Comparative Characterization of Two Luteoviruses: Beet Western Yellows Virus and Barley Yellow Dwarf Virus

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ABSTRACT

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A legume isolate of beet western yellows virus (BWYV-CA) and a vector nonspecific isolate of barley yellow dwarf virus (BYDV-PAV-IL) were characterized. An associated top component (BWYV-CA-TC) was isolated with BWYV-CA. BWYV-CA-TC had a maximum absorbance at 275 nm, an $s_{20,w}$ of 62 S, buoyant densities of 1.31 g/ml in CsCl and 1.28 g/ml in Cs_2SO_4. No nucleic acid was detected in BWYV-CA-TC and the particles were not infectious. BWYV-CA had a maximum absorbance at 259 nm, an $s_{20,w}$ of 114 S, and buoyant densities of 1.42 g/ml in CsCl and 1.36 g/ml in Cs_2SO_4. BYDV-PAV-IL had a maximum absorbance at 258 nm, an $s_{20,w}$ of 106 S, and buoyant densities of 1.41 g/ml in CsCl and 1.35 g/ml in Cs_2SO_4. The relative molecular masses (M_r) of the major proteins of BWYV-CA-TC, BWYV-CA, and BYDV-PAV-IL were 24,000, 24,000, and 22,500, respectively. The M_r of the undenatured RNA species of BWYV-CA was 1.90×10^6 and of BYDV-PAV-IL was 1.85×10^6. In negatively stained preparations of purified particles from sucrose density gradients, particle diameters of BWYV-CA-TC, BWYV-CA, and BYDV-PAV-IL were 23 ± 2, 25 ± 1, and 25 ± 1 nm, respectively.

Luteoviruses cause yellowing diseases of many small grains, dicotyledonous crops, and weeds (28). The viruses are circulative in and persistently transmitted by their aphid vectors and are sometimes vector specific (14,25). Generally, lengthy acquisition and inoculation access periods are required for efficient transmission (10,14,25). With some vectors, transmission is dependent on the presence of another virus (26). Luteoviruses are not sap transmissible and the about 25-nm icosohedral particles apparently are confined to phloem and phloem parenchyma tissue (8,11,15,16,21), suggesting considerable tissue or physiological specificity.

Typically, yields of purified virus are very low and sufficient material for characterization usually is not available. Luteoviruses are excellent antigens and most investigators understandably use their meager yields to produce antisera. While developing purification procedures for BWYV-CA and BYDV-PAV-IL (9), a top component (BWYV-CA-TC) was found associated with BWYV-CA, which contained no nucleic acid. This intriguing property led to a comparative physiochemical study of these two economically important viruses. The objectives were to obtain physical and chemical information required for experimental manipulation, to provide insights about the viruses' relationships to other viruses, and to add to the growing body of information needed to better define the luteovirus group concept and perhaps to contribute to the eventual formulation of subgroup concepts.

MATERIALS AND METHODS

A California isolate of BWYV obtained from J. E. Duffus and an Illinois vector nonspecific (PAV-like) isolate of BYDV from H. Jedlinski were used for characterization studies. Virus propagation, purification methods, and infectivity assays have been described (9). When BWYV-CA-TC, BWYV-CA, and BYDV-PAV-IL were compared with known standards, a standard curve was plotted or, using the same data, a regression coefficient was generated. Physiochemical properties were then determined using the standard curve or the regression equation. The numerical values reported for each physiochemical property are averages of at least three determinations.

Ultraviolet absorbance profiles and spectra. Spectrophotometric properties of preparations containing 0.1–0.5 mg/ml of each particle type were determined in a Beckman 34 scanning spectrophotometer. After correction for light scattering, absorption ratios were calculated, and the average of 10 preparations was determined.

Sedimentation coefficients. Sedimentation coefficients were estimated by linear log sucrose density gradient centrifugation (5) using methods described earlier (18).

