Physicochemical Characterization of Maize Mosaic Virus

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ABSTRACT

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Virions of maize mosaic virus (MMV) were purified from greenhouse-grown inoculated maize plants and the physicochemical properties of the virions were determined. The best purification procedure was a modified method of those used for sonchus yellow net virus and potato yellow dwarf virus. Electron microscopy of purified preparations showed virions with dimensions of $224 \pm 21 \times 68 \pm 9$ nm. Purified preparations injected into *Peregrinus maidis*, the planthopper vector of MMV, were infectious to maize test plants. MMV had a sedimentation coefficient of 774 as estimated by linear-log sucrose density gradient centrifugation. Analysis of virion proteins by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) revealed three major virion proteins of relative mass (Mr) 75,000 ± 2,000 (75K), Mr 54,000 ± 3,200 (54K) and Mr 30,000 ± 1,000 (30K). The 75K protein stained for carbohydrate and both the 75K and 30K proteins were solubilized by treatment of virions with nonidet P-40 (NP-40) suggesting that these are G and M proteins, respectively. The 54K protein was not solubilized by NP-40 and is interpreted as an N protein. MMV was also found to have a ss-RNA of ~Mr 4.2×10^6. From these data we conclude that MMV should be placed in the lettuce necrotic yellows virus subgroup of plant rhabdoviruses.

Additional key words: glycoprotein, *Peregrinus maidis*, rhabdoviridae.

Maize mosaic has been a serious disease in the tropics and subtropics (2) for many years. The causal agent of maize mosaic was thought to be a virus, and in 1960 Herold et al (7) reported the association of particles typical for the plant rhabdovirus group with maize mosaic. Lastra and Acosta (14) purified maize mosaic virus (MMV) and confirmed that it was a rhabdovirus.

In the summer of 1982 a new disease of maize, transmitted by *Peregrinus maidis*, was observed in south Florida (J. H. Tsai, *unpublished*). The symptoms of this disease differed from those of the endemic maize stripe (19), which also is transmitted by *P. maidis*. Sap from infected plants reacted by immunodiffusion and ELISA tests with antiserum to the Venezuelan isolate of MMV (kindly supplied by R. Lastra). Because there is very little information on the physicochemical properties of the MMV virion and its relationships to other members of the Rhabdoviridae, we characterized the purified virions of the Florida isolate of MMV and here report the properties.

MATERIALS AND METHODS

Plants, vectors, and viruses. *Peregrinus maidis* Ashmead, the planthopper vector of MMV, was reared as previously described (19). MMV was transmitted using the methods used for maize stripe virus (19). Adult *P. maidis* were given a 72-hr acquisition access period on infected plants and serially transferred at 3- to 4-day intervals to healthy maize seedlings (*Zea mays* L. Guardian) to produce a series of infected plants.

The SYDV-NY isolate of potato yellow dwarf virus (PYDV) was maintained as previously described (6). Isolates of tobacco mosaic virus (TMV) and brome mosaic virus (BMV) were those used previously (5).

Virion purification. Virions of MMV were purified using modifications of methods used previously for SYDV-NY (6), sonchus yellow net virus (SYNV) (9), and MMV (14). In some experiments, the buffers used by Lastra and Acosta (14) for MMV (referred to here as buffers C and D for 0.1 M glycine, 0.01 M $MgCl_2$, 0.001 M DIECA, pH 8.0; and 0.1 M glycine, 0.01 M $MgCl_2$, and 0.001 M DIECA, pH 7.0, respectively) were compared with buffers A and B (0.1 M tris, 0.01 M $MgCl_2$, 0.04 M Na_2SO_3, pH 8.4; and 0.1 M tris, 0.01 M $MgCl_2$ and 0.04 M Na_2SO_3, pH 7.5, respectively). Freshly harvested tissues, taken from maize plants 3 to 4 wk after inoculation, were used in all experiments. One part tissue was mixed with four parts buffer (w/v) and homogenized in a Waring blender. The homogenate was filtered through cheesecloth and the Celite filtration and differential centrifugation conditions described previously (6) were used in all cases. After rate-zonal sucrose density gradient centrifugation, gradients were fractionated in an ISCO model 640 fractionator. The virion zone was removed and virions were pelleted using the Beckman type 40 rotor at 40,000 rpm for 35 min, resuspended in 1 ml of buffer B and recentrifuged in 5–30% (w/v) rate-zonal sucrose density gradients using the Beckman SW28 rotor for 25 min at 28,000 rpm. The gradients were fractionated and virions were again pelleted and finally resuspended in buffer B.

The sedimentation coefficient of MMV was estimated by using linear-log sucrose gradients (1). Purified virions of MMV, TMV (190S), and SYDV-NY (900S) were centrifuged on linear-log sucrose gradients in the Beckman SW28 rotor. Run conditions were 5 C, 15 min each at 28,000 rpm.

Analysis of virion proteins and nucleic acid. MMV virion proteins were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (11). Electrophoresis was done on 0.8-mm-thick gels composed of a 10-mm-high 5% acrylamide stacking gel and a 55-mm-high 12 or 10% acrylamide (depending on the experiment) resolving gel. After reducing and alkylating proteins (12), they were labeled with 2-methoxy-2,4-diphenyl-3 (2H)-furanone (MDPF; kindly supplied by W. E. Scott) at pH 10 (13). Electrophoresis was done using a "mini slab" unit (IDEA Scientific®) at 200 V constant voltage for 1 hr at room temperature. When electrophoresis was complete, proteins were visualized by exposing gels to ultraviolet light (302 nm) and gels were photographed by using a Wratten 9 filter and type 665 P/N Polaroid film.

Virion proteins were stained for carbohydrate using the method of Fairbanks et al (4). After electrophoresis, gels were soaked in a

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solution of 25% isopropanol and 10% acetic acid overnight. The following day, gels were soaked first in a solution of 10% isopropanol and 10% acetic acid for 6 hr, followed by 10% acetic acid overnight. Gels were then treated with the following sequence of solutions: 0.5% periodic acid for 2 hr; 0.5% sodium metabisulfite and 5% acetic acid for 2 hr; 0.1% sodium metabisulfite and 5% acetic acid, twice for 20 min each; 5% acetic acid for 20 min; and Schiff reagent (fuchsin sulfurous acid) overnight.

Virion proteins of MMV and SYDV-NY were selectively solubilized in nonidet P-40 (NP-40) as previously described (3). Purified virions were incubated for 1 hr in solutions of: buffer only; 1% NP-40; 2% NP-40; 4% NP-40; 1% NP-40 + 0.5 M NaCl; or 2% NP-40 + 0.5 M NaCl. The samples were then layered on a 3-ml cushion of 20% sucrose in buffer B and centrifuged for 1 hr at 35,000 rpm in the Beckman type 40 rotor. The supernatants and pellets were separated and saved. The supernatant proteins were precipitated with 10% trichloroacetic acid (TCA). The high-speed pellet and TCA pellet proteins were resuspended in distilled water and all proteins were analyzed by SDS-PAGE.

Nucleic acids were extracted from purified virions of MMV, SYDV-NY, TMV, and BMV with the SDS-phenol method used previously (5). Nucleic acids were precipitated with ethanol and analyzed by electrophoresis on horizontal 1% agarose slab gels using the glyoxal-DMSO denaturing system (16) except that buffers and the gel contained 0.02 M sodium phosphate, pH 7.0, and 1 mM disodium ethylenediamine tetraacetate (EDTA). Electrophoresis was at room temperature for 2 hr at 5 V/cm. Nucleic acids were stained with ethidium bromide (400 ng/ml) after the gels were first treated for 20 min in 0.05 M NaOH, 20 min in 0.5 M ammonium acetate, and 1 hr in 0.1 M ammonium acetate (15). Gels were placed on a transilluminator (302 nm) and photographed using Polaroid type 665 P/N film and Wratten 9 and 23A filters.

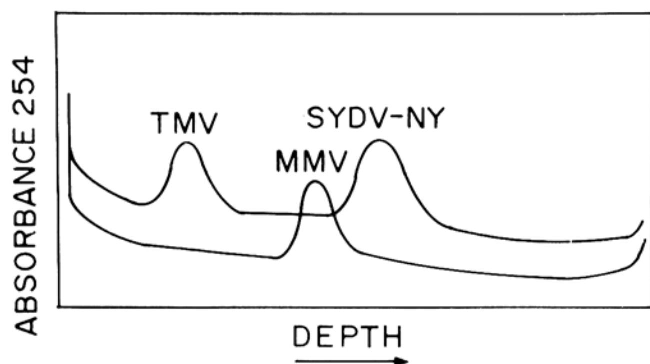

Fig. 1. Comparison of the sedimentation of maize mosaic virus (MMV), the SYDV-NY isolate of potato yellow dwarf virus, and tobacco mosaic virus (TMV) in 5–30% rate-zonal sucrose density gradients. Samples are 250 μg TMV and 200 μg of SYDV-NY and MMV. Centrifugation was for 25 min at 28,000 rpm in the SW 28 rotor at 5 C.

RESULTS

Virion purification. Virions of MMV were purified as previously described (6). In some experiments buffers described for purifying Venezuelan MMV (glycine buffers C and D) were compared with the tris buffers used for SYNV and PYDV. The glycine buffers gave initially cleaner preparations. However, yields of purified virions of MMV were about one-third the yields obtained by using the tris buffers. Therefore, the tris buffers were routinely used, and contaminating green pigments were removed by two or three cycles of rate-zonal sucrose density gradient centrifugation and high-speed centrifugation.

MMV virions did not sediment as far in 5–30% rate-zonal sucrose density gradients as did SYDV-NY virions (Fig. 1). The sedimentation coefficient of MMV was estimated using purified virions of TMV (190S) and SYDV-NY (900S) as markers. In three separate experiments the sedimentation coefficient of MMV was estimated to be 774S.

When ultraviolet absorbing material collected from rate-zonal sucrose density gradients was examined using electron microscopy, typical rhabdovirus particles were seen (Fig. 2). Dimensions were 224 ± 21 × 68 ± 9 nm, which are slightly smaller than the dimensions given for MMV previously (10), but are well within the ranges of sizes for plant rhabdoviruses. Purified MMV from the density gradients also was infectious when injected into adult *P. maidis* by using micro-capillary needles. Mortality of injected planthoppers was high in two of seven experiments but the percentage of injected insects that survived and then transmitted

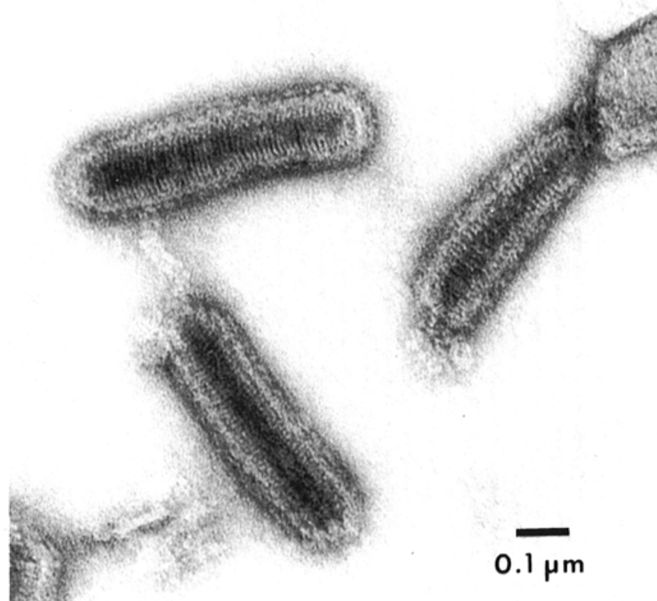

Fig. 2. Electron micrograph of purified virions of maize mosaic virus. Virions were stained with 1.0% uranyl acetate in water.

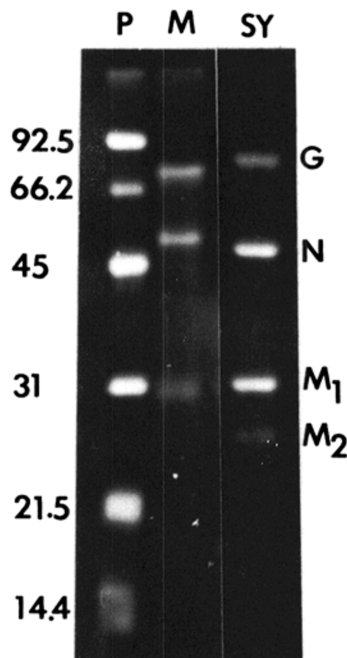

Fig. 3. An SDS-polyacrylamide (12% acrylamide) slab gel showing the virion proteins of maize mosaic virus (MMV). P shows the molecular weight markers: phosphorylase B, bovine serum albumin, ovalbumin, carbonic anhydrase, soybean trypsin inhibitor and lysozyme in order of decreasing relative mass (Mr). Mr values are shown to the left. M shows MMV virion proteins and SY shows the virion proteins of the SYDV-NY isolate of potato yellow dwarf virus. The SYDV-NY proteins are labeled G, N, M_1, and M_2 according to standard rhabdovirus terminology (see text).

MMV varied from 5 to 95%, depending on the experiment. In later experiments, transmission efficiency of injected planthoppers was consistently within the range from 75 to 95%.

Analysis of virion proteins and RNA. Three major MMV proteins were resolved by SDS-PAGE on 10 or 12% polyacrylamide gels. No proteins were found in similar preparations from healthy plants. The protein patterns differed significantly from SYDV-NY proteins electrophoresed in the same gel (Fig. 3). The SYDV-NY virion proteins are labeled G, N, M_1, and M_2 in order of decreasing relative mass (Mr) according to standard rhabdovirus terminology (8,10).

The molecular weights for the MMV virion proteins were estimated from 10 separate 12% polyacrylamide slab gels by using phosphorylase B, bovine serum albumin, ovalbumin, carbonic anhydrase, soybean trypsin inhibitor, and lysozyme as molecular weight markers (molecular weights are given in Fig. 3) in the same gels. Molecular weights for the MMV proteins were estimated to be Mr 75,000 with a standard deviation of 2,000 (75K), Mr 54,000 with a standard deviation of 3,000 (54K), and Mr 30,000 with a standard deviation of 1,000 (30K) in order of increasing mobility. Some virus preparations also contained three minor bands. One faint band appeared above the 75K protein, another was immediately below the 54K protein, and a faint band was sometimes observed which comigrated with SYDV-NY M_2 protein, just below the 30K protein. Because not all virus preparations contained the minor bands, we believe they may be co-purifying host proteins.

When the virion proteins were stained for carbohydrate, only the 75K MMV protein and the control SYDV-NY glycoprotein gave positive reactions (*unpublished*), indicating that the 75K MMV protein is a glycoprotein.

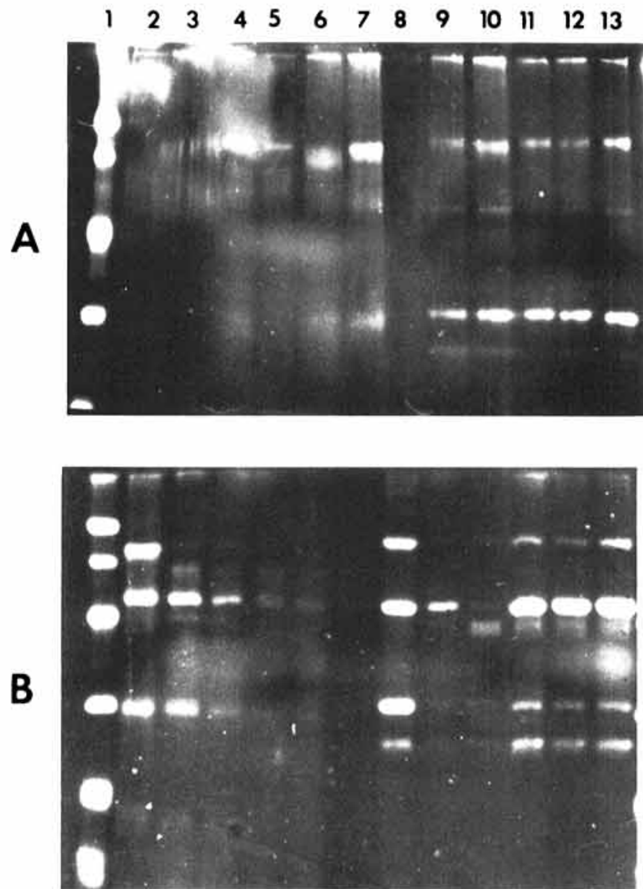

Fig. 4. Solubilization of the virion proteins of maize mosaic virus (MMV) and the SYDV-NY isolate of potato yellow dwarf virus using nonidet P-40 (NP-40). **A,** Lane 1 shows the molecular weight markers (see Fig. 3). Lanes 2–7 and 8–13 are the soluble proteins of MMV and SYDV-NY, respectively, for the following treatments: buffer only, 1% NP-40, 2% NP-40, 4% NP-40, 1% NP-40 + 0.5 M NaCl, and 2% NP-40 + 0.5 M NaCl, respectively. **B,** Nucleocapsid proteins show in the order given for A.

Nonionic detergent treatments of purified virions of MMV and SYDV-NY selectively solubilized virion proteins. In all NP-40 treatments the MMV 75K protein and the MMV 30K protein were found mainly in the soluble fractions while the MMV 54K protein was found mainly in the nucleocapsid fractions (Fig. 4). The 75K protein was more readily solubilized by NP-40 and was easily detected in the soluble fraction. A majority of the 30K protein was still seen in the nucleocapsid fraction after 1% NP-40 treatment, but was solubilized by higher concentrations. Two new bands were seen between the 75K and 54K proteins in the nucleocapsid fractions in some of the NP-40 treatments. However, the origins of these bands presently is unknown. The addition of sodium chloride to the NP-40 treatments solubilized all MMV virion proteins. Interestingly, for the SYDV-NY control, lower concentration NP-40 treatments completely solubilized virion proteins. The 4% NP-40 treatment, and when NaCl was added to the 1 and 2% NP-40 treatments, more selectively solubilized the SYDV-NY G, M_1, and M_2 proteins. The M_1 protein appeared to be more effectively solubilized by these treatments than was the M_2, but traces of both M_1 and M_2 proteins remained with the nucleocapsid fractions.

The RNA extracted from purified virions of MMV migrated slightly ahead of the ss-RNA of SYDV-NY in 1% agarose gels using the glyoxal-DMSO denaturing system (Fig. 5). By comparing the mobility of MMV RNA to SYDV-NY, TMV, and BMV RNAs, the molecular weight of MMV RNA was estimated to be ~Mr 4.2×10^6, slightly less than the 4.6×10^6 ss-RNA of SYDV-NY (18).

DISCUSSION

The properties shown here for MMV are consistent with those of the rhabdovirus group and extend our knowledge of the physicochemical properties of MMV. We have identified three major virion proteins for MMV. Based on the work of Dale and Peters (3), MMV is more likely a member of the lettuce necrotic yellows (LNYV) subgroup of the plant rhabdoviruses than the sowthistle yellow vein virus (SYVV) subgroup which contains four major virion proteins. The SYDV-NY isolate used as a control in these studies is a member of the SYVV subgroup (3) and was shown here to differ significantly from MMV.

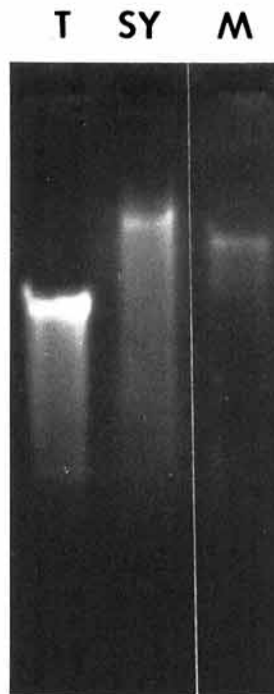

Fig. 5. A 1% agarose horizontal slab gel showing: tobacco mosaic virus ss-RNA (T), SYDV-NY ss-RNA (SY), and maize mosaic virus ss-RNA (M). RNAs were denatured with glyoxal and DMSO, electrophoresed for 2 hr at 5V/cm and stained with ethidium bromide.

Glycoprotein staining and NP-40 solubilization of MMV proteins show that MMV virion proteins can be described using conventional rhabdovirus terminology. The MMV 75K protein stained for carbohydrate and was readily solubilized by NP-40 treatment and therefore most likely is a membrane associated 'spike' glycoprotein (G protein) as with other rhabdoviruses. Because it is a glycoprotein, the 75K estimate given here for this protein may not be correct as some glycoproteins migrate anomalously in acrylamide gels (17). The MMV 30K protein also was solubilized by NP-40 treatment and is consistent in size for a rhabdovirus M protein. The MMV 54K protein was not solubilized by NP-40 treatment, but was still associated with the nucleocapsid and therefore is an N protein. It's size is consistent with that of N proteins of other rhabdoviruses. We could not identify an L protein for MMV, but suspect that MMV virions have one which we failed to detect.

The dimensions of MMV virions are similar to those reported for MMV previously (10), and similar to those of other plant rhabdoviruses. MMV sediments slightly slower than SYDV-NY and has a ss-RNA similar in mass to that of SYDV-NY. As was stated by Jackson et al (10) "studies of the various physicochemical properties of rhabdoviruses of the Gramineae are especially scant." Properties of MMV reported here for this potentially serious maize pathogen will provide a basis for further studies to identify and compare properties of rhabdoviruses infecting maize. The only other characterized maize rhabdovirus, wheat striate mosaic virus, also appears to be a member of the LNYV subgroup of plant rhabdoviruses (3).

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