Properties of Soybean Mosaic Virus Ribonucleic Acid

J. H. Hill and H. I. Benner

Department of Plant Pathology, Seed, and Weed Science, Iowa State University, Ames, 50011.
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ABSTRACT

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Infectious single-stranded RNA isolated from purified soybean mosaic virus has an S value of 39.7 before, and 25.4 after, formaldehyde denaturation. Nucleic acid molecular-weight determinations made by linear log sucrose density gradient centrifugation gave values of 3.46×10^6 and 3.18×10^6, respectively, for native and formaldehyde-treated RNA. Electrophoresis on polyacrylamide gels gave molecular weight values of 3.02×10^6 both before and after formaldehyde denaturation. No evidence for virion-associated low molecular weight RNAs was obtained. Thermal denaturation profiles showed at T_m of 49 C in 0.1 M sodium phosphate, pH 7.0, and a hyperchromicity of 30.4%. Nucleotide composition was 29.9% adenylic acid, 24.3% guanylic acid, 14.9% cytidylic acid, and 30.9% uridylic acid. The RNA is 5.3% of the virus particle.

Additional key word: potyvirus.

Soybean mosaic virus (SMV), a virus with properties characteristic of the potato Y virus group (6,13) causes a common disease of soybeans. Considerable information is available on the disease and reduction in seed quality caused by the virus (18,26), but there is little information on the biochemical properties of the virus particle (13). Because nucleic acid analyses provide some of the most important information for plant virus classification, we report properties of the nucleic acid isolated from SMV.

MATERIALS AND METHODS

Virus purification. The viruses used in this study, SMV (Ia 75-16-1), bromegrass mosaic virus (BMV) (ATCC-PV180), and tobacco mosaic virus (TMV) (common strain), were purified as described by Hill and Benner (13), Bockstahler and Kaesberg (2,3), and Whitfeld and Williams (31), respectively.

Isolation of viral nucleic acids. Viral RNAs of TMV and BMV were prepared by phenol disruption of the virions as described (12). SMV RNA was obtained by the ammonium carbonate method of Brakke and Van Pelt (8) as described by Hill and Benner (12), the two-phase phenol procedure as used for TMV and BMV (12), or disruption of the virus by guanidine hydrochloride in the presence of lithium chloride (15). All RNA preparations in appropriate solvents were used immediately or stored at −20 C until use. RNA concentrations were determined spectrophotometrically by using an extinction coefficient of 25 $cm^2\ mg^{-1}$ at 260 nm.

Analytical techniques. Orcinol tests for the presence of RNA were made as described by Shatkin (27), and diphenylamine tests for DNA were as described by Burton (9).

Phosphorous content of the purified virus particle was determined by using the procedure of Nakamura (23). Virus solutions were dialyzed exhaustively against glass-distilled water, lyophilized, placed in a vacuum oven at 100 C for 72 hr, cooled, and stored in a vacuum desiccator over phosphorous pentoxide Weighed amounts were dissolved in a measured amount of 60% (v/v) perchloric acid, and portions were taken for analysis.

Nucleotide composition of SMV RNA was determined by hydrolyzing RNA, prepared by guanidine hycrochloride-lithium chloride degradation of the virus, in 0.4 N NaOH for 24 hr at 37 C. The nucleotides were separated on ammonium sulphate-impregnated Whatman No. 1 paper by descending chromatography in 76% ethanol (20). The separated nucleotides were detected under a UV lamp, eluted in 0.01 N HCl, and concentrations were determined with extinction coefficients (19).

Rate zonal density gradient experiments to estimate sedimentation coefficients and the molecular weight of SMV RNA were performed in linear log gradients (7) as described by Hill et al (14). BMV and TMV RNAs were used as standards. Results were calculated as previously described (14).