Buoyant density of the particles. Buoyant densities were determined by equilibrium centrifugation in a SW 55 Ti rotor. Cesium chloride was dissolved in 0.1 M sodium phosphate buffer, pH 6, containing approximately 10 μg of BWYV-CA, BWYV-CA-TC, and BYDV-PAV-IL at initial densities of 1.400, 1.300, and 1.400 g/ml, respectively. Cesium sulfate was prepared in the same manner, except the initial densities were 1.360, 1.280, and 1.360 g/ml. The preparations were centrifuged to equilibrium at 40,000 rpm for 18 hr at 4 C. Immediately thereafter the gradients were scanned at 254 nm and fractionated. Refractive indices of representative 0.2-ml fractions were measured with a Bausch & Lomb Abbe refractometer at 25 C. After the refractive indices of the fractions containing BWYV-CA-TC, BWYV-CA, and BYDV-PAV-IL were determined, the refractive indices were converted to buoyant densities (20).

Percent RNA and extinction coefficients of the infectious particles. Percent nucleic acid of BWYV-CA and BYDV-PAV-IL was calculated from buoyant density data with the formula developed by Sehgal et al (30). Extinction coefficients were calculated from the percent nucleic acid with the formula developed by Gibbs and Harrison (13).

Nucleic acids. Viral nucleic acid was prepared by incubating

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intact particles in TMS dissociation buffer [0.1 M Tris, pH 8, 5% 2-mercaptoethanol, and 4% sodium dodecyl sulfate (SDS)] at 37 C for 1 hr. Tobacco mosaic virus (TMV) RNA, relative molecular mass (M_r) 2.04×10^6, cowpea mosaic virus (CPMV) RNA, M_r 2.0 and 1.4×10^6, and brome mosaic virus (BMV) RNA, M_r 1.1, 1.0, 0.7, and 0.3×1^6 were used as markers and ssRNA controls. Hind III digested lambda DNA was a dsDNA control. Electrophoresis was in 1%, 10- × 13-cm × 3-mm agarose gels (Seakem, FMC Bioproducts, Rockland, ME) for 4 hr at 50 mA in a horizontal apparatus at room temperature. The buffer used for electrophoresis was TBE (0.089M Tris, 0.089M boric acid, and 0.025M disodium EDTA, pH 8.3). After electrophoresis, the gels were incubated in 0.5 μg/ml of ethidium bromide in deionized water for 1 hr or overnight. Gels were destained in deionized water for 1 hr before examination with UV light. A modification of the method described by Morris and Dodds (22) was used to determine the nature of the nucleic acids. First, the gel was incubated in 0.03 M $MgCl_2$ and 30 μg/ml of DNAse I (Sigma, St. Louis, MO) for 2–3 hr at 25 C. After three 1-hr rinses in distilled water, half of the gel was treated for 1 hr with ribonuclease A (Sigma) at a concentration of 50 μg/ml, while the other half of the gel was incubated in the same concentration of ribonulease A in 0.3 M NaCl.

Protein subunits. The M_r of the protein subunits were estimated by SDS-polyacrylamide gel electrophoresis. Protein markers (Bio-Rad Laboratories, Rockville Centre, NY) and dissociated virus protein subunits were boiled for 3 min in TMS buffer and applied to a 13 × 11 cm × 1.5 mm three-layer discontinuous slab gel system (6). Electrophoresis was at room temperature at 50 mA for 5 hr. After electrophoresis, the gels were fixed and stained overnight in 25% methanol, 8% acetic acid, and 0.2% Coomassie brilliant blue R. The gels were destained in the same solution without the stain for 3 hr.

Electron microscopy. Purified virus and top component preparations were negatively stained with a saturated solution of uranyl acetate and viewed in a JOEL JEM-100C electron microscope at 60 or 80 kV.

RESULTS

Isolation of BWYV-CA top and bottom components and BYDV-PAV-IL. Rate-zonal density gradient centrifugation separated partially purified BWYV-CA preparations into two bands, a slowly sedimenting upper band and a faster sedimenting lower band (Fig. 1). The ratio of empty particles to infectious particles in this isolate appeared to be rather high and remained at a high level for some time (Fig. 2). Electron microscope examination revealed the presence of isometric 23 ± 2 nm particles in the top band and 25 ± 1 nm particles in the lower band (Fig. 3). The negative stain penetrated nearly all the particles in the upper band but only a few particles in the lower band. When nonviruliferous *Myzus persicae* Sulz. were allowed to feed through a membrane on top or bottom band preparations containing 20% sucrose, only the bottom band was infectious. In contrast, no evidence of a top component was found for BYDV-PAV-IL (Fig. 1). Partially purified BYDV-PAV-IL was characterized by a single, fast sedimenting band containing a homogeneous population of 25 ± 1 nm particles (Fig. 1).

