Etiology

Some Biological and Physicochemical Properties of Bean Rugose Mosaic Virus

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ABSTRACT

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Characterization of a bean rugose mosaic virus (BRMV) isolate from Cali, Colombia, confirmed that this virus belongs to the comovirus group. In sucrose density gradients BRMV separated into three centrifugal components after rate zonal sedimentation and into four or five density components after CsCl equilibrium sedimentation, depending on the type of buffer. The native nucleoprotein particles also resolved into one or two electrophoretic forms depending on the type of buffer used for purification. Unfractionated BRMV, as well as the individual nucleoprotein particles, contained two (39.5×10^3 and 24.5×10^3 daltons) or three (39.5×10^3, 24.5×10^3 and 22×10^3 daltons) proteins that could be separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The number of proteins depended on the type of buffer used for the virion purification. From symptomatology and biophysical characteristics the Colombian isolate seemed to be closely related to the BRMV-Costa Rica strain but somewhat different from the BRMV-Ampollado strain from El Salvador.

Additional key words: electron microscopy, serological relationships, ultrastructural studies.

The disease of beans caused by bean rugose mosaic virus (BRMV) was described first at Turrialba, Costa Rica (11,12). A similar disease was observed later in Guatemala (3,4) and El Salvador (16). This beetle-transmitted virus was partially characterized and considered a new member of the comovirus group (8,12), although in some reports the virus did not have the three centrifugal components typical of this group (8,9). Ultrastructural studies and morphological changes of bean leaf tissues infected with BRMV have been reported (6,19). In this paper we describe some biological and physicochemical properties of a BRMV isolate from Cali, Colombia.

MATERIALS AND METHODS

Virus propagation and purification. Bean leaves infected with the Cali isolate of BRMV were donated by Dr. G. E. Gálvez (Centro Internacional de Agricultura Tropical, Cali, Colombia). The virus was grown in *Phaseolus vulgaris* L. '27-R' or 'Guaŕi'. Infected tissue was homogenized in 0.01 M potassium phosphate buffer, pH 7.0, containing Carborundum (600 mesh) and inoculated by rubbing fully expanded primary leaves. One hundred grams of infected leaves, with symptoms of the disease, were homogenized in 100 ml of 0.01 M potassium phosphate buffer, pH 7.0, or in 0.01 M sodium-EDTA buffer, pH 7.8. The homogenate was filtered through Miracloth and centrifuged at 12,000 *g* for 10 min. The supernatant was emulsified with 35% chloroform/butanol 1:1 (v/v), or with 25% chloroform and 10% amyl alcohol (11,14). The emulsion was centrifuged at 12,000 *g* for 10 min. The upper aqueous phase was centrifuged at 120,000 *g* for 3.5 hr in a Beckman 50.1 rotor. Alternatively the virus was precipitated with 8% polyethylene glycol (PEG-8000 Baker) and 0.2 M NaCl. Purification was continued by resuspension in 0.01 M potassium phosphate buffer, pH 7.0, or in 0.01 M sodium-EDTA buffer, pH 7.8, and centrifugation at 12,000 *g* for 15 min. The virus suspension was layered onto 10–40% or 10–50% sucrose density gradients made in the same buffer in which the virus had been previously resuspended, and centrifuged at 130,000 *g* for 3–5 hr in a Beckman SW 27 rotor. The fractions containing each of the separate nucleoprotein components of the virus were pooled, diluted, and concentrated by centrifugation at 150,000 *g* for 4 hr or by PEG-precipitation. After resuspension in the same buffer as before they were subjected to a second cycle of sucrose density gradient centrifugation in the same conditions. Virus concentration was estimated by assuming an extinction coefficient of 8 $(mg/ml)^{-1}$ cm^{-1} at 260 nm (26). The RNA content of the

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different nucleoprotein components of BRMV was determined using the A_{280}/A_{260} ratio (28). All operations were carried out at 4 C. Infectivity was determined with local lesion assays on Linea 32 bean.

Serological relationships. Serological studies were conducted in Ouchterlony double-diffusion tests (23) (0.9% ion agar No. 2 in 0.05 M Tris-Cl, pH 7.5, 0.85% NaCl, 0.02% NaN_3). Antisera to cowpea severe mosaic virus-Arkansas strain (CSMV-Ark), cowpea mosaic virus-Surinam strain (CPMV-Sb), southern bean mosaic virus-bean strain (SBMV-Sb), southern bean mosaic virus-cowpea strain (SBMV-cp), bean pod mottle virus (BPMV), blackgram mosaic virus (BGMV), bean yellow stipple virus (BYSV), and quailpea mosaic virus (QPMV) were donated by Dr. H. A. Scott (Department of Plant Pathology, University of Arkansas). The sap of infected tissue or purified virus was used in serological tests.

Electron microscopy. Virus purified by two cycles of sucrose density gradient centrifugation was dialyzed against 0.01 M potassium phosphate buffer, pH 7.0, and negatively stained with KOH neutralized 2% phosphotungstic acid on grids covered with a carbon-coated formvar film. More than 100 particles were measured at several magnifications; southern bean mosaic virus was used as an internal standard. Ultrastructural studies were done by fixing 2-mm infected pieces of leaves in 2% glutaraldehyde in Milloning buffer overnight at 4 C. Postfixation was in 2% osmium tetroxide in Milloning buffer for 24 hr, followed by dehydration with an acetone series. The tissue was embedded in Epon 812 and ultrathin sections were made with a Porter Blum MT-2 ultramicrotome. Sections were collected with grids covered with a carbon-coated formvar film and stained with 2% uranyl acetate.

Equilibrium sedimentation in CsCl. Virus suspensions in either 0.01 M potassium phosphate, pH 7.0, or in 0.01 M sodium-EDTA, pH 7.8, were made 40% (w/w) in CsCl and centrifuged at 208,000 *g* for 48 hr in a Beckman SW 41 Ti rotor or in a Beckman SW 65 rotor at 300,000 *g* for 15 hr. Fractions from the gradients were collected manually from the bottom of the tube and their absorbance pattern monitored at 260 nm. The density of the fractions was determined using a Bausch & Lomb Abbe 3L refractometer. The RNA content of the nucleoprotein components was estimated from the A_{280}/A_{260} ratio (28) or the correlation between buoyant density and ribonucleic acid content (25).