SMV RNA was examined by using previous methods (12) in polyacrylamide gels containing 0.5% agarose. Gel concentrations

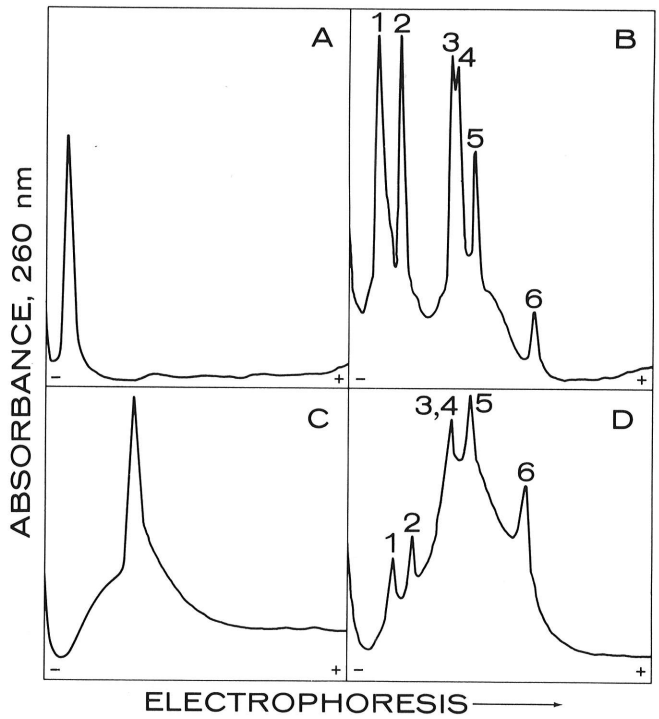

Fig. 1. Absorbance scans of polyacrylamide gels containing 0.5% agarose. Electrophoresis of **A,** SMV RNA and **B,** SMV RNA (peak 1) with standard RNAs of TMV (peak 2), BMV_1 (peak 3), BMV_2 (peak 4), BMV_3 (peak 5), and BMV_4 (peak 6) in 2.4% polyacrylamide gels. Electrophoresis of **C,** formylated SMV RNA, and **D,** formlyated SMV RNA (peak 1) with formalin-denatured standard RNAs of TMV (peak 2), BMV_1 (peak 3), BMV_2 (peak 4), BMV_3 (peak 5), and BMV_4 (peak 6) in 2.0% gels. Gels in (C) and (D) contained 1.1 M formaldehyde. Electrophoresis was for 6 hr at 5 mA/gel.

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generally were 2.4% polyacrylamide containing 0.12% bisacrylamide except when lowered to 2.0% to facilitate entry of RNA molecules denatured by formylation. Gel concentrations of 5.0, 7.5, and 10.0% without incorporation of agarose were used to detect possible low-molecular-weight RNAs that might be associated with the virion (1,11).

Because RNA molecular weights are most accurately determined when hydrogen bonding within the molecule and between RNA subunits is prevented (4,5) and the integrity of an RNA molecule can be determined only after denaturation (12), SMV RNA preparations were denatured with formaldehyde (12). Examination of formylated RNA by polyacrylamide gel electrophoresis and density gradient centrifugation in linear log gradients has been described (12).

Thermal denaturation of SMV RNA isolated by the ammonium carbonate method was done by using a Gilford Model 2527 thermoprogrammer with a programmed temperature gradient of 1 C/min. SMV RNA preparations in 0.1 M sodium phosphate, pH 7.0, were examined in the native configuration as well as after denaturation by formaldehyde (12).

To study the infectivity of SMV RNA after isolation by the ammonium carbonate procedure, RNA was divided into four equal parts and precipitated by addition of 1.5 volumes of cold isopropanol and sufficient 3.0 M sodium acetate, pH 5.0, to yield a final concentration of 0.1 M. After centrifugation of the four samples, they were resuspended in 1 × SSC (0.15 M NaCl, 0.015 M sodium citrate), pH 7.0; 1 × SSC, pH 7.7; 0.1 M sodium phosphate, pH 7.0; or 0.1 M sodium phosphate, pH 7.7. Concentrations of RNA were determined spectrophotometrically and adjusted to equivalent OD_{260nm} values by addition of the appropriate buffer containing sufficient Celite to yield a final concentration of 10 mg/ml. Infectivity was determined by applying four drops of nucleic acid solution to a detached half-leaf of the local lesion host *Phaseolus vulgaris* 'Top Crop' (22) previously dusted with 22-μm (600-mesh) Carborundum and rubbing the solution into the leaf with the flat end of an autoclaved vial. The other half of the