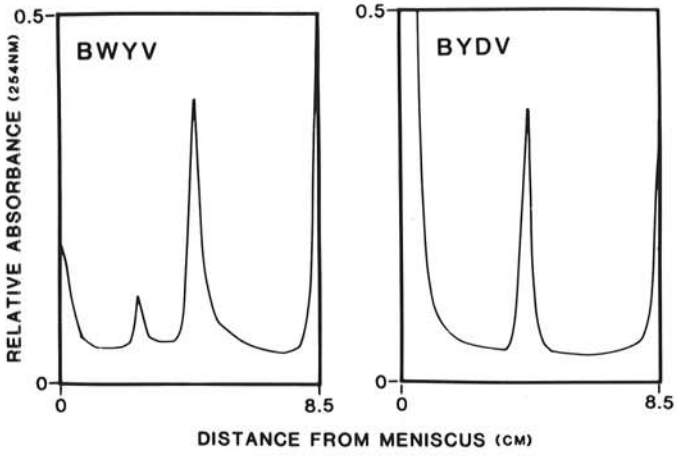

Fig. 1. Ultraviolet absorption profiles of purified virus preparations. Top (TC) and bottom (BWYV) components of BWYV-CA were purified from 100 g of shepherd's purse shoots and BYDV-PAV-IL (BYDV) from 24 g of Coast Black oats. Both viruses were isolated from linear (10–40%) sucrose gradients in 0.1 M sodium phosphate, pH 6 (BWYV-CA) or pH 7 (BYDV-PAV-IL), after centrifugation at 24,000 rpm for 5 hr in a Beckman SW27 rotor at 4 C.

Ultraviolet absorption spectra. Ultraviolet absorbance profiles of BWYV-CA top and bottom components were typical of protein and nucleoprotein preparations, respectively (Fig. 4). The top component maximum and minimum absorbances occurred at 276 and 249 nm. The profile for the infectious particle was quite different, having a maximum at 259 and a minimum at 239 nm. Maximum and minimum absorbances for BYDV-PAV-IL were 258 and 238 nm. $A_{260/280nm}$ absorbance ratios were 0.62 ± 0.02 for BWYV-CA top component, 1.65 ± 0.05 for the bottom component, and 1.90 ± 0.01 for BYDV-PAV-IL.

