Preparation and Properties of Maize Dwarf Mosaic Virus Ribonucleic Acid

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ABSTRACT

An infectious, single-stranded, ribonucleic acid (RNA) was prepared from purified maize dwarf mosaic virus (MDMV). The sedimentation coefficient of MDMV-RNA was about 38.9 S, as determined in linear-log sucrose density gradients. The RNA was susceptible to ribonuclease degradation and to alkali hydrolysis, resistant to deoxyribonuclease, and sedimented at 23.0 S after formaldehyde denaturation. The estimated molecular weight of MDMV-RNA is 2.7×10^6.

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Maize dwarf mosaic virus (MDMV) is a flexuous rod ca. 750 nm in length (1, 8). Wheat streak mosaic virus (WSMV) has a structure similar to MDMV, measuring about 700 nm (2). An infectious, single-stranded RNA, with a sedimentation coefficient of 40 S, was prepared from WSMV without the use of phenol (3). The similarity of MDMV and WSMV suggested that methods suitable for the preparation of WSMV-RNA might also be useful in the preparation of nucleic acid from MDMV. We report here the isolation of a single-stranded infectious RNA from MDMV with a sedimentation coefficient of 38.9 S.

The MDMV culture was a Nebraska isolate identified as MDMV-B on the basis of failure to systemically infect Johnson grass (*Sorghum halepense* [L.] Pers.). Greenhouse-grown sweet corn plants (*Zea mays* L. 'Golden Hammer' or 'Golden Giant') were manually inoculated, and the leaves harvested 10-14 days later. The virus was purified by grinding leaves in a blender with 2 ml of 0.01 M $(NH)_2HPO_4$, 0.8% polyvinylpyrrolidone, and 0.4% sodium thioglycolate/g tissue, followed by in situ calcium phosphate and chloroform clarification. The virus was precipitated by polyethylene glycol 6000 (6%), resuspended, clarified by a low-speed centrifugation, and then pelleted through a layer of 300 mg sucrose/ml (2.5 to 3.0 hr at 92,000 *g*) in a Spinco No. 30 (Beckman Instruments, Palo Alto, Calif.) rotor. The extinction coefficient for the morphologically similar tobacco etch virus ($A_{261}^{0.1\%}$nm = 2.4) (6) was used to estimate concentration of the nucleoprotein.

About 100 μg MDMV were added to an equal volume of 0.2 M ammonium carbonate, pH 9.0, 0.002 M disodium ethylenediamine tetraacetate (Na_2-EDTA) with 2% sodium dodecyl sulfate (SDS), and 200 μg EDTA-treated bentonite (5) per ml, as described by Brakke & Van Pelt (3). The solution was incubated for 4.5 to 24 hr at 4 C prior to layering on gradients. Brome mosaic virus (BMV) RNA was prepared by dissolving 2 mg BMV in 1.0 ml of 0.02 M Tris [tris (hydroxymethyl) amino methane], pH 9.0, 0.1 M NaCl, containing 2 mg bentonite (3). Tobacco mosaic virus (TMV) RNA was prepared by adding 2 mg TMV to a final volume of 1.0 ml of 0.02 M Tris-HCl, pH 9.0, 0.001 M Na_2-EDTA, containing 2 mg bentonite (3).

All nucleic acid preparations were layered on linear-log sucrose density gradients designed for the Beckman SW41 rotor (4), using 0.15 M sodium chloride, 0.015 M sodium citrate, pH 7.0 (SSC) as a buffer. The gradients were placed at 4 C overnight before use, and were run in the Beckman L265B ultracentrifuge at 259,000 *g* (max) for 6 hr at 14 C, or for 7.5 hr at 162,000 *g* (max). The gradients were fractionated with an ISCO (Instrumentation Specialties, Co., Lincoln, Nebr.) Model 640 density gradient fractionator coupled to a Model UA-2 ultraviolet analyzer.

The log S of MDMV nucleic acid was determined by plotting log depths against log S values from marker RNA species and drawing a straight line through the points. Values of sedimentation coefficients for BMV-RNA species were assumed to be 13.8, 20.5, and 25.3 S, whereas TMV-RNA was assumed to be 31.1 S (3). These values are for 14 C in sucrose in SSC corrected to the viscosity and density of water at 20 C.