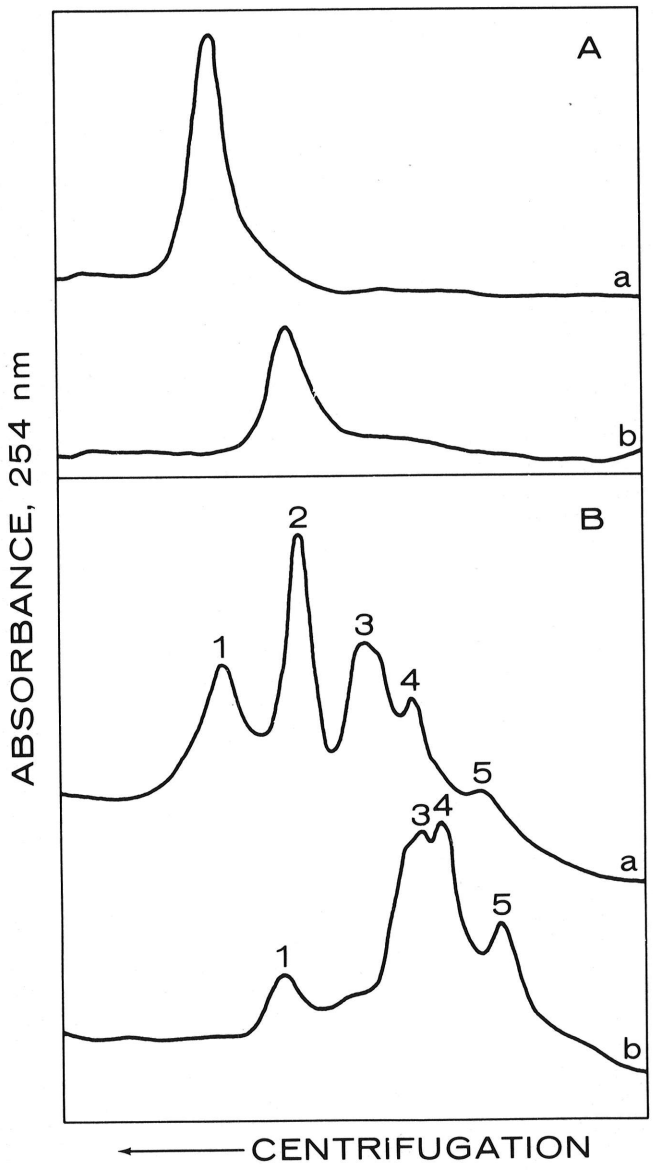

Fig. 2. Ultraviolet absorbance profiles of viral RNA species centrifuged in linear log sucrose density gradients in 1 × SSC, pH 7.0 Centrifugation was at 14 C and 25,000 rpm for 15 hr in a Beckman SW27 rotor. RNAs are **A**, SMV and **B**, SMV (peak 1), TMV (peak 2), $BMV_{1 \text{ and } 2}$ (peak 3), BMV_3 (peak 4), and BMV_4 (peak 5). RNAs are (a) native and (b) formaldehyde denatured. Migration distances of RNA species from various runs are not absolutely comparable.

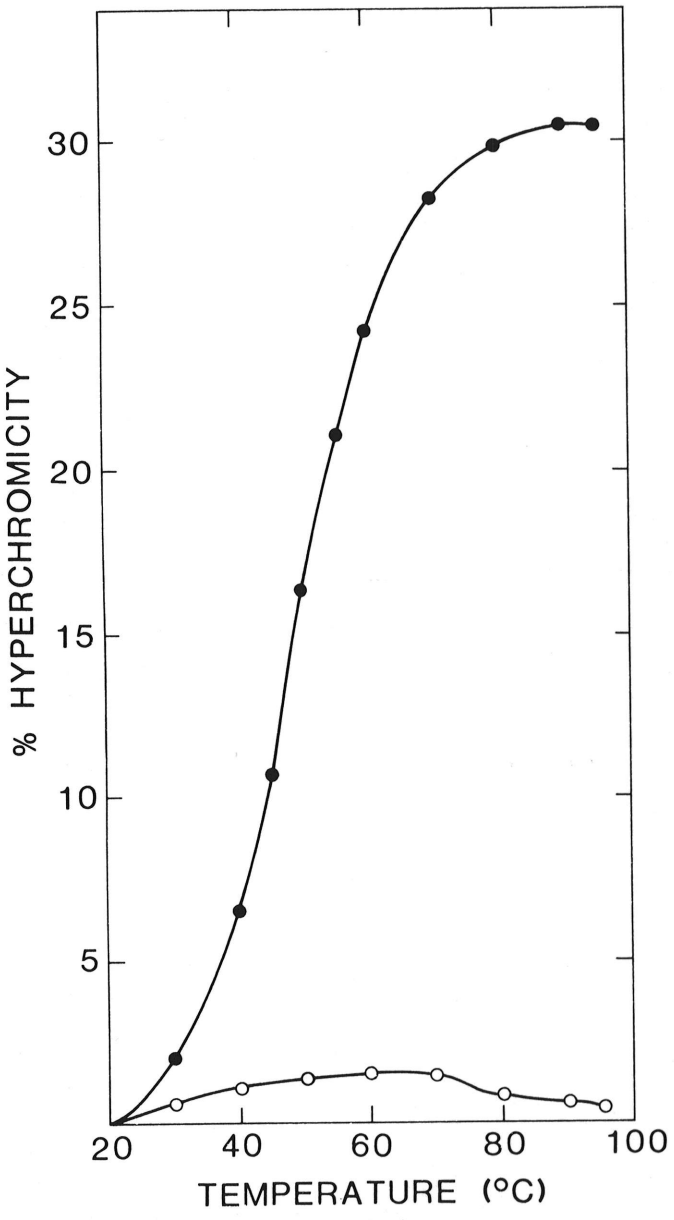

Fig. 3. Thermal denaturation profiles of SMV RNA (•—• and formalin-denatured SMV RNA (o—o) in 0.1 M sodium phosphate, pH 7.0. Data were corrected for thermal expansion of the solvent by using a hypoxanthine solution with the same extinction as the sample. Curves were derived from the mean values of 12 experiments using native RNA and 10 experiments using formalin-denatured RNA employing two different RNA preparations.

detached leaf was inoculated with a standard consisting of a semipurified SMV prepartion in 0.05 M sodium phosphate, pH 7.0. Detached leaves were placed in petri dishes lined with moist filter paper at 30 C under low light intensity for 48 hr, after which local lesions were counted.

RESULTS

Properties of SMV nucleic acid. Nucleic acid in SMV reacted positively with orcinol and negatively with diphenylamine indicating that the nucleic acid in SMV is RNA. Nucleotide analyses of alkaline-hydrolyzed RNA (eight trials) gave mean and standard deviation values of 29.9 ± 3.4% adenylic acid, 24.3 ± 3.3% guanylic acid, 14.9 ± 2.9% cytidylic acid, and 30.9 ± 3.6% uridylic acid. Only R_f values corresponding to those of authentic samples of the four nucleotides were detected. Phosphorus analyses of three different virus preparations gave a mean value and standard deviation of 0.49 ± 0.03% phosphorus by weight. From these data, the RNA content of the virus was calculated at 5.32 ± 0.33%.

TABLE 1. Half-leaf infectivity assay of soybean mosaic virus RNA isolated by ammonium carbonate degradation in four buffers

Buffer	Mean no. of local lesions[a]		Percent of Standard
	Standard[b]	Treatment[c]	
0.1 M $NaPO_4$, pH 7.7	35.6	21.6	60.7
1 × SSC[d], pH 7.7	31.1	14.2	45.7
1 × SSC, pH 7.0	44.6	11.7	26.2
0.1 M $NaPO_4$, pH 7.0	52.4	12.9	24.6

[a] Mean number of local lesions on 17 half-leaves of *Phaseolus vulgaris* 'Top Crop' from three different experiments.
[b] A preparation of semipurified soybean mosaic virus in 0.05 M $NaPO_4$, pH 7.0, was applied to each half-leaf.
[c] Four drops of a solution of soybean mosaic virus RNA (0.04 mg/ml) in the indicated buffer was applied to each half-leaf.
[d] 1 × SSC = 0.15 M NaCl, 0.015 M sodium citrate.

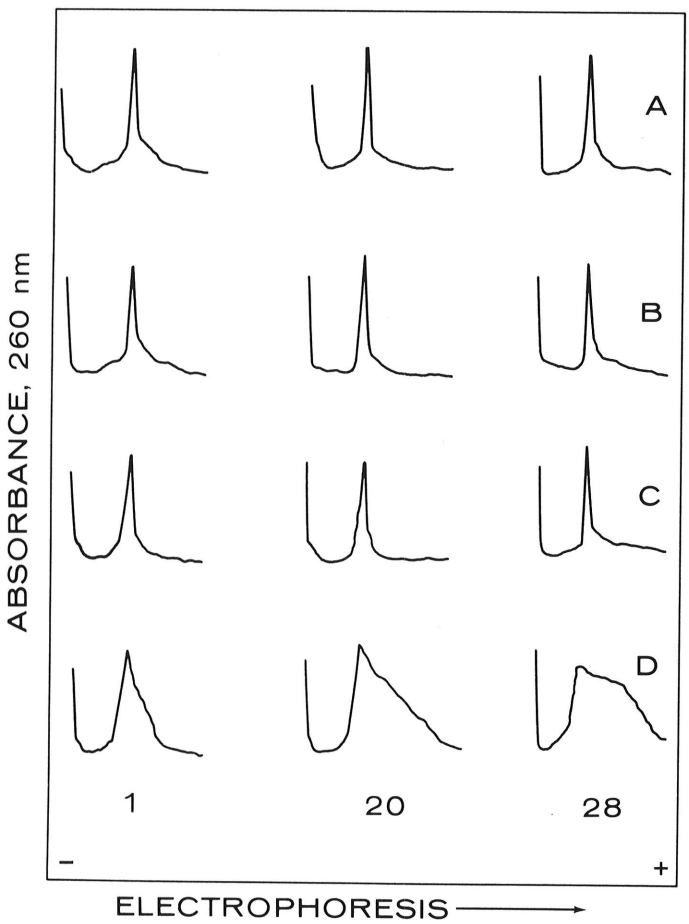

Fig. 4. Absorbance scans of formylated SMV RNA in 2.0% polyacrylamide gels containing 1.1 M formaldehyde and 0.5% agarose. Samples in row **A**, 1 × SSC (0.15 M NaCl, 0.015 M sodium citrate), pH 7.0; row **B**, 1 × SSC, pH 7.7; row **C**, 0.1 M sodium phosphate, pH 7.0; and row **D**, 0.1 M sodium phosphate, pH 7.7, were electrophoresed for 6 hr at 5 mA/gel at 1, 20, and 28 days (columns) after preparation.

Determination of the molecular weight of SMV RNA. The RNA of SMV isolated by ammonium carbonate virus degradation appeared as a single well-defined homogeneous peak both before and after formaldehyde modification when analyzed on polyacrylamide gels, which were scanned at 260 nm (Fig. 1A, 1C). The RNA molecular weight determined by coelectrophoresis with standard RNAs (Fig. 1 B,D) was 3.02 ± 0.09 × 10^6 before formylation and 3.02 ± 0.28 × 10^6 (mean and standard deviation from results of 10 and 17 experiments, respectively) after formaldehyde modification.