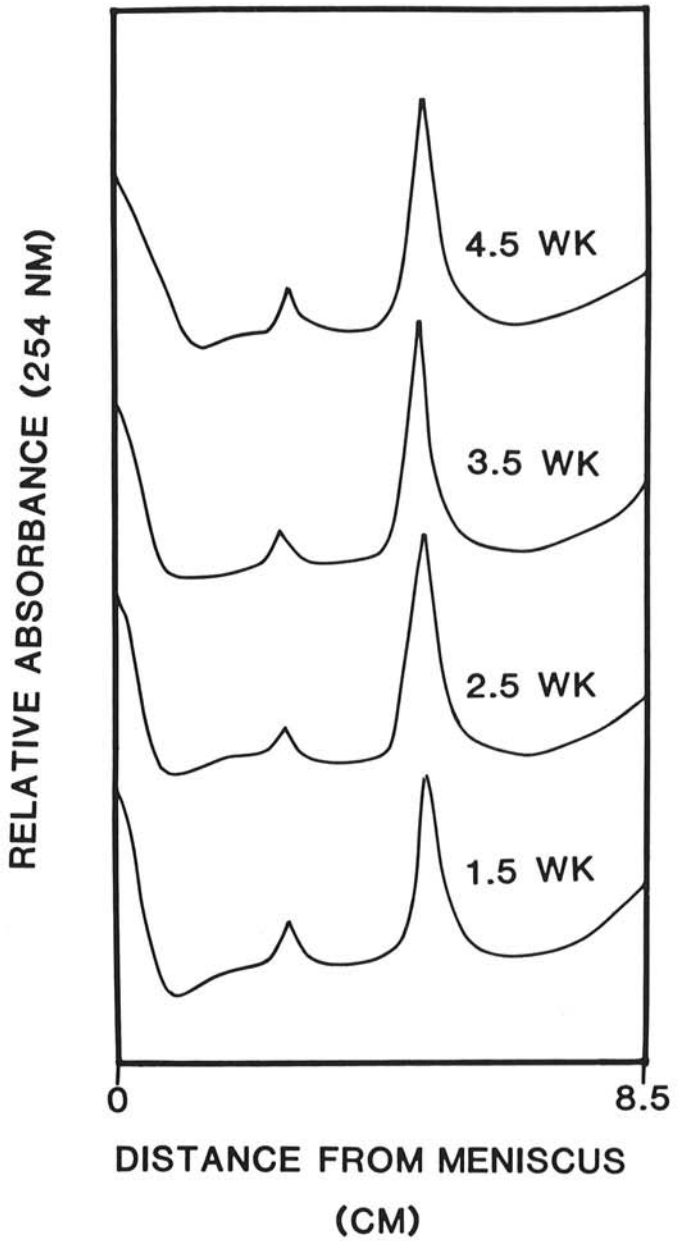

Fig. 2. Ultraviolet absorption profiles of BWYV-CA top and bottom components from 50 g of shepherd's purse shoots harvested 1.5, 2.5, 3.5, and 4.5 wk after inoculation.

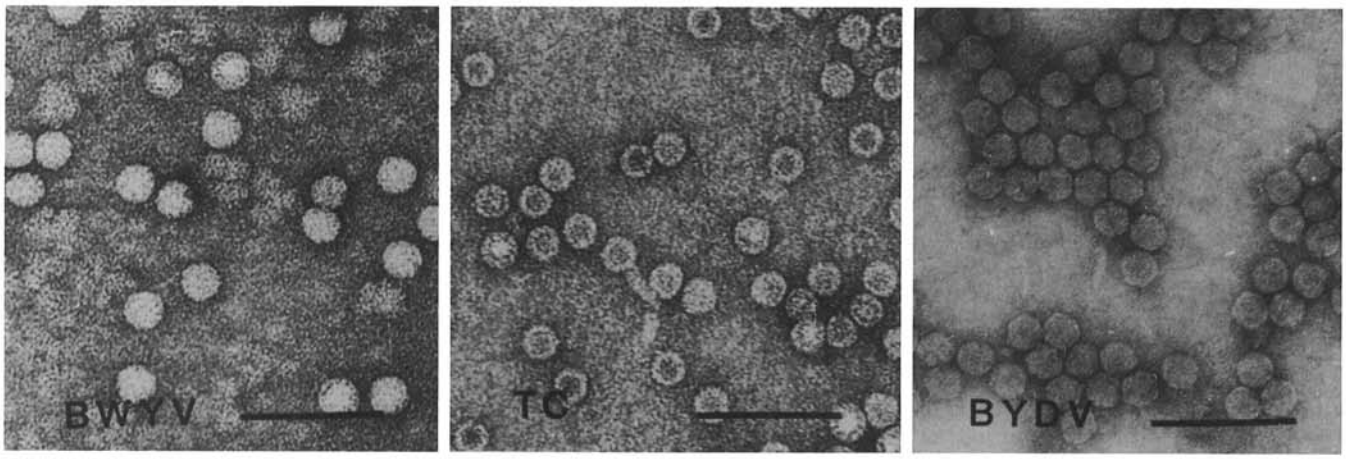

Fig. 3. Electron micrographs of BWYV-CA, BWYV-CA-TC, and BYDV-PAV-IL. Particles from partially purified preparations were negatively stained with a saturated solution of uranyl acetate and viewed in a JOEL JEM-100C electron microscope at 60 kV. Bars represent 100 nm.