Polyacrylamide gel electrophoresis of virus particles. Electrophoresis of intact virus was performed in a Tris-borate-EDTA system at pH 8.3 in 2.8% polyacrylamide gels prepared in 6-mm (i.d.) tubes (21). Gels were scanned at 260 nm or stained with 0.2% Coomassie brilliant blue in methanol:water:glacial acetic acid (5:5:1) and destained in a solution containing 25% methanol and 7% acetic acid.

Polyacrylamide gel electrophoresis of virus protein. The procedure used for polyacrylamide gel electrophoresis was essentially the same as that described by Laemmli (20), except that the gel and buffer chamber contained 0.1% 2-mercaptoethanol. Samples were prepared by addition of 2× treatment buffer (0.125 M Tris-Cl, pH 6.8, 4% sodium dodecyl sulfate, 20% glycerol, 10% 2-mercaptoethanol, 10 M urea, 0.001% bromophenol blue) to an equal volume of viral suspension and incubation in a boiling water bath for 3 min. Electrophoresis of samples was carried out in a Hoefer Scientific vertical slab chamber (SE 600) through a 1.5-mm-thick 12.5% polyacrylamide gel or through 6-mm (i.d.) columns of 7.5% polyacrylamide. The gels were stained and destained as for those with virus particles. The cylindrical gels were scanned with a Varian Techtron spectrophotometer at 550 nm and the molecular weight of the viral proteins was determined by comparison with standard proteins.

RESULTS

Symptoms. On the bean cultivars Guañ and 27-R, BRMV-Cali produced a severe mosaic, regular rugosity, and dwarfing on the first trifoliate leaf, and very irregular rugosity and malformation of the second trifoliate leaf (Fig. 1A). When plants were naturally coinfected with BRMV and bean mild mosaic virus (BMMV) (Acosta, *unpublished*), leaf malformation became even more severe (Fig. 1B). After BRMV infection, the Linea 32 bean cultivar developed dark brown local lesions with an average diameter of 1.5 mm. On Top-crop bean the necrotic local lesions were a very intense brown and had an average diameter of 1.25 mm. In both cases the necrotic lesions appeared about 4–5 days postinoculation and were measured at the same age.

Properties in vitro. The BRMV in sap was still infectious after being heated for 10 min at 65 C, but not at 70 C, or when diluted to 10^{-4}, but not to 10^{-5} in 0.01 M potassium phosphate buffer, pH 7.0. The virus was infective after 3 but not after 4 days at 25 C.

Serological relationships. In sap of systemically infected plants, BRMV reacted strongly with antisera to CSMV-Ark and BPMV (Fig. 2A and B) at dilutions higher than 1/64, but reactions with antiserum to QPMV (Fig. 2A) were only detectable up to a dilution of 1/4. The same reactions were observed with purified nucleoprotein components of BRMV at a concentration of 1.5–1.8 mg/ml. All other antisera tested gave negative results.

Purification and electron microscopy. All purification procedures gave three virus components, top (T), middle (M), and bottom (B), after rate zonal sucrose gradient centrifugation, irrespective of the buffer used (Fig. 3). Relative concentrations of the virus components were variable from one purification to another. Yield of virus was about 0.350 g/kg of leaf tissue from 27-R bean plants. The RNA contents were 5% (T), 25% (M), and 37% (B) as determined from ultraviolet (UV) absorption spectra.

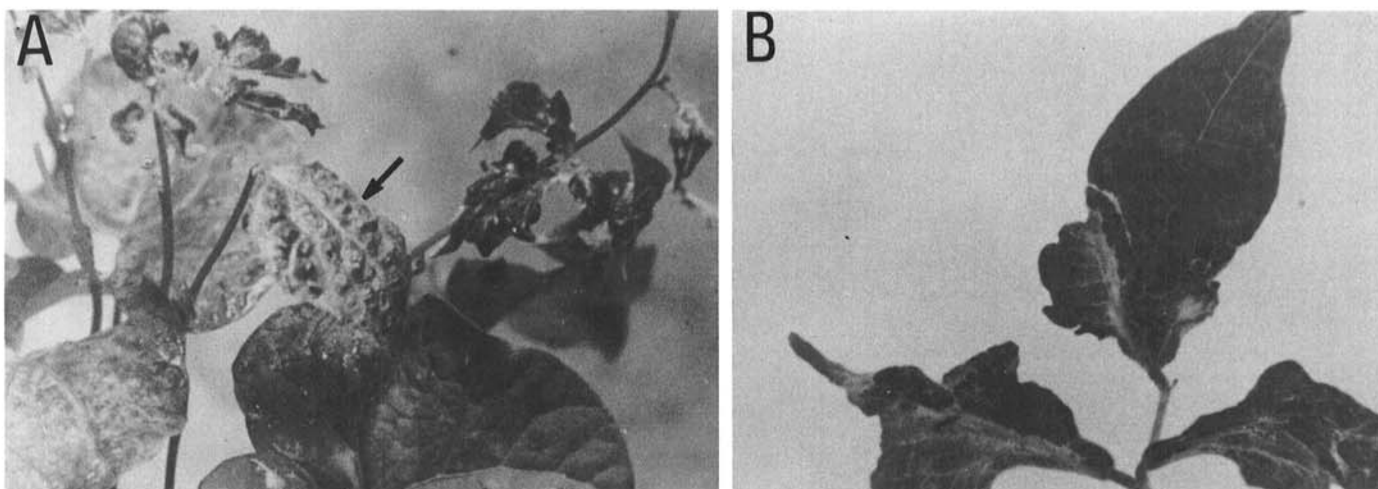


Fig. 1. Symptoms of bean rugose mosaic virus (BRMV) on leaves. **A,** Systemically infected leaves of 27-R bean showing blistering, rugosity, and malformations. Arrow indicates first trifolium. **B,** First trifolium of 27-R bean doubly infected with BRMV and bean mild mosaic virus (BMMV) showing severe malformation.

When observed in the electron microscope, the top component purified by two cycles of density gradient centrifugation consisted mostly of electron-dense shells (Fig. 4A), whereas particles of the middle and bottom components were not penetrated by the negative stain (Fig. 4B and C). All particles showed a hexagonal profile with an average diameter of 30 nm.