Sedimentation experiments with SMV RNA showed a single sedimenting zone before and after formylation of the RNA (Fig. 2A). Mean sedimentation coefficients obtained by sedimenting marker RNA species in the same gradient with SMV RNA (Fig. 2B) were 39.7 ± 0.6 and 25.4 ± 0.6 S (mean and standard deviation from results of 10 and 21 experiments, respectively) at 14 C in sucrose in 1 × SSC, pH 7.0, corrected to the viscosity and density of water at 20 C before and after formylation of the RNA, respectively. With use of the formula derived by Hull et al (17), a molecular weight of 3.18 × 10^6 was calculated from the S value of nonformylated RNA. Similarly, a molecular weight of 3.47 × 10^6 was calculated from sedimentation experiments with formylated RNA by using the equation of Brakke and Van Pelt (8).

Because the ammonium carbonate method of RNA isolation involved collection of the monodisperse sedimenting RNA zone from a density tube by tube fractionation, a remote possibility existed that potential small amounts of low-molecular-weight RNAs associated with the virion (1,11), which may not be detected in the density gradients, could be excluded from our analyses of the viral RNA. Therefore, we isolated RNA from SMV by using two-phase phenol degradation of the virus. Although yields were less than those obtained by the ammonium carbonate procedure, migration of the RNA obtained was indistinguishable from that isolated by the ammonium carbonate procedure when analyzed in 2.4% gels or when formylated and examined in 2.0% gels containing formaldehyde. No smaller-molecular-weight components were observed. When the RNA preparations were further analyzed on 5.0, 7.5, and 10.0% gels, the viral RNA remained at the top, and no low-molecular-weight components were evident.

Configuration of SMV RNA. The calculated reduction of 36.0% in S value of SMV RNA after formaldehyde modification reflects the degree of secondary structure in the molecule. Similarly, the reduction of 22.2 ± 3.6% (mean and standard deviation from results of 11 experiments) in electrophoretic mobility obtained in coelectrophoresis experiments of nonformylated and formylated SMV RNA in 2.0% gels containing formaldehyde also demonstrates the secondary structure of the molecule.

The melting behavior of SMV RNA was characteristic of single-stranded RNA having some secondary structure (Fig. 3). The T_m of the molecule was 49 C in 0.1 M sodium phosphate, pH 7.0, and hyperchromicity was 30.4%. Assuming 50% hyperchromicity for a completely base-paired polynucleotide (10), the measurements suggest that 60% of the bases of SMV RNA are involved in hydrogen bonding in this buffer system.

Because the reliability of RNA molucular-weight determinations are contingent upon demonstration that the secondary structure of RNAs utilized in an analytical system is virtually eliminated, the melting behavior of SMV RNA was examined after reaction with formaldehyde. Data indicated that residual hyperchromicity was below the 5% that may be associated with single-stranded base stacking (Fig. 3) (5).

Stability and infectivity of SMV RNA in different buffers. The RNA of tobacco etch virus, another member of the potato Y group of plant viruses, has been shown to have various degrees of stability

when stored at −20 C in 0.1 M sodium phosphate, pH 7.0 or pH 7.7; or 1 × SSC, pH 7.0 or pH 7.7. Greatest stability occurred in 1 × SSC, pH 7.0, with least stability in 0.1 M sodium phosphate, pH 7.7 (12). SMV RNA obtained by ammonium carbonate degradation was resuspended in 1 × SSC, pH 7.0 or pH 7.7; or 0.1 M sodium phosphate, pH 7.0 or pH 7.7 after cold isopropanol precipitation from sucrose gradients. The RNA was stored at −20 C. Preceding analysis at 1, 20, and 28 days after isolation on 2.0% polyacrylamide gels containing 0.5% agarose and formaldehyde, the preparations were reacted with formaldehyde. Results indicated that the RNA remained stable over the test period in 1 × SSC, pH 7.0 or pH 7.7; and 0.1 M sodium phosphate, pH 7.0. Preparations in 0.1 M sodium phosphate, pH 7.7, however, showed some evidence of polydispersity 1 day after isolation, with increasing degradation continuing over the 28-day test period (Fig. 4).

SMV RNA preparations in the above buffers also were tested for infectivity immediately after isolation by using a half-leaf assay on the local lesion host *P. vulgaris* 'Top Crop.' The greatest apparent infectivity occurred with RNA in 0.1 M sodium phosphate, pH 7.7 (Table 1) for which some polydispersity was evident in gel tests. RNA preparations in all buffers were infectious, however.

DISCUSSION

The values obtained for the molecular weight of SMV RNA by sedimentation and gel electrophoresis analyses are in good agreement with those reported for other members of the potyvirus group (12,16,21,24,25). The variation between molecular weights of formylated RNAs obtained by sedimentation analysis (3.47×10^6) and gel electrophoresis (3.02×10^6) is somewhat greater than we desire, although work with other flexuous rod-shaped plant viruses sometimes has shown similar variations (30). The variation cannot be attributed to presence of residual secondary structure after formylation because melting experiments of formylated SMV RNA demonstrated little hyperchromicity. At present, we regard the molecular weight estimate of 3.25×10^6 calculated from the mean of electrophoretic and sedimentation analyses of formalinized SMV RNA to be the most reliable.

Reduction in S value and electrophoretic mobility after denaturation are reflections of the degree of secondary structure in an RNA molecule (12,14). The 36% reduction in S value for SMV RNA is consistent with the 36–39% reduction in sedimentation coefficient reported for the RNAs of other potyviruses (12), but the 22.2% reduction in electrophoretic mobility is somewhat lower than the 29–35% previously reported for other potato Y group members (12). This may reflect anomalous behavior of the RNA in these experiments as, for example, the electrophoretic behavior of a nonformylated RNA molecule in a gel containing formaldehyde. Melting behavior of SMV RNA is consistent with similar data for other potyviruses (12). Therefore, SMV RNA seems to possess a degree of secondary structure similar to other viruses in its group.

We calculate a molecular weight of 6.13×10^7 for the virus particle, based on the percentage of RNA in the particle and its molecular weight. Assuming a protein subunit molecular weight of 28,300 (13), SMV contains approximatley 2,166 subunits. According to the calcualtions outlined by Veerisetty (29), a pitch of 34 Å (28) and a virion length of 746 nm (6), the radial location of the RNA is at 3.7 nm. There are approximately 4.9 nucleotides per subunit, 9.4 subunits per turn, and 10,613 nucleotides in the particle. These values are consistent with those previously calculated for other members of the potyvirus group (29). Further direct experimentation is needed to verify these calculated values.

LITERATURE CITED

1. BEACHY, R. N., and M. ZAITLIN. 1977. Characterization and in vitro translation of the RNAs from less-than-full-length, virus related, nucleoprotein rods present in tobacco mosaic virus preparations. Virology 81:160-169.
2. BOCKSTAHLER, L. E., and P. KAESBERG. 1962. The molecular weight and biophysical properties of bromegrass mosaic virus. Biophys. J. 2:1-9.
3. BOCKSTAHLER, L. E., and P. KAESBERG. 1965. Isolation and properties of RNA from bromegrass mosaic virus. J. Mol. Biol. 13:127-137.
4. BOEDTKER, H. 1968. Dependence of the sedimentation coefficient on molecular weight of RNA after reaction with formaldehyde. J. Mol. Biol. 35:61-70.
5. BOEDTKER, H. 1971. Conformation independent molecular weight determinations of RNA by gel electrophoresis. Biochim. Biophys. Acta 240:448-453.
6. BOS, L. 1972. Soybean mosaic virus. No. 93 in: Descriptions of Plant Viruses. Commonw. Mycol. Inst., Assoc. Appl. Biol., Kew, Surrey, England, 4 pp.
7. BRAKKE, M. K., and N. VAN PELT. 1970. Linear-log sucrose gradients for estimating sedimentation coefficients of plant viruses and nucleic acids. Anal. Biochem. 38:56-64.
8. BRAKKE, M.K., and N. VAN PELT. 1970. Properties of infectious ribonucleic acid from wheat streak mosaic virus. Virology 42:699-706.
9. BURTON, K. 1956. A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. Biochem. J. 62:315-323.
10. DOTY, P., H. BOEDTKER, J. R. FRESCO, R. HASELKORN, and M. LITT. 1959. Secondary structure in ribonucleic acids. Proc. Nat. Acad. Sci. USA 45:482-499.
11. HIGGINS, T. J. V., P. R. WHITFELD, and R. E. F. MATTHEWS. 1978. Size distribution and in vitro translation of the RNAs isolated from turnip yellow mosaic virus nucleoproteins. Virology 84:153-161.
12. HILL, J. H., and H. I. BENNER. 1976. Properties of potyvirus RNAs: Turnip mosaic, tobacco etch, and maize dwarf mosaic viruses. Virology 75:419-432.
13. HILL, J. H., and H. I. BENNER. 1980. Properties of soybean mosaic virus and its isolated protein. Phytopathol. Z. (In press).
14. HILL, J. H., H. I. BENNER, and R. J. ZEYEN. 1977. Properties of hydrangea ringspot virus ribonucleic acid. J. Gen. Virol. 34:115-125.
15. HILL, J. H., and R. J. SHEPHERD. 1972. Biochemical properties of turnip mosaic virus. Virology 47:807-816.
16. HINOSTOZA-ORIHEULA, A. M. 1975. Some characteristics of infectious RNA from potato virus Y. Virology 67:276-278.
17. HULL, R., M. W. REES, and M. N. SHORT. 1969. Studies on alfalfa mosaic virus. I. The protein and nucleic acid. Virology 37:404-415.
18. KENNEDY, B. W., and R. L. COOPER. 1967. Association of virus infection with mottling of soybean seed coats. Phytopathology 57:35-37.
19. KNIGHT, C. A. 1963. Chemistry of viruses. Protoplasmatologia Band IV. Springer-Verlag, Vienna.
20. LANE, B. G. 1963. The separation of adenosine, guanosine, cytidine, and uridine by one-dimensional filter paper chromatography. Biochim. Biophys. Acta 72:110-112.
21. MAKKOUK, K. M., and D. J. GUMPF. 1974. Isolation and properties of potato virus Y ribonucleic acid. Phytopathology 64:1115-1118.
22. MILBRATH, G. M., and M. M. SOONG. 1976. A local lesion assay for soybean mosaic virus using *Phaseolus vulgaris* L. cv. 'Top Crop.' Phytopathol. Z. 87:255-259.
23. NAKAMURA, G. R. 1952. Microdetermination of phosphorous. Anal. Chem. 24:1372.
24. PALIWAL, Y. C., and J. H. TREMAINE. 1976. Multiplication, purification, and properties of ryegrass mosaic virus. Phytopathology 66:406-414.
25. PRING, D. R., and W. G. LANGENBERG. 1972. Preparation and properties of maize dwarf mosaic virus ribonucleic acid. Phytopathology 62:253-255.
26. ROSS, J. P. 1977. Effect of aphid-transmitted soybean mosaic virus on yields of closely related resistant and susceptible soybean lines. Crop Sci. 17:869-872.
27. SHATKIN, A. J. 1969. Colorimetric reactions for DNA, RNA, and protein determinations. Pages 231-237 in: K. Habel and N. P. Salzman, eds. Fundamental Techniques in Virology. Academic Press, New York.
28. VARMA, A., A. J. GIBBS, R. D. WOODS, and J. T. FINCH. 1968. Some observations on the structure of the filamentous particles of several plant viruses. J. Gen. Virol. 2:107-114.
29. VEERISETTY, V. 1978. Relationships among structural parameters of virions of helical symmetry. Virology 84:523-529.
30. VEERISETTY, V., and M. K. BRAKKE. 1977. Differentiation of legume carlaviruses based on their biochemical properties. Virology 83:226-231.
31. WHITFELD, P. R., and S. WILLIAMS. 1963. On the ribonuclease activity associated with tobacco mosaic virus preparations. Virology 21:156-161.