV globulin solution (Fig. 5) are characteristic X-bodies associated with DMV infection. In the epidermal strips of infected leaves treated first with unconjugated anti-CaMV globulin and then post-stained with conjugated globulin, no staining was detected either on the X-bodies or on the other cell organelles. Thus, the immunocytological observations in situ support the evidence that there are serological relationships between CaMV and DMV (1, 2). It should be further studied whether anti-CaMV globulin adsorbed not only to DMV particles in or on X-bodies but also whether it adsorbed to the matrix of X-bodies.

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