Ultrastructural studies. Viruslike particles formed clusters or were scattered in the cytoplasm and vacuoles of infected cells (Fig. 5A and B). When the viruslike particles were scarce they were almost indistinguishable from ribosomes. The formation of numerous vesicles was evident in the cytoplasm and rarely in the chloroplasts (Fig. 5C and D). In some cases it was possible to observe viruslike particles within vesicles (Fig. 5E) or closely associated with their external membranes (Fig. 5F). This location could be related to some event of the viral replication cycle. In some vesicles it was possible to observe a fibrous material (Fig. 5C). Crystalline arrays of virus particles were never found.

Electrophoretic analysis of components separated in sucrose gradients. After electrophoresis in nondenaturing conditions, the nucleoprotein components (purified and separated by two cycles of sucrose density gradients centrifugation in 0.01 M sodium-EDTA buffer, pH 7.8) generally gave a single, slow electrophoretic band (Fig. 6A), but the top component occasionally gave a second, fast electrophoretic band (Fig. 6A, curve B). When 0.01 M potassium phosphate buffer, pH 7.0, was used in the purification and separation of nucleoprotein particles, top, middle, and bottom components all gave two electrophoretic bands, slow and fast (Fig. 6B). When a single slow electrophoretic band of virus particles

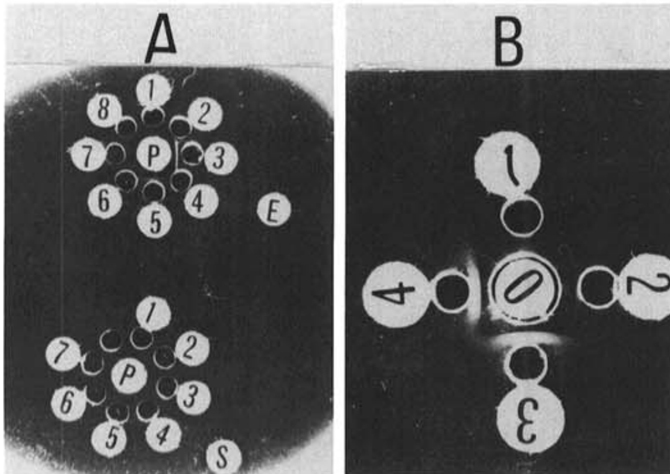


Fig. 2. Double immunodifusion patterns of bean rugose mosaic virus (BRMV) upon reaction with the antisera prepared against some leguminous viruses. **A,** The sap of the BRMV-infected tissue was placed in the center well (E). Outer wells contained antiserum to CPMV-sb (1), SBMV-cp (2), BPMV (3), SBMV-sb (4), QPMV (5), SBMV-cp (6), BYSV (7), BGMV (8). The sap of healthy tissue is in the center well (S). Outer wells contained the same antisera as in (E). **B,** The antiserum to CSMV-Ark is contained in the center well. The outer wells contained juice from BRVM-infected bean (wells 3 and 4) and juice from the healthy bean (wells 1 and 2).

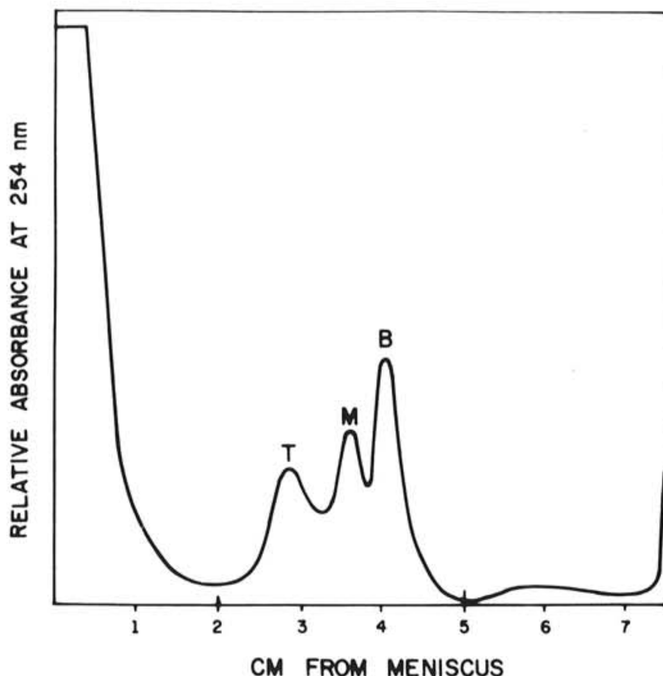


Fig. 3. Sucrose gradient profile of bean rugose mosaic virus (BRMV). Sample was layered onto a 10–50% sucrose gradient in 0.01 M sodium-EDTA buffer, pH 7.8, and centrifuged for 4 hr at 208,000 *g* in a Beckman SW 41 rotor at 4 C. The gradients were monitored at 254 nm with an ISCO gradient fractionator. T = top component, M = middle component, B = bottom component.

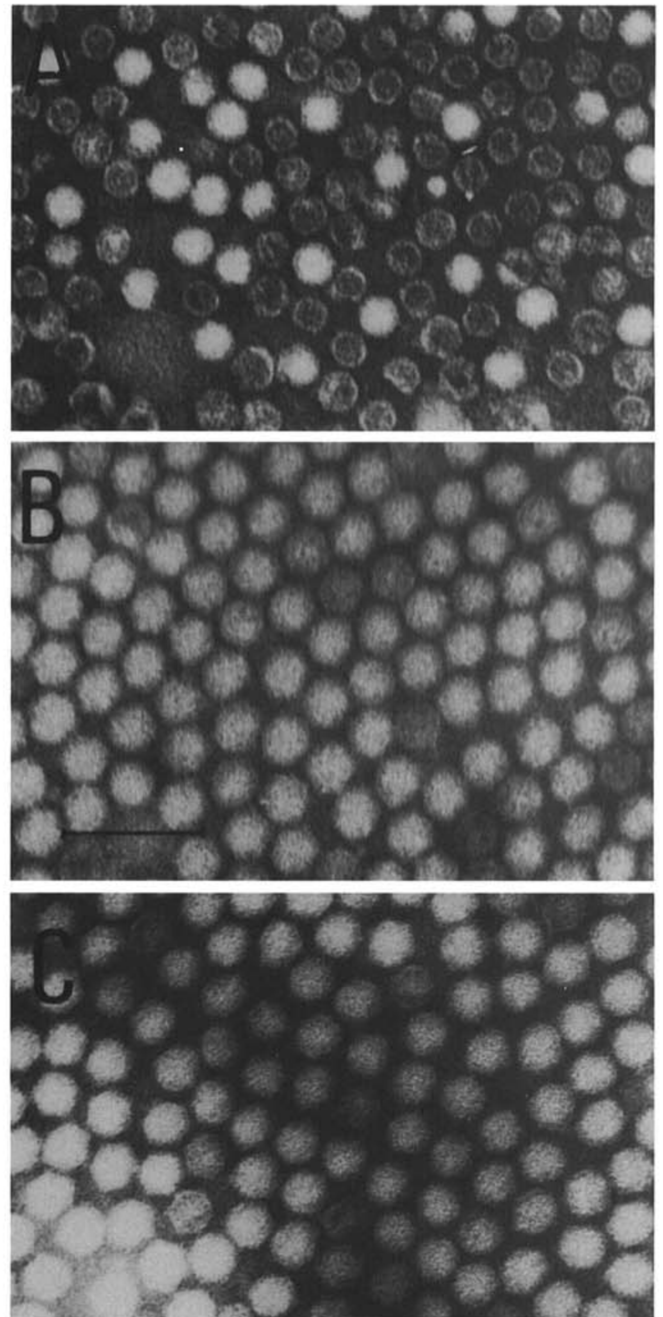


Fig. 4. Electron microscopy of centrifugation components of bean rugose mosaic virus (BRMV). **A,** Top (T) component. **B,** Middle (M) component. **C,** Bottom, (B) component. Staining was with 2% phosphotungstic acid. Bar = 90 nm.

appeared, denaturation of each of the three centrifugal components and separation of the proteins by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions (0.1% 2-mercaptoethanol in all systems) gave two protein bands (39.5×10^3 and 24.5×10^3 daltons) (Fig. 7A). When two electrophoretic bands of virus particles were present, slow and fast, SDS-PAGE in the same conditions separated three proteins (39.5×10^3, 24.5×10^3 and 22×10^3 daltons) (Fig. 7B). After storage of infected tissue at 20 C for six or more months and purification in 0.01 M potassium phosphate buffer, pH 7.0, only two virus proteins with molecular mass of 39.5×10^3 and 22×10^3 daltons were observed in SDS-PAGE (Fig. 8). Similar behavior has been reported for other members of the comovirus groups (14,30), indicating that after storage the 24.5×10^3 dalton protein

TABLE 1. RNA content of BRMV nucleoprotein components

Density components[a] (g/c^3)	RNA content[b] (%)	
	A_{280}/A_{260}	Buoyant density
1.305 ± 0.0035	5	0
1.398 ± 0.0020	25	29
1.412 ± 0.0025	30	31
1.428 ± 0.0021	34	33
1.470 ± 0.0028	40	38

[a] Named according to density in CsCl centrifugation in 0.01 M sodium buffer, pH 7.8. Results obtained from three different preparations.
[b] Obtained according to ultraviolet absorbance ratios or the buoyant density.

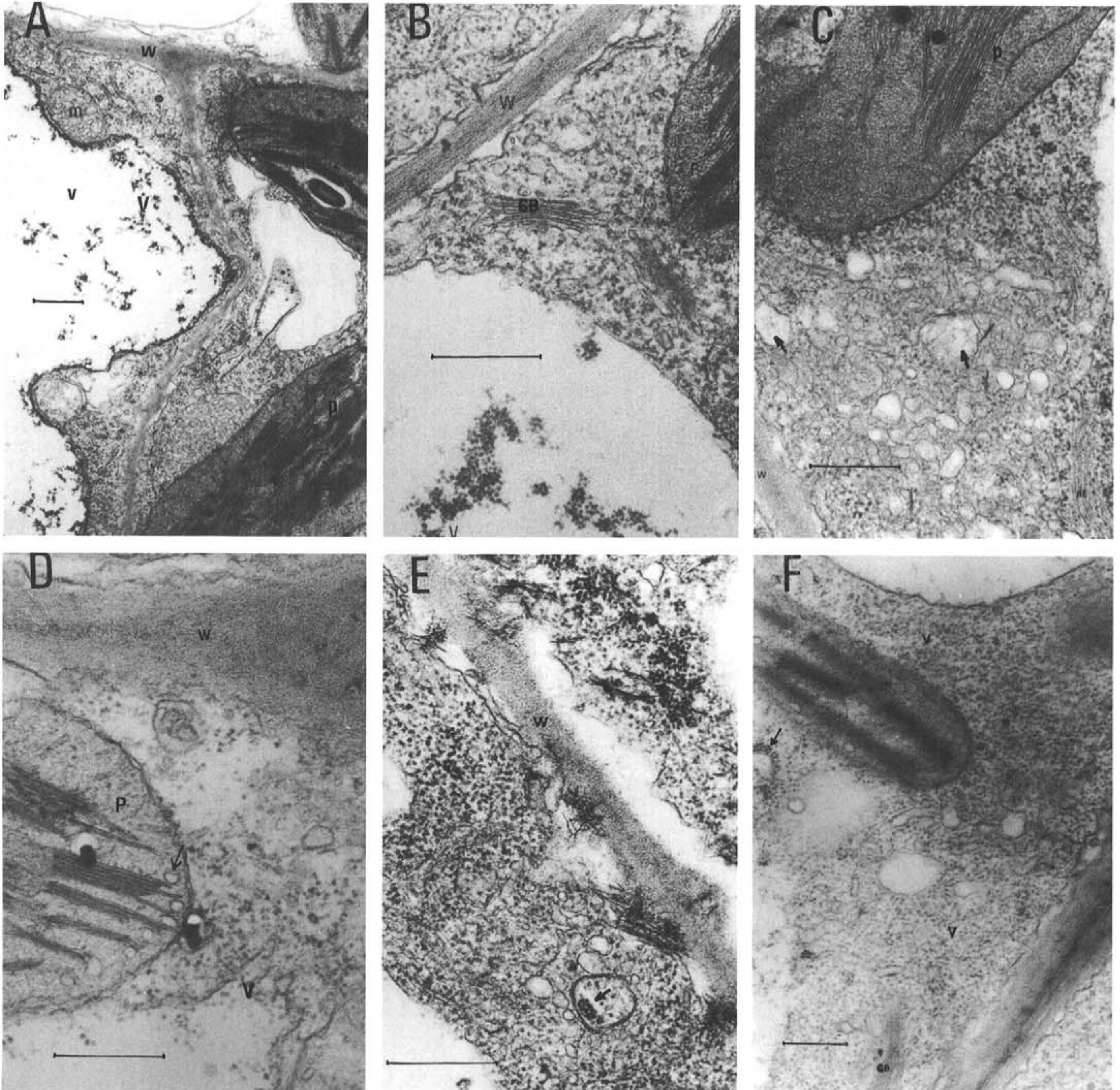
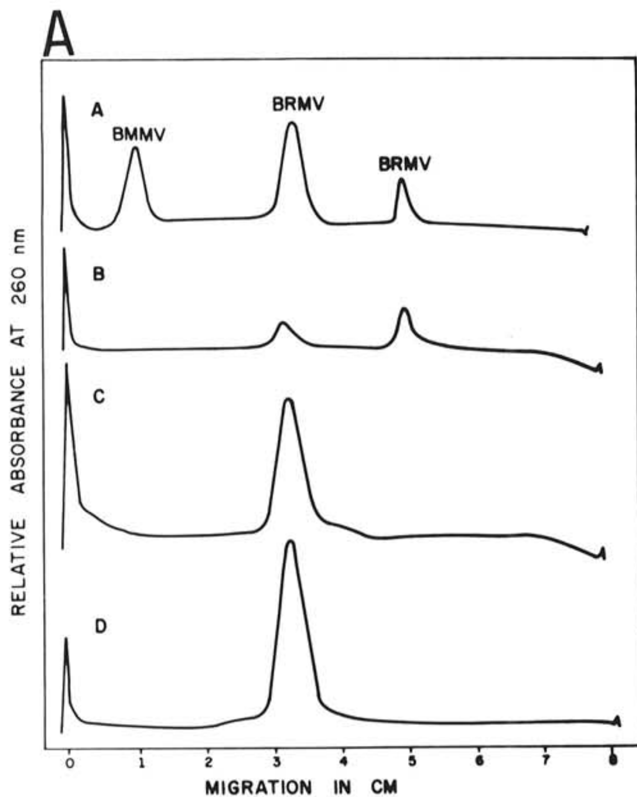


