Further Examination of the RNA and Coat Protein of Spring Beauty Latent Virus

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Approved for publication by the director of the Louisiana Agricultural Experiment Station as manuscript no. 90-38-4390.
Accepted for publication 1 November 1990 (submitted for electronic processing).

ABSTRACT

Valverde, R. A., and Glascock, C. B. 1991. Further examination of the RNA and coat protein of spring beauty latent virus. Phytopathology 81:401-404.

Spring beauty latent virus (SBLV) has been proposed as a new member of the bromovirus group. Four dsRNAs (M_r 2.2, 2.0, 1.4, and 0.5 $\times$ 10^6) with a profile similar to those of the bromoviruses were obtained when plant tissue infected with SBLV was analyzed. A fifth dsRNA (M_r 1.2 $\times$ 10^6) with nucleic acid similarity to the genomic RNA also was obtained. Molecular hybridization with cDNA to SBLV RNA did not show nucleic acid similarity between SBLV and four bromoviruses. Infectivity tests with SBLV ssRNAs indicated that it has a tripartite genome because RNAs 1, 2, and 3 were required for infectivity. When RNAs 1, 2, 3, and 4 were translated, 95, 48, and 23 kDa proteins were obtained. Translation of RNA 4 yielded only a 23 kDa protein that comigrated and was serologically related to SBLV and brome mosaic virus coat proteins. Western blot analysis revealed relationships among SBLV coat proteins and those of four bromoviruses. Results presented here provide further information of the properties of SBLV RNA and coat protein as well as evidence that supports placing SBLV in the bromovirus group.

Bromoviruses have been extensively investigated as models for plant virus genetic studies. Members of the bromovirus group include brome mosaic virus (BMV), cowpea chlorotic mottle virus (CCMV), and broad bean mottle virus (BBMV) (10). Melandrium yellow fleck virus (MYFV) is a putative member of this group (6). Cassia yellow blotch virus (CYBV) (2) and spring beauty latent virus (SBLV) (17) were proposed as members of the bromovirus group based on similar physicochemical properties.

SBLV is an isometric virus found to infect spring beauty (*Claytonia virginica* L.) in Arkansas (17). When infected, this perennial plant is symptomless, and virus is best isolated from flowers. Little is known about the natural transmission of this virus, although mechanical transmission is probably the main way for dissemination (17). SBLV is serologically related to BMV and CCMV (17). Other properties, such as number and molecular weight of the RNAs and sedimentation coefficients, are similar to those of other bromoviruses. Therefore, it has been proposed as a new member of the bromovirus group (17).

The purpose of this investigation was to partially characterize the RNA and protein coat of SBLV, to compare them with those of four other bromoviruses, and to determine if these properties support placing SBLV in the bromovirus group.

MATERIALS AND METHODS

Virus isolates and purification. The type isolate of SBLV (ATCC, PV-369) originally isolated from spring beauty in Arkansas was used throughout this study. Other viruses used for comparison were: BMV-type (ATCC, PV-47), BMV-1 (16), CCMV (ATCC, PV-299), BBMV (ATCC, PV-111), and MYFV (supplied by A. A. Brunt). SBLV was propagated in *Gomphrena globosa* L. and purified by using a modification of Steere's chloroform-butanol method as described previously (17). BMV was propagated in *Triticum aestivum* L. 'McNair 1003', CCMV in *Phaseolus vulgaris* L. 'Pinto', BBMV and MYFV in *Pisum sativum* L. 'Little Marvel'. Virus purification was done by using published methods for each one of these viruses (6,9,15).

Purification and electrophoretic analysis of viral-associated RNA. Viral RNA (ssRNA) from all of the viruses was isolated by using the sodium-perchlorate method (20). The same plant species used for virus purifications were also used for dsRNA analysis. dsRNAs were extracted from 7.0 g of infected plant tissue by two cycles of fractionation on columns of cellulose powder (Whatman CF-11) as described by Morris and Dodds (12). Simultaneously, 7.0 g of healthy tissue from each plant species was similarly processed. Aliquots of 30 μl (approximately 0.5 μg) were loaded on 6% polyacrylamide gels (40:1, acrylamide/bisacrylamide) in a vertical slab gel apparatus. Electrophoresis was at a constant voltage of 100 V for 3 hr. Gels were stained with 20 ng/ml of ethidium bromide. Molecular weight markers were dsRNAs from tobacco mosaic virus (M_r 4.3 and 0.4 $\times$ 10^6) and tobacco necrosis virus (M_r 2.3, 0.8, and 0.6 $\times$ 10^6).

Infectivity tests of the ssRNAs. Purified SBLV ssRNA was run in 1.2% agarose gels in TAE buffer (0.04 M Tris, 0.02 M sodium acetate, 0.001 M EDTA). Gels were stained briefly with ethidium bromide (20 ng/ml), placed on a UV transilluminator, and ssRNA bands were excised and electroeluted in one-fifth the strength of TAE buffer. RNAs 1 and 2 were eluted together and RNAs 3 and 4 eluted individually. Eluted ssRNA was recovered in the solution and ethanol was precipitated. Aliquots (1-ml) of each RNA preparation in 1 mM EDTA, pH 7.4, and 0.1% celite were mechanically inoculated alone and in different combinations (each RNA at a final concentration of approximately 0.1 mg/ml) to six half-leaves of *Chenopodium amaranticolor* Coste and Reynier. EDTA alone was used as the control. Successful infections of SBLV RNA were determined by recording the number of necrotic local lesions on half-leaves.

Molecular hybridization. Aliquots of approximately 1.0 μg (in double-distilled H_2O) of ssRNA from all five viruses were denatured by heating at 60 C for 15 min in 50% formamide and spotted on nitrocellulose membranes for molecular hybridization with a dot-blot apparatus. CDNA from SBLV ssRNA (total RNA) was transcribed with reverse transcriptase (Amersham, Arlington Heights, IL) in the presence of [^{32}P]-dCTP as described by Maniatis et al (11). Hybridizations were conducted as described previously (18). Polyacrylamide gels containing dsRNAs from plants infected with SBLV were denatured and electroblotted to nylon membranes, as described (18). To determine if these dsRNAs had nucleic acid similarity with SBLV genomic RNA, molecular hybridization was done by using the cDNA probe to SBLV ssRNA, as described.

In vitro translation of SBLV RNA. In vitro translations were done by using a rabbit reticulocyte lysate translation kit (Promega

Corporation, Madison, WI). Reactions were obtained according to the supplier's instructions. Proteins were separated by polyacrylamide-gel electrophoresis (8), followed by fluorography. RNAs used in the translation experiments included SBLV RNAs 1, 2, 3, and 4 (combined), SBLV RNA 4, SBLV RNAs 4 and 3, and BMV RNAs as the positive control.

Immunoprecipitation. For immunoprecipitation experiments, metallic beads coated with goat-anti-rabbit IgG (Advanced Magnetics, Cambridge, MA) were used. One milliliter of bead suspension was added to 40 μl of SBLV polyclonal antiserum (undiluted), mixed, and stored at room temperature for 30 min. The beads were then collected by centrifugation (10,000 *g*) for 1 min. The supernatant was aspirated and beads resuspended in 250 μl of phosphate-buffered saline (PBS). Twenty-five microliters of bead suspension was added to the in vitro translation products. Samples were incubated for 30 min at room temperature and then centrifuged at 10,000 *g* for 1 min. Pellets were washed twice with 100 μl of PBS and electrophoresed in polyacrylamide gel as described.

Protein analysis and western blots. Purified virus preparations were dissociated according to Laemmli (8). Samples (approximately 5–10 μg) were loaded on a sodium dodecyl sulfate polyacrylamide gel. Electrophoresis was done in a vertical gel apparatus (9 cm × 8 cm × 1.5 mm) at 100 V for 3 hr. Western blots were done as described by Falk and Elliot (3). Antiserum to SBLV was the same as used previously (17). Other antisera used were provided as follows: BMV, CCMV, and BBMV by H. A. Scott; and MYFV by A. A. Brunt. Titer of all five antisera were adjusted to 1:512 (as determined by a tube-precipitin test). Antisera solutions (1:500 dilutions in PBST [phosphate-buffered saline, 0.05% Tween 20]) were added to the blots and incubated with shaking for 1.5 hr.

RESULTS

RNA infectivity. The results of the infectivity test are shown in Table 1. When RNAs 1–4 were mechanically inoculated to *C. amaranticolor*, necrotic local lesions were observed. Similar results were obtained when RNAs 1, 2, and 3 were inoculated. With the exception of a few occasional local lesions, lesions were not observed when any other combination of RNAs or EDTA alone were inoculated. Pure preparations of RNA 1 or 2 could not be obtained because of the similar mobility of the bands in the gels.

dsRNA analysis. All bromoviruses had at least four dsRNA bands (Fig. 1). In some cases, such as with SBLV and MYFV other less intense dsRNA bands were also present. The molecular weights of SBLV dsRNAs were: 2.2, 2.0, 1.4, and 0.5 × 10^6 for dsRNAs 1, 2, 3, and 4, respectively. A fifth dsRNA, of about M_r 1.2 × 10^6 was consistently obtained from SBLV-infected tissue. However, a corresponding ssRNA was not detected. Bromoviruses could be differentiated by the relative mobility of the dsRNA bands. MYFV had a distinct pattern that consisted of six dsRNA bands. dsRNA was not obtained when uninoculated plants were analyzed.

Molecular hybridization. Under high stringency conditions, cDNA to SBLV hybridized with its homologous ssRNA, but not to ssRNA from BMV, CCMV, BBMV, or MYFV.

In vitro translation of SBLV RNA. Translation products directed by SBLV RNAs are shown in Figure 2. Translation of RNA 4 yielded a 23 kDa protein that comigrated with the coat protein. Other proteins (95 and 48 kDa) were also obtained when RNAs 1, 2, and 3 were co-translated. Two proteins (48 and 23 kDa) were obtained when RNAs 4 and 3 were translated. Anti-

TABLE 1. Infectivity of spring beauty latent virus ssRNA components

Mixture of ssRNA components[a]	Lesions per half-leaf of *Chenopodium amaranticolor*[b]
1 + 2	1.5
1 + 2 + 3	58.0
1 + 2 + 4	2.0
1 + 2 + 3 + 4	49.5
3 + 4	0
3	0
4	0
EDTA	0

[a] The ssRNA components (approximately 0.1 mg/ml of the respective ssRNA) were mechanically inoculated to half-leaves of *C. amaranticolor*.
[b] Average number of lesions on six random half-leaves.

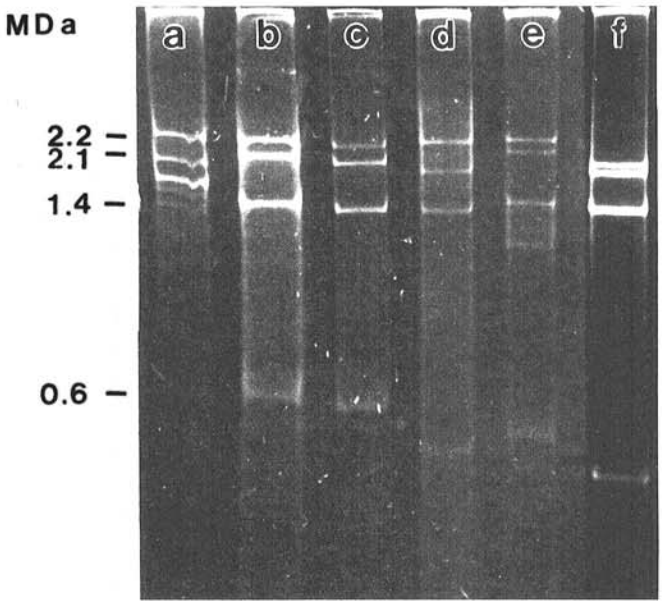

Fig. 1. Polyacrylamide-gel electrophoresis (6%) of dsRNAs extracted from different plant species infected with five bromoviruses. Lane **A,** dsRNA from *Pisum sativum* 'Little Marvel' infected with melandrium yellow fleck virus; lanes **B** and **C,** *Triticum aestivum* 'McNair 1003' infected with brome mosaic virus-1 and brome mosaic virus-type, respectively; lane **D,** *Phaseolus vulgaris* 'Pinto' infected with cowpea chlorotic mottle virus; lane **E,** *Gomphrena globosa* infected with spring beauty latent virus; and lane **F,** *P. sativum* 'Little Marvel' infected with broad bean mottle virus.

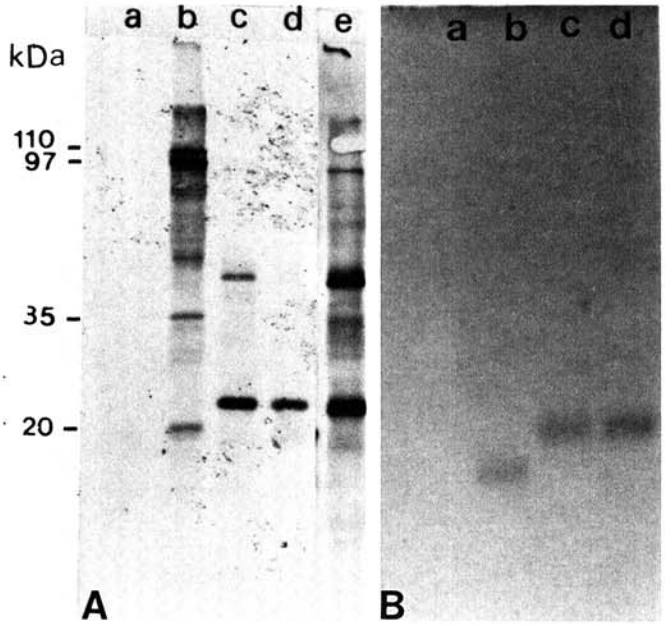

Fig. 2. Polyacrylamide-gel electrophoresis of protein products synthesized in rabbit reticulocyte lysates. **A,** Lane A, no RNA added; lane B, brome mosaic virus RNA; lane C, spring beauty latent virus (SBLV) RNA 3 and 4; lane D, SBLV RNA 4; and lane E, SBLV RNAs 1, 2, 3, and 4. **B,** Immunoprecipitation of in vitro translation products in lanes A–D with SBLV-specific antiserum.

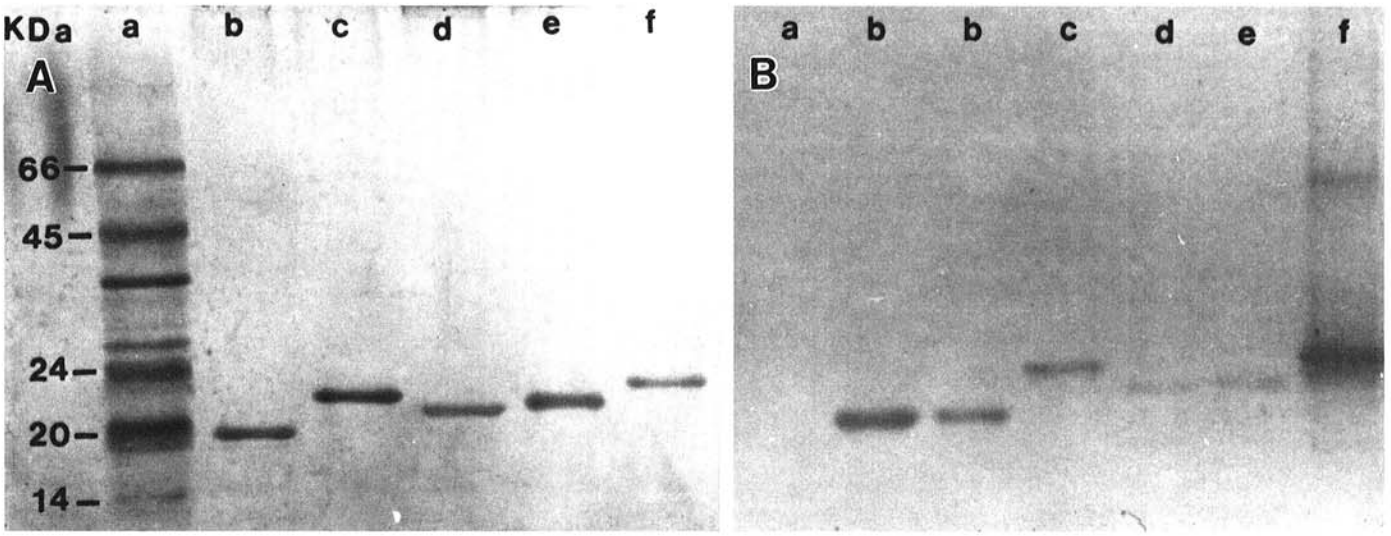

Fig. 3. A, Silver-stained sodium dodecyl sulfate polyacrylamide gel (12%) of the coat proteins from five bromoviruses. Lane A, molecular weight standards in order of decreasing mass: bovine serum albumin, egg albumin, glyceraldehyde-3-phosphate dehydrogenase, carbonic anhydrase, trypsinogen, trypsin inhibitor, and lactalbumin; lane B, brome mosaic virus-type (BMV-type); lane C, cowpea chlorotic mottle virus; lane D, broad bean mottle virus; lane E, melandrium yellow fleck virus; and lane F, spring beauty latent virus (SBLV). **B,** Western blot of a similar gel electroblotted and probed with antiserum to SBLV. Letters on blot lanes correspond to those of the silver-stained gel. Electrophoresis was conducted at 100 V for 3 hr. Protein loads ranged between 5 and 10 μg.

serum to SBLV precipitated the 23 kDa protein product of RNA 4 and the BMV capsid protein (Fig. 2).

Western blot analysis. Results of western blot analysis using SBLV antiserum are shown in Figure 3. Polyclonal antisera to each bromovirus cross-reacted with heterologous antigens. Different degrees of intensity, perhaps depending on the serological relatedness, were obtained among the different bromoviruses (results not shown). Apparent coat protein sizes of the different bromoviruses were variable; SBLV had the coat protein with the least mobility.

DISCUSSION

The serological relatedness among BMV, CCMV, and BBMV is well established (13–15). Serological relationships among SBLV, BMV, and CCMV have been reported (13,15,17). However, earlier attempts to find relationships among SBLV, BBMV, and MYFV were unsuccessful (17). Similarly, other authors have failed to detect serological relationships between MYFV or CYBV and BMV, CCMV, or BBMV (2,6). This was probably because of the low sensitivity of the tests used. Results obtained in this investigation revealed extensive serological relationships among the different bromoviruses by western blot analysis. The serological relationship of SBLV and MYFV with other bromoviruses is strong evidence for regarding all of these viruses as related.

In vitro translation products of SBLV RNAs were similar to those reported for other bromoviruses (10). The results obtained here indicate that RNA 4 codes for the 23 kDa coat protein, RNA 3 for a 48 kDa protein, and RNAs 1 and/or 2 for a 95 kDa protein. Whether or not the 95 kDa protein is a single product or two distinct products of similar molecular weight needs to be determined by translating RNAs 1 and 2 individually.

SBLV has a tripartite genome, RNAs 1, 2, and 3 were required for infectivity. Furthermore, translation of RNA 4 indicated that it encodes the coat protein gene. These properties are shared by the bromoviruses and cucumoviruses.

Patterns of dsRNA in the bromoviruses were distinct. These patterns could be used to diagnose bromoviruses. Two strains of BMV showed only a slight difference in the mobility of the dsRNA bands. Although a fifth dsRNA was detected in plant extracts infected with SBLV, a corresponding ssRNA was not detected in ssRNA preparations from purified virus. This fifth dsRNA hybridized with a probe to virus ssRNA. Therefore, this dsRNA could be either a fragment of the replicative forms of SBLV RNA or a replicative form of a subgenomic ssRNA. Haber and Hamilton (5) found a similar fifth dsRNA for a strain of BMV isolated in Canada, but were unable to detect the corresponding ssRNA form.

In spite of the serological relationship between SBLV and all bromoviruses tested, SBLV did not have detectable nucleic acid similarities after hybridization under high stringency. This, together with the serological data, provides strong evidence that supports placing SBLV as a distinct member of the bromovirus group.

The bromoviruses and cucumoviruses have often been used for genetic studies of plant viruses (19). The bromoviruses share many physical and chemical properties with the cucumoviruses (1,4,7), among them, number and size of RNA components and similar 3′ terminal nucleotides for the four RNA species. It has been suggested that these two groups may be related by divergent evolution from a common ancestor (7). SBLV is an unusual bromovirus; its host range includes six families of dicots and one monocot (17). Furthermore, SBLV like MYFV is very stable at neutral pH (6,17). All other bromoviruses are stable at low pH and unstable at high pH (4,7,10). Some properties of SBLV, such as stability at neutral pH, size of the coat protein, dsRNA pattern, and wide host range are similar to those of members of the cucumovirus group. Other properties, such as serology, high stability, and sedimentation coefficient are typical of the bromovirus group. Comparisons of the RNA sequences of SBLV with that of other bromoviruses and cucumoviruses may help to better understand the relationship between these two groups and the unique properties of this virus.

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