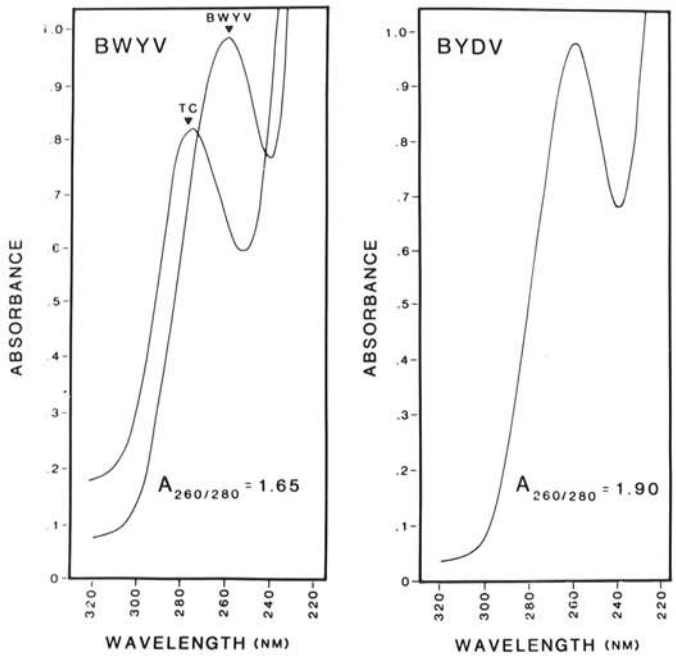

Fig. 4. Ultraviolet absorption spectra of purified BWYV-CA top (TC) and bottom components (BWYV) and BYDV-PAV-IL. After final purification, pelleted particles were allowed to resuspend in 0.05 M phosphate buffer, pH 6 (BWYV-CA) or pH 7 (BYDV-PAV-IL) for 18 hr at 4 C. Then each preparation was scanned in a Beckman Model 34 spectrophotometer.

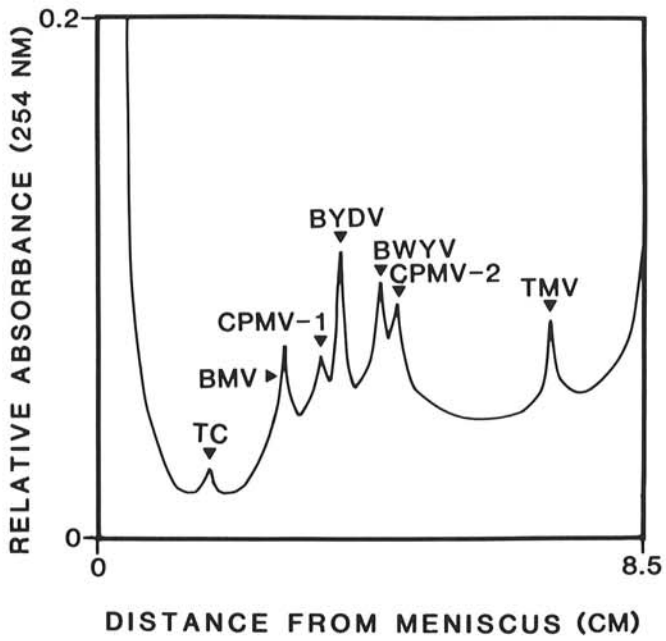

Fig. 5. Ultraviolet absorption profiles of purified BWYV-CA, BWYV-CA-TC, BYDV-PAV-IL, BMV, CPMV, and TMV. Sedimentation coefficients were estimated by scanning linear log sucrose gradients with known internal standards. Calculated sedimentation coefficients were: 62 S (BWYV-CA-TC), 114 S (BWYV-CA), and 106 S (BYDV-PAV-IL).

Sedimentation coefficients. Sedimentation coefficients ($s_{20,w}$) for BWYV-CA top and bottom components and BYDV-PAV-IL were 62, 114, and 106 S, respectively (Fig. 5).

Buoyant densities, percent RNA, and extinction coefficients. Purified BWYV-CA top and bottom components and BYDV-PAV-IL had buoyant densities in CsCl of 1.31, 1.42, and 1.41 and in Cs_2SO_4 of 1.28, 1.36, and 1.35 g/ml, respectively. Using buoyant density in CsCl the percent RNA of the infectious particles was estimated as 32% for BWYV-CA and 31% for BYDV-PAV-IL. Calculated extinction coefficients for BWYV-CA and BYDV-PAV-IL were 8.1 and 7.8 $(mg/ml)^{-1}$ cm^{-1} at 260 nm.