Fig. 5. Ultrastructural studies of cytopathic effects in bean rugose mosaic virus-infected bean mesophyll cells. **A,** At low magnification viruslike particles are observed as dense clusters within vacuoles. (× 15,800). **B,** At higher magnification particles with virus polyhedral shape are distinguisable in the vacuole and the cytplasm. (× 36,200). **C,** Numerous vesicles are seen in the cytoplasm. Some contain filamentous material (arrow). (× 29,500). **D,** Vesicles in the chloroplast were rarely observed (arrow). (× 36,400). **E,** Viruslike particles in the vesicles (arrow). (× 36,300). **F,** Viruslike particles around the vesicle membrane (arrow). (× 21,200). Viruslike particles (V), cell wall (W), chloroplast (P), mitochondrion (m), vacuole (v), Golgi body (GB). Bar = 0.5 μm.



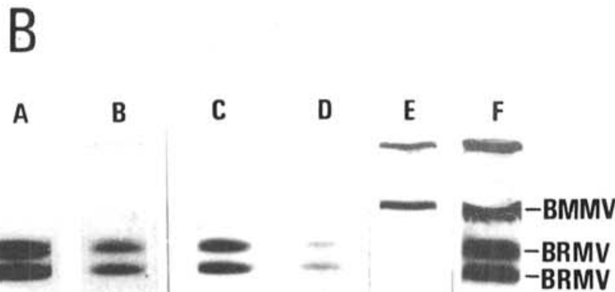


Fig. 6. A, Absorbance profiles of bean rugose mosaic virus (BRMV)-centrifugation components following gel electrophoresis in nondenaturing condition after purification using 0.01 M sodium-EDTA buffer, pH 7.0. The cylindrical gels were 2.9% polyacrylamide and were run 15 hr at 25 V followed by scanning at 260 nm. (Curve A) Sample containing unfractionated BRMV. BMMV is included as a reference. (Curve B) BRMV-top component. (Curve C) BRMV-middle component. (Curve D) Bottom component. **B,** Polyacrylamide gel electrophoresis of BRMV components purified and separated using 0.01 M potassium phosphate buffer, pH 7.0. The electrophoresis was under nondenaturing conditions in cylindrical gels containing 2.9% polyacrylamide for 6 hr at 150 V and then the gels were stained with Coomassie brilliant blue. (Lane A) Unfractionated BRMV. (Lane B) BRMV-Bottom component. (Lane C) BRMV-Middle component. (Lane D) BRMV-Top component. (Lane E) BMMV. (Lane F) Unfractionated BRMV and BMMV.

loses a short peptide to form the 22×10^3 dalton protein and produce the fast electrophoretic virion form.

Separation of the nucleoprotein components by equilibrium sedimentation in CsCl. When virions prepared in potassium phosphate buffer were centrifuged in the same buffer containing CsCl, they banded at four densities, 1.308, 1.401, 1.429, and 1.470 g/c^3 (Fig. 9). When sodium-EDTA buffer was used, the virion banded at five densities, 1.305, 1.398, 1.412, 1.428, and 1.470 g/c^3 (Fig. 10). The components separated in potassium phosphate buffer, showing densities of 1.429 and 1.470 g/c^3, cosedimented as the bottom component in sucrose density gradients, whereas the 1.401 g/c^3 density component sedimented at the middle position. The relationships between CsCl density components in sodium-EDTA and sucrose sedimentation components were not studied in

TABLE 2. Infectivity of middle (M) and bottom (B) centrifugation components of bean rugose mosaic virus

Local lesions[a]				Relative infectivity[b]	
M[c]	M + B	B[c]	M + B	M/M + B	B/M + B
32	81	20	105	0.39	0.19
8	73	42	98	0.10	0.42
10	85	68	140	0.11	0.48
11	75	48	61	0.14	0.78
14	77	80	92	0.18	0.86
25	93	65	132	0.26	0.49
19	71	53	114	0.26	0.46
15	47	60	95	0.31	0.63
18	69	55	98	0.26	0.56
11	78	67	105	0.14	0.63
Mean				0.21	0.55

[a] Corresponding to opposite half-leaves. Half-leaves were inoculated with each separate component, M or B, then the corresponding opposite half-leaves were inoculated with the mixture M + B.

