Properties of Sweet Potato Feathery Mottle Virus RNA and Capsid Protein

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SUMMARY

Sweet potato feathery mottle virus (SPFMV) shares many biological and
cytopathological characteristics with viruses in the potyvirus group. However, the
native virion lengths for strains of SPFMV range from 810 to 865 nm. This is similar to
the length attained by other potyvirus virions after reaction with divalent cations. In
this paper we show that the SPFMV genome is an infective, polyadenylated, probably
single-stranded RNA molecule with a molecular weight of 3.65 x 10^6 and that the coat
protein has a molecular weight of 3.8 x 10^5. These properties also resemble those of
potyviruses.

Sweet potato feathery mottle virus (SPFMV) has elongated particles (810 to 865 nm) (Cali &
Moyer, 1981; Campbell et al., 1974; Moyer & Kennedy, 1978; Nome et al., 1974), induces
cytoplasmic, pinwheel inclusions (Lawson et al., 1971) and is non-persistently transmitted by
aphids (Kennedy & Moyer, 1982; McLean, 1959). These properties have led to the suggestion
that SPFMV should be classified as a potyvirus (Hollings & Brunt, 1981).

Potyvirus particles are filamentous, 680 to 900 nm long and contain polyadenylated, plus-
sense, single-stranded RNA with a molecular weight of 3.2 x 10^6 to 3.65 x 10^6 and a capsid of a
single polypeptide species with a molecular weight of 3.2 x 10^4 to 3.65 x 10^4 (Hollings & Brunt,
1981; Matthews, 1979). Particles of most potyviruses are 700 to 750 nm long. However, particles
of some can be longer than 800 nm following relaxation with divalent cations (Edwardson, 1974;
Govier & Woods, 1971; Moghal & Francki, 1981) and a few (less than 5%) have particles longer
than 800 nm even when measured without adding divalent cations (Edwardson, 1974). The
extra length of these particles could encapsidate 10 to 15% more RNA than normal-length
potyvirus particles which could code for an additional polypeptide. If true, this would suggest a
basic genetic difference between viruses such as SPFMV and other potyviruses. None of these
potyviruses with long particles has been examined to determine if the genome size is consistent
with the observed virion length. In this paper we report the sizes of the capsid protein and RNA
of SPFMV and compare them to those of the protein and RNA of other potyviruses.

The common (SPFMV-C) and russet crack (SPFMV-RC) strains of SPFMV (Cali & Moyer,
1981) were propagated in Ipomoea nil 'Scarlet O'Hara'. The properties of the protein and RNA
of particles of the two strains were indistinguishable, and all the data shown are for the RC
strain. The strains of SPFMV and strain NC-57 of potato virus Y (PVY), which was grown in
Nicotiana tabacum 'Burley 21', were purified for RNA isolation as described by Cali & Moyer
(1981), except that the resuspension buffer used prior to CsCl isopycnic centrifugation
contained 50 mM-MgCl2 instead of Triton X-100. Brome mosaic virus (BMV) virions were
extracted from Hordeum vulgare 'Clayton' as previously described (Bockstahler & Kaesberg,
1962) and purified for RNA extraