Purification of Maize Dwarf Mosaic Virus by Equilibrium Centrifugation in Cesium Chloride

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Missouri Agricultural Experiment Station Journal Series No. 5680. Project No. 579.

Maize dwarf mosaic virus (MDMV) was purified by clarifying corn (*Zea mays* L.) leaf extract with chloroform or acidification-diethylaminoethyl (DEAE) cellulose treatment, differential centrifugation, and sucrose density-gradient centrifugation (5, 7). Although satisfactory results have been obtained in MDMV purification by rate zonal centrifugation in sucrose density-gradient columns, difficulties have occasionally been encountered in separating MDMV from host constituents by this procedure. Furthermore, MDMV does not always form discrete bands in sucrose density-gradient columns, a difficulty which has also been encountered in purifying other flexuous plant viruses, including sugarcane mosaic virus (4, 7). Our attempts to substitute the sucrose density-gradient centrifugation step by adsorption chromatography or gel-filtration to separate MDMV from host impurities have been unsuccessful. We now report a satisfactory procedure for separating MDMV from nonviral constituents by equilibrium centrifugation in CsCl.

The MDMV-A isolate used was originally recovered from corn plants in Missouri (5). The virus was propagated in young Golden Giant sweetcorn seedlings. Infectivity assays for MDMV were done on third and fourth leaves of 2-week-old *Sorghum bicolor* Moench 'AKS 614' plants (2). Tobacco mosaic virus strain U_1 (TMV-U_1) was multiplied in *Nicotiana tabacum* L. 'Samsun', and infectivity assays were done on *N. tabacum* 'Xanthi-nc' (6).

MDMV was partially purified from corn leaves (120-140 g fresh wt) by chloroform (7) or acidification-DEAE cellulose treatment (5). After high-speed centrifugation to concentrate the virus, the pellets were dissolved in 2-3 ml of neutral phosphate buffer containing 10 mM glutathione. The virus preparations were centrifuged at 5,000 *g* for 20 min prior to equilibrium centrifugation. Extract of healthy corn leaves was similarly clarified. TMV-U_1 was purified by the procedure previously described (6).

Isopycnic centrifugations were done in 3 M CsCl (Pierce Chemical Company, Rockford, Ill.) solution. Eleven ml of CsCl solution ($\rho = 1.3809$) was introduced into 14.5 × 96 mm centrifuge tubes, and aliquots of clarified healthy corn leaf extract, MDMV preparation, and TMV (about 700 µg) were layered on top of the CsCl solution. The tubes were centrifuged at 150,000 *g* at 10 C for 30-36 hr. After centrifugation, the visible bands were removed by a hypodermic syringe, or the density-gradient column was fractionated with an ISCO density-gradient fractionator (Model D), and the various fractions were monitored at 254 mµ with a UA-2 analyzer. Refractive indices of the various fractions were determined in a Bausch & Lomb Abbe 3L Refractometer, and were converted into density. To assess infectivity, aliquots of the various fractions were diluted with distilled water (1:10 for MDMV; 1:10-1:50 for TMV), and Celite (50 mg/ml) was added to the solutions prior to bioassay.

In some experiments, the samples removed from the visible zones were dialyzed overnight at 4 C against distilled water, and aliquots of these preparations appropriately diluted and their ultraviolet light absorption spectra characterized.

Following isopycnic centrifugation, visual examination revealed that all the material in clarified healthy leaf extracts remained at the top of the CsCl density-gradient column. In MDMV preparations, in addition to the material remaining at the top, two distinct bands, one at 1.25 cm and the other at 1.85 cm below the meniscus, were observed. TMV-U_1 formed a sharp light-scattering band 1.85 cm below the meniscus.

In several experiments performed, MDMV was readily separated from the host contaminants by equilibrium CsCl centrifugation, and this procedure proved fairly reproducible. The results of a typical experiment after isopycnic centrifugation and fractionation of the density-gradient column using healthy leaf extract showed a major peak, (I) ($\rho = 1.2880$) at the meniscus (Fig. 1-A). In the MDMV preparation, two additional peaks, (II) ($\rho = 1.3103$) and (III) ($\rho = 1.3245$), were observed; bioassays of the various fractions showed that infectivity was associated only with peak III (Fig. 1-B). Electron micrographs also confirmed presence of MDMV in peak III. TMV-U_1 appeared at a $\rho = 1.3248$ (Fig. 1-C).