[b] Number of local lesions produced by the component/number of local lesions produced by M + B mixture.

[c] M and B components were obtained by centrifuging twice in sucrose density gradients the 1.401 g/c^3 density component (M) and a mixture of 1.429 g/c^3 and 1.470 g/c^3 density components (B). The separate components were inoculated at a concentration of 1.25 $A_{260\ nm}$ or at 0.75 $A_{260\ nm}$ each when inoculated in combination. The components were purified and inoculated using 0.01 M potassium phosphate buffer, pH 7.0.

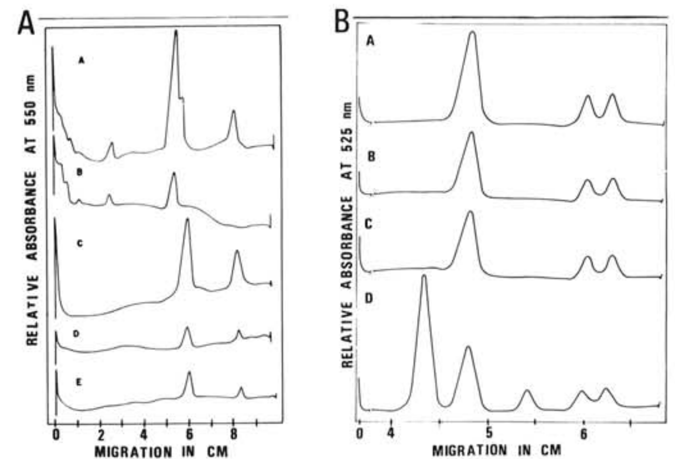


Fig. 7. A, Absorbance profile of Coomassie brilliant blue stained 7.5% cylindrical gels following sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and scanning at 550 nm. Bean rugose mosaic virus (BRMV) was purified in 0.01 M sodium-EDTA buffer, pH 7.8. (Curve A) BRMV and BMMV. (Curve B) BMMV (44,500 and 93,000 daltons). (Curve C) Nonfractionated BRMV (39,500 and 24,500 daltons). (Curve D) BRMV-Middle component. (Curve E) BRMV-Bottom component. **B,** Scanning pattern of 7.5% polyacrylamide gels following SDS-PAGE and Coomassie brilliant blue staining. The gels were scanned at 525 nm. The BRMV was purified in 0.01 M potassium phosphate buffer, pH 7.0. (Curve A) Nonfractionated BRMV. (Curve B) BRMV-Middle component. (Curve C) BRMV-Middle component. (Curve D) BRMV, BMMV (44,500 daltons) and SBMV (29,000 daltons).

detail. The RNA content of the components separated by CsCl equilibrium sedimentation was in good agreement by both methods used (Table 1). The small amount of RNA estimated for the 1.305 g/c^3 density component by the UV absorbance ratio was attributed to contamination with full particles. Electron microscopy of each density component separated in sodium-EDTA buffer revealed particles with identical size and shape (Fig. 11) although the top component (Fig. 11A) showed an electron-dense core.

Electrophoretic analysis of components separated by equilibrium sedimentation in CsCl. After separation by CsCl centrifugation in sodium-EDTA buffer, virus particles of each

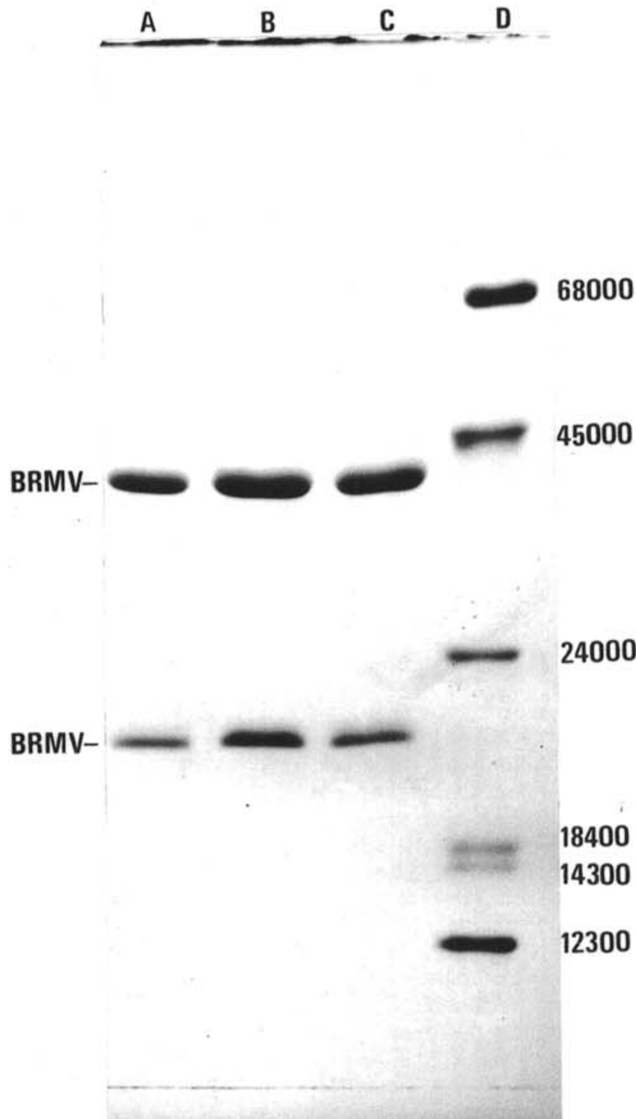


Fig. 8. Polyacrylamide gel electrophoresis with sodium dodecyl sulfate of BRMV purified in 0.01 M potassium phosphate buffer, pH 7.0, after storage for 8 mo at −20 C. The slab gel contained 12.5% polyacrylamide and was stained with Coomassie brilliant blue. (Lane A) BRMV. (Lane B) BRMV-Middle component. (Lane C) BRMV-Bottom component. (Lane D) Bovine serum albumin (68,000 daltons), ovoalbumin (45,000 daltons), trypsinogen (24,000 daltons), β-lactoglobulin (18,400 daltons), lysozyme (14,300 daltons), and cytochrome C (12,300 daltons).