In 11 experiments, MDMV nucleic acid sedimented as a single zone (Fig. 1-A, left) with a mean sedimentation coefficient of 38.9 S and a range of 37.8 to 43.0 S. In many preparations, some degree of degradation was apparent as evidenced by an increase of absorbance ahead of the main zone, as seen in Fig. 1-A, left. The peak absorbance near the meniscus in Fig. 1-A, left, is viral protein, as judged by removal of the zone by phenol deproteinization after the viruses had been buffer-deproteinized for 24 hr at 4 C. In two experiments, MDMV nucleic acid

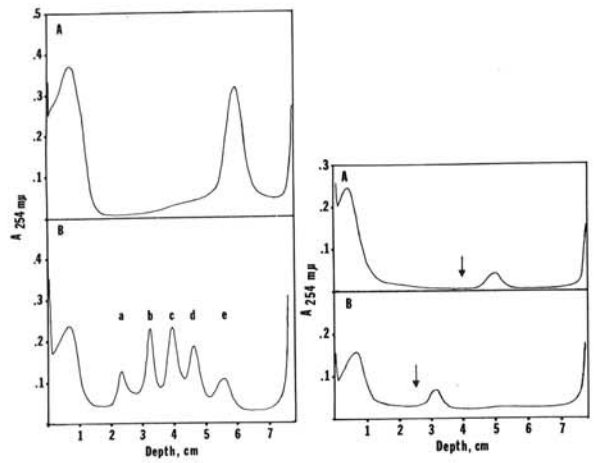

Fig. 1. (Left) Photometric scanning patterns of RNA species centrifuged in linear-log sucrose density gradients at 14 C at 259,000 *g* (max) in Beckman SW41 rotor. **A)** Maize dwarf mosaic virus (MDMV) RNA centrifuged for 6.0 hr. **B)** Brome mosaic virus-RNA species of 13.8 S (a), 20.5 S (b), and 25.3 S (c); tobacco mosaic virus-RNA (31.1 S) (d), and MDMV-RNA (38.9 S) (c) centrifuged for 5.5 hr. (Right) Photometric scanning patterns of maize dwarf mosaic virus (MDMV) RNA centrifuged in linear-log sucrose density gradients at 14 C for 6.0 hr at 259,000 *g* (max) in the Beckman SW41 rotor. **A)** Native MDMV-RNA (38.9 S); **B)** formaldehyde-treated MDMV-RNA (23.0 S). Arrows mark depth of native TMV-RNA (A) and formaldehyde-treated TMV-RNA (B).

was layered on an SW41 tube with TMV-RNA and BMV-RNA. Excellent separation of the five zones was apparent (Fig. 1-B, left), with MDMV nucleic acid perhaps more polydisperse than the other four RNA species.

Maize dwarf mosaic virus nucleic acid was subjected to ribonuclease (Sigma, 1A, 0.1 μg/ml, SSC, 30 min at 37 C) and to deoxyribonuclease (Sigma DN-EP, 20 μg/ml, 0.001 M $MgCl_2$, 30 min at 25 C). The MDMV zone was absent after ribonuclease treatment, whereas deoxyribonuclease treatment had no effect. No MDMV nucleic acid zone was present after exposing the nucleic acid to 0.1 M NaOH for 30 min at 25 C. Viral RNA was also subjected to formaldehyde treatment. About 100 μg MDMV was allowed to incubate in the ammonium carbonate system for 6 to 12 hr at 4 C. The preparation was then placed in a dialysis bag and incubated in 1.1 M formaldehyde, 0.001 M Na_2-EDTA, pH 7.0, for 7 to 15 hr at 40 C (3). A control (TMV-RNA) was included in each experiment. Each of six trials with MDMV-RNA yielded a single zone (Fig. 1, right) with about a 41% reduction in S value to a mean of 23.0 S with a range of 21.4 to 24.4 S. Formaldehyde-treated WSMV-RNA sedimented at 23.4 S (3). Maize dwarf mosaic virus-RNA is therefore a single-stranded, single component RNA with an S value very close to that of WSMV-RNA, before or after formaldehyde treatment.

Substitution of the 23.0 S value into the formula $S = 0.083\ M^{0.38}$ (3) gives a molecular weight for MDMV-RNA of 2.68×10^6, or slightly less than the 2.8×10^6 value for WSMV-RNA (3). The S value of formaldehyde-treated TMV-RNA had deviated from the 20.7 S value previously reported (3); hence the estimate of the molecular weight of MDMV-RNA must be interpreted with caution.

The specific infectivity of MDMV-RNA was low. In 12 trials, the MDMV-RNA zone was collected, bentonite (100-500 μg) was added, and the Golden Cross Bantam, Golden Giant, Golden Hammer, or Ohio 28 corn cultivars were inoculated using Celite (Johns-Manville diatomaceous silica) as an abrasive. No plants became infected in any of the trials. In addition, MDMV-RNA zones were collected, precipitated in 70% ethanol, 0.1 M sodium acetate, pH 5.5, and the RNA was resuspended in a variety of buffers containing bentonite. In none of these trials did any inoculated plants become infected.

In one experiment, MDMV-RNA from 6.8 mg virus was layered on a 75 to 300 mg/ml sucrose gradient in a 2.54- X 8.89-cm tube made in SSC which was run at 104,000 *g* (max) at 5 C for 12 hr in a Beckman SW27 rotor. The viral RNA zone was collected and divided in two, and ethanol precipitated. One preparation was taken up in 0.5 ml SSC, and the other in a buffer found useful in infectivity studies with other viral RNA species (0.035 M K_2HPO_4, 0.05 M glycine, pH 9.2) (M. K. Brakke, *unpublished data*). Five days after inoculation, symptoms were apparent in the base of new leaves of two of seven plants inoculated with an estimated 100 μg MDMV-RNA/ml in the phosphate-glycine buffer, and one plant was infected from the SSC inoculations. Samples collected above or below the RNA zone were not infectious. Sap expressed from the infected plants reacted with MDMV-B antiserum in agar diffusion plates.

In another infectivity test, MDMV-RNA was resuspended in 0.035 M K_2HPO_4, 0.050 M glycine, pH 9.2, with 100 μg bentonite/ml. At a concentration of about 90 μg/ml, 8 of 9 Golden Cross Bantam plants became infected. At about 45 μg MDMV-RNA/ml, 4 of 9 plants, and at 4 μg/ml, 2 of 10 plants became infected. Symptoms were apparent at 90 hr after inoculation in a growth room at 25 C, with about 1,200 ft-c light intensity at plant height for 13 hr/day. No noninoculated plants kept in the same room became infected. In this particular experiment, purified MDMV was placed in dissociation buffer 8 hr after grinding the tissue; previous experiments required up to 36 hr. It may be suggested that the apparent lability of MDMV or its RNA could have been responsible for the lack of infectivity of the RNA in earlier experiments. No differences in the shape or S value of the MDMV-RNA zones were apparent in the experiments.

Several buffer systems were utilized in an attempt to isolate MDMV-RNA. These included the TMV and BMV "systems" described above, which failed to release MDMV-RNA. Suspension of purified MDMV in 0.25 sodium orthoborate, pH 9.0, 0.002 M Na_2EDTA, with 100 μg bentonite/ml and 0.002% N-lauroyl sarcosine (Sigma), yielded a very small

quantity of MDMV-RNA compared to the ammonium carbonate system.

In one experiment, the use of phenol with the ammonium carbonate buffer system failed to produce MDMV-RNA. Since Sehgal (7) was able to detect an orcinol-positive nucleic acid from MDMV using phenol, other phenol-buffer systems may be suitable. The ease with which WSMV-RNA (3) and MDMV-RNA are released in the ammonium carbonate system indicates that this procedure may prove helpful in the preparation of nucleic acids from other long, flexuous rod-shaped viruses without the time-consuming procedures required for phenol deproteinization.

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