The ultraviolet light absorption spectra of the dialyzed MDMV and TMV after isopycnic centrifugation were typical for nucleoproteins. The characteristic hump present at 290 mµ in TMV (due to tyrosine and tryptophan residues in the viral coat protein) was absent in MDMV (Fig. 2). The corrected 280:260 ratio for purified MDMV was 0.85, indicating presence of about 5-6% nucleic acid (3). The ultraviolet light absorption spectra of fractions under peaks I and II showed presence of much light-scattering material. The identity of the component present in peak II was not ascertained, but it appears to be a virus-related product because of its absence in healthy leaf extract. The material under peak I consisted primarily of host proteins, as indicated by its low density.

In some experiments, by employing preformed CsCl gradients (1), the centrifugation time to achieve equilibrium in the density-gradient columns was reduced to 12-15 hr without affecting the viral separation.

Density determinations provide useful information on the nucleic acid:protein ratio of nucleoproteins (9). Our estimate of the density of TMV-U_1 which consists of 95% protein and 5% nucleic acid is similar to that reported by Siegel & Hudson (8), and resembles that of MDMV. It is likely that in MDMV the extent of packing of protein subunits around the nucleic acid,

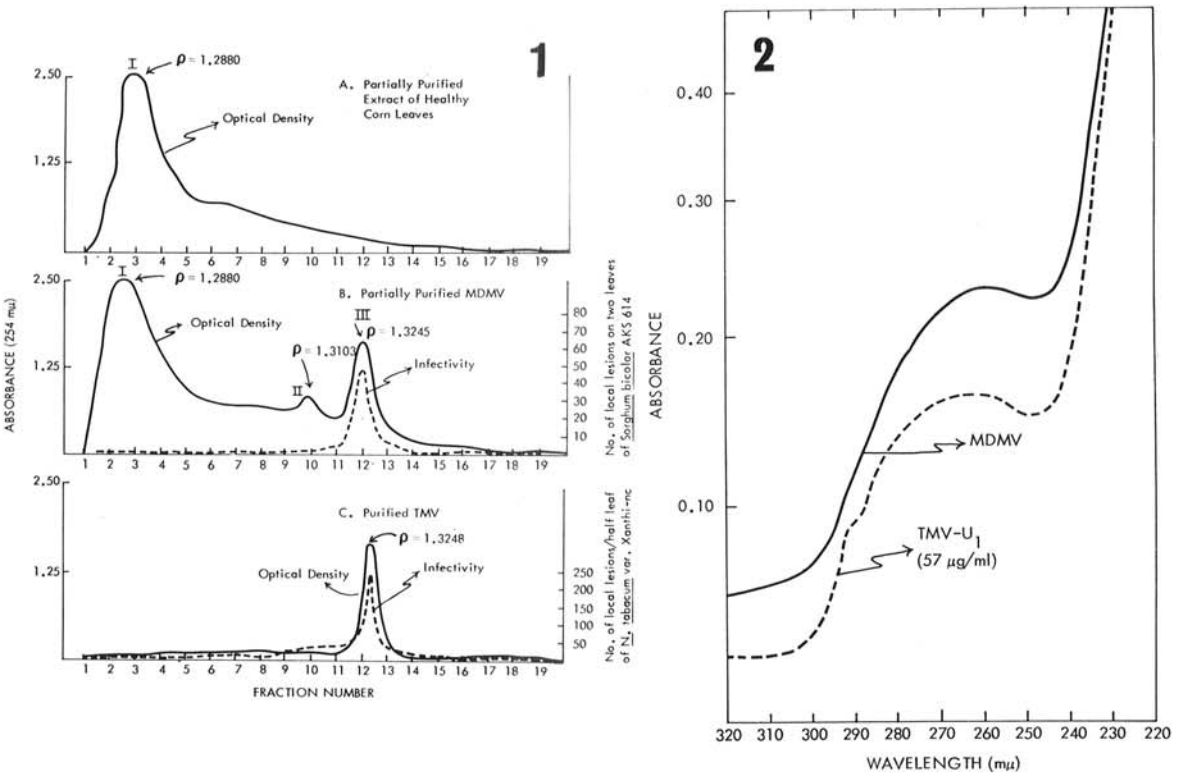

Fig. 1-2. **1)** Fractionation of the CsCl density-gradient column after isopycnic centrifugation. **A)** Clarified extract of healthy corn leaves. **B)** Maize dwarf mosaic virus preparation. **C)** Tobacco mosaic virus strain U_1. **2)** Ultraviolet light absorption spectra of dialyzed maize dwarf mosaic virus and tobacco mosaic virus after isopycnic centrifugation in CsCl.

and the protein to nucleic acid ratio, may be similar to that of TMV.

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