Nucleic acid. After incubation in TME buffer and agarose gel electrophoresis of BWYV-CA bottom component and BYDV-PAV-IL in separate lanes, a single nucleic acid was observed for each virion type. BWYV-CA and BYDV RNAs had M_r of 1.90 and 1.85×10^6, respectively (Fig. 6). No nucleic acid bands were found in BWYV-CA-TC after dissociation and electrophoresis in the same manner. Treatment of the gels after electrophoresis with RNAse-free DNAse and RNAse demonstrated stability to DNAse and sensitivity to RNAse, establishing the RNA nature of both genomes. The nucleic acids were found to be unstable in both high and low ionic strength RNAse, which indicates that the RNAs are single-stranded.

Protein subunits. Single major protein bands between trypsin inhibitor and carbonic anhydrase were observed after dissociation and electrophoresis of BWYV-CA, BWYV-CA-TC, and BYDV-PAV-IL (Fig. 7). The M_r of these proteins were 24,000 for BWYV-CA and BWYV-CA-TC and 22,500 for BYDV-PAV-IL. No differences in the M_r of BWYV-CA and BWYV-CA-TC major bands were observed although several minor protein bands were present occasionally in the virion, and frequently in the BWYV-CA-TC lanes. Two or three minor protein bands were found sometimes in the BYDV-PAV-IL lanes. The M_r of several of these additional bands were close to multiples of the major bands and may have resulted from aggregation of protein subunits.

DISCUSSION

In many respects, the isolates investigated here are typical

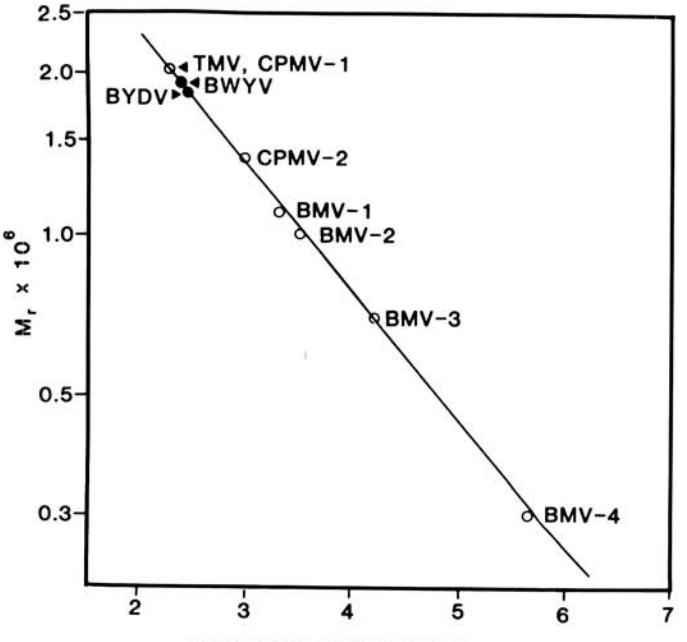

Fig. 6. Plot of the log of the relative molecular masses of BWYV-CA, BYDV-PAV-IL, and marker RNAs against the distance migrated. Markers and their (M_r) were TMV RNA (2.04×10^6), CPMV RNA, 2.0 and 1.4×10^6, and BMV RNA, 1.1, 1.0, 0.7, and 0.3×10^6. Calculated RNA M_r were: 1.9×10^6 (BWYV-CA) and 1.85×10^6 (BYDV-PAV-IL). No RNA was isolated from BWYV-CA-TC.

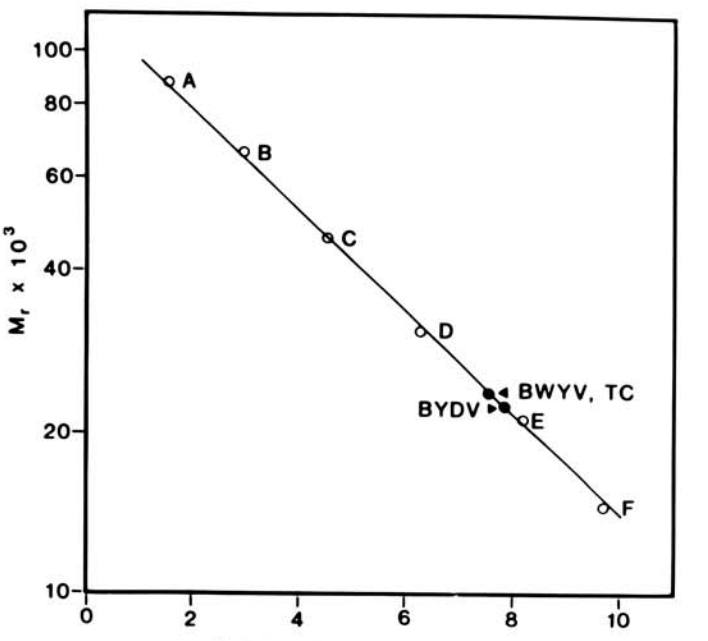

Fig. 7. Plot of the log of the relative molecular masses of BWYV-CA-TC, BWYV-CA, and BYDV-PAV-IL and marker coat protein subunits. Markers and their M_r were: phosphorylase B, 92,500 (A); bovine serum albumin, 66,200 (B); ovalbumin, 45,000 (C); carbonic anhydrase, 31,000 (D); soybean trypsin inhibitor, 21,500 (E); lysozyme, 14,400 (F). Calculated protein subunit M_r were: BWYV-CA-TC and BWYV-CA (24,000) and BYDV-PAV-IL (22,500).

luteoviruses. However, certain physiochemical properties of group members vary considerably and these differences may be significant. Reported sedimentation coefficients for the group range from 127 for potato leafroll virus (PLRV) (29) to 104 for carrot redleaf virus (CRLV) (32). Sedimentation coefficients for different isolates of the same virus also may vary. Three values for PLRV have been reported, 127 S (29), 115 S (31), and 112 S (32). Isolates of the yellowing and dwarfing strains of soybean dwarf virus have sedimentation coefficients of 114 and 108, respectively (18). Similarly, sedimentation coefficients of BYDV isolates range from 120 (24) to 106 for the BYDV-PAV-IL isolate from Illinois with intermediate values of 115–118 S (27) and 115 S (17). The only reported sedimentation coefficient for BWYV is 114 reported here, placing this isolate in the middle of the group range.

The BYDV-PAV-IL absorbance ratio ($A_{260/280nm}$) is high (1.90) and, where this property is known, typical of most luteoviruses (1,4,23,31). In contrast, BWYV-CA $A_{260/280nm}$) is 1.65 and like CRLV (32), and a PAV-like isolate of BYDV (24) is low compared with other members of the group. This figure may reflect the composition of the protein subunits, the relative quantities of protein and nucleic acid that compose the virus particle or it may reflect a value distorted by the presence of top component in the virion fraction. Like BWYV-CA, the BYDV isolate characterized by Proll et al (24) has a top component. Electron micrographs of purified BWYV-CA virions indicate that complete separation of the two particle types has not been accomplished in sucrose gradients. Isopycnic centrifugation effects good separation but both BWYV-CA particle types and BYDV-PAV-IL appear to be less stable in cesium salts than in sucrose.

Electron microscope examination of two other BWYV isolates, one in situ (11) and the other after purification (7), has revealed the presence of two populations of similar-sized virus-like particles. Although different staining procedures were used, both studies show that one population of particles does not absorb stain, whereas the second population clearly does. Two populations of particles have been observed in an early in situ electron microscope study of BYDV-infected barley (21). Also, Proll et al (24) found that the top component of their BYDV isolate absorbed stain, whereas the bottom component did not.

The presence of a noninfectious top component devoid of nucleic acid has not been reported for any luteovirus until recently (19,24). Initial attempts to purify BWYV-CA did not reveal the presence of the top component because yields were too low for detection or it was masked by contaminants in the upper portion of the gradient. If top components are associated with other luteoviruses, these same difficulties might explain why they have escaped detection by others. Other luteoviruses may produce top components that are not sufficiently stable to withstand the purification procedure or the presence of a top component may typify some or only a few isolates in the luteovirus group.

The presence or absence of a top component represents fundamental differences in the type and strength of protein-protein and protein-nucleic acid interactions that provide stability to the virion (2,3). Whether these differences are important in classifying the luteoviruses cannot be resolved until more members are purified in sufficient quantity to be characterized physiochemically. The group, however, may be more variable than early characterization studies suggest. Recently, a BWYV isolate with a satellite RNA (12) was described. BWYV and BYDV each have at least one isolate with a top component, a characteristic that, most likely, will be observed in other luteoviruses. In the future, the concept of the physiochemical properties of the group may have to be expanded to include the presence of an associated satellite or top component.

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