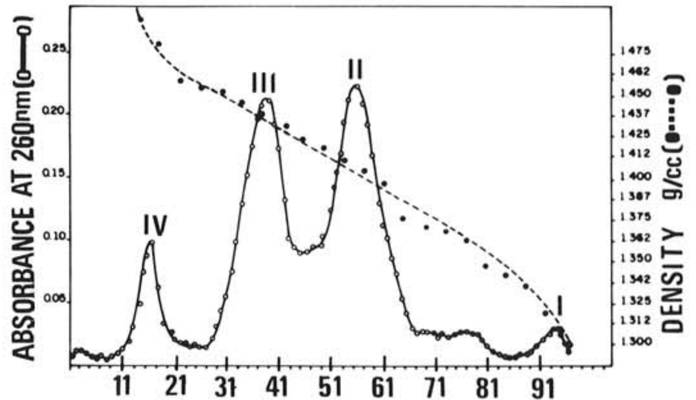


Fig. 9. Absorbance profile of the equilibrium sedimentation in CsCl of bean rugose mosaic virus (BRMV) using 0.01 M potassium phosphate buffer, pH 7.0. The centrifugation was carried out at 300,000 *g* for 15 hr in a Beckman SW 65 rotor at 15 C. Fractions were collected from the tube bottom and the A_{260nm} and the density of each fraction was determined. I, 1.308 g/c^3; II, 1.401 g/c^3; III, 1.429 g/c^3; IV, 1.470 g/c^3.

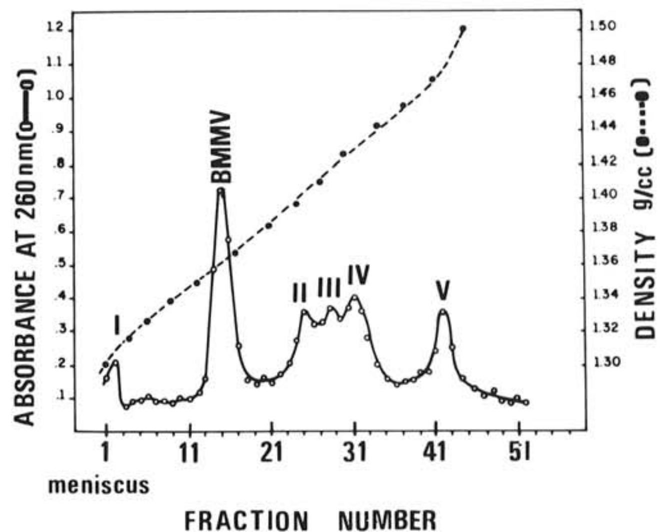


Fig. 10. Absorbance profile of the equilibrium sedimentation in CsCl of bean rugose mosaic virus (BRMV) using 0.01 M sodium-EDTA buffer, pH 7.8. Centrifugation was carried out at 208,000 *g* for 48 hr in a Beckman SW 41 rotor at 15 C. Fractions were collected from the bottom of the tube and the A_{260nm} and the density of each fraction was determined. I, 1.305 g/c^3; II, 1.398 g/c^3, III, 1.412 g/c^3; IV, 1.428 g/c^3; V, 1.470 g/c^3. BMMV appears banding at 1.360 g/c^3 as a density reference.

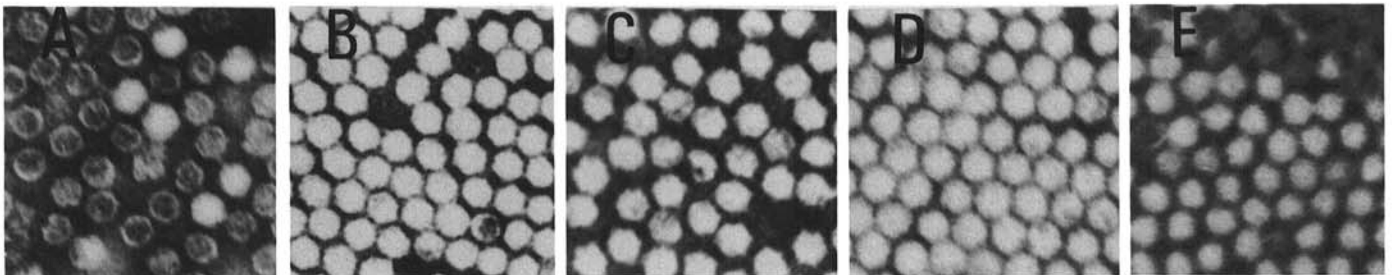


Fig. 11. Electron microscopy of BRMW density components separated by CsCl centrifugation in 0.01 M sodium-EDTA buffer, pH 7.8, and negatively stained with 2% phosphotungstic acid. **A,** 1.305 g/c^3 BRMV density component. **B,** 1.398 g/c^3 BRMV density component. **C,** 1.412 g/c^3 BRMV density component. **D,** 1.428 g/c^3 density component. **E,** 1.470 g/c^3 BRMV density component.

density component showed the same electrophoretic pattern with a single, slow band except for the 1.305 g/c^3 density component, which had an additional fast electrophoretic band, similar to the pattern obtained with purified virus particles in sucrose gradients with the same buffer (Fig. 6A). SDS-PAGE of the same density components revealed two structural proteins with molecular weights of 39.5×10^3 and 24.5×10^3, confirming the results obtained with the particles separated by sucrose gradient sedimentation (Fig. 7A). In contrast, all density components of virus particles separated by equilibrium centrifugation in potassium phosphate buffer consisted of two electrophoretic species. SDS-PAGE resolved three structural proteins with molecular weights of 39.5×10^3, 24.5×10^3, and 22×10^3. These observations are consistent with the results obtained by electrophoresis after sucrose gradient sedimentation (Figs. 6B and 7B).

Infectivity of nucleoprotein components. Mixtures of the centrifugation components M and B were more infectious than each one separately (Table 2). Infectivity of the components separated by CsCl gradient centrifugation was always lower than that of nonfractionated BRMV (Table 3). The residual infectivity of the separated M and B fractions may be interpreted as contamination with adjacent components. The infectivity data strongly suggest that BRMV has a divided genome.

DISCUSSION

The symptoms produced by BRMV on infected plants were similar to those produced by 2,4-dichlorophenoxyacetic acid (9). The severity of symptoms was enhanced when plants were coinfected with BMMV.

Serological tests showed strong reactions with antisera to CSMV-Ark and BPMV, and a weak reaction with antiserum to QPMV. Except for the last reaction, the other reactions have already been reported. However BRMV-Ampollado (9) reacted weakly with both CSMV-Ark and BPMV antisera, whereas BRMV-Costa Rica (13) reacted weakly with CSMN-Ark and strongly with BPMV antisera.

The properties described in vitro were similar to those reported for other isolated of BRMV (9,10,13).

All purification procedures, including the one reported by Gálvez (11) were satisfactory and produced three centrifugal components, T, M, and B after sucrose density gradient centrifugation, although their relative concentration was variable from one purification to another. Strains of CPMV reportedly differ in the amount of the T component (1) and red clover mottle virus loses this component during purification (24). The presence of three centrifugal components of BRMV is in good agreement with the report for BRMV from Costa Rica (13), but not with the report for the Ampollado strain from El Salvador, which generally contains two components and only occasionally three (9–11). The M and B fractions almost exclusively contained particles that resisted penetration by negative stain and contained RNA. On the other hand, the T fraction had many electron-dense particles. According to their UV spectra they have a low RNA content, which could be an artifact resulting from contamination with particles from the other fractions. According to their buoyant density, the T component should be devoid of RNA (Fig. 11, Table 1). These particles were mostly found in the middle component of the Ampollado strain from El Salvador (9–11).

The ultrastructural studies of the infected tissue showed that the virus particle arrangement of BRMV-Cali within cells was very similar to that of BRMV-Strain A_2 (19) and the Ampollado strain (8).

The patterns obtained in polyacrylamide gel electrophoresis both under nondenaturing and denaturing conditions were in good agreement with various observations made on other members of the comovirus group (22). The conversion of the 24.5×10^3 dalton protein to the 22×10^3 dalton protein could be a step in the proteolytic processing of the M-RNA coded precursor proteins reported for some members of the comovirus groups (5,7). The use of 0.01 M sodium-EDTA buffer, pH 7.8, in the purification procedures appears to prevent such a conversion and the corresponding appearance of the fast electrophoretic component of virus particles.

We have found that the BRMV B component banded into two different density fractions during CsCl centrifugation in potassium phosphate buffer. Similar behavior has been reported for the bottom component of CPMV in the same buffer (2,27). Buoyant density changes in the bottom component of CPMV at different pH values probably result from irreversible structural changes at alkaline pH that affect the amount of the cesium binding and/or hydration (29). However, the spectrophotometric data indicated that the BRMV 1.470 g/c^3 density component had a greater RNA content than that at 1.429 g/c^3, suggesting the the protein/nucleic acid ratio may also play some role in the density differences without visibly affecting the sedimentation in sucrose density gradients. Nevertheless, gel electrophoresis showed a homogeneous pattern for all density components, indicating that the different density components are identical electrophoretic species. Bruening has reported that the lower density of the B component may also reflect a higher content of polyamines, especially spermidine (3).

When equilibrium sedimentation was conducted in sodium-EDTA buffer an additional component banding at 1.412 g/c^3 was observed. In this case the relationships between the separated components in sucrose and CsCl gradients were not studied. All components exhibited identical electrophoretic patterns, except for the top component, whereas electron microscopy showed the same size and shape for all components. It has been reported that alkaline and chelating buffers produce heterogeneity in CsCl density sedimentation (15,18). Some plant viruses have also been reported to be unstable in concentrations of CsCl commonly used to form gradients (17). This result offers additional evidence that the heterogeneity in CsCl centrifugation could be related in some

TABLE 3. Infectivity of the BRMV density components separated by CsCl centrifugation

Local lesions[a]												Relative infectivity[b]					
I[c]	NFV	II	NFV	III	NFV	I+II	NFV	I+III	NFV	II+III	NFV	I/NFV	II/NFV	III/NFV	I+II/NFV	I+III/NFV	II+III/NFV
7	62	18	59	2	45	50	82	24	65	29	69	0.11	0.30	0.40	0.60	0.36	0.42
10	50	22	68	0	68	42	70	28	61	37	72	0.20	0.32	0.00	0.60	0.45	0.51
8	68	29	71	5	55	50	71	38	70	25	65	0.11	0.40	0.09	0.84	0.54	0.38
7	49	25	70	3	57	38	62	45	54	31	73	0.14	0.35	0.05	0.60	0.83	0.48
11	55	23	55	1	61	45	65	42	68	40	61	0.20	0.41	0.01	0.60	0.61	0.65
5	58	25	67	0	75	35	68	35	50	31	75	0.08	0.37	0.00	0.51	0.70	0.41
10	65	26	77	4	61	61	60	51	63	24	61	0.15	0.34	0.06	1.01	0.80	0.39
4	59	30	62	4	69	54	69	43	57	28	58	0.06	0.48	0.02	0.91	0.75	0.42
Mean												0.13	0.37	0.03	0.70	0.63	0.43

[a] Corresponding to opposite half-leaves. Eight half-leaves were inoculated with each separate component or the mixture, followed by inoculation with nonfractionated BRMV on the corresponding opposite half-leaves.

[b] Number of local lesions produced by the component or the mixture/number of local lesions produced by the nonfractionated purified BRMV.

[c] I. 1.401 g/c^3 BRMV density component; II. 1.429 g/c^3 BRMV density component; III. 1.470 g/c^3 density component. All single components and nonfractionated BRMV at 1.0 $A_{260\ nm}$. Mixture of components contained 0.5 $A_{260\ nm}$ of each component. All steps of purification were made in 0.01 M potassium buffer, pH 7.0. NFV = Nonfractionated purified BRMV.

way to pH and/or ion type.

Our results confirm that BRMV belongs to comovirus group (13) and suggest that our isolate is a strain closely related to the Costa Rica strain (13) but more distantly related to the Ampollado strain from El Salvador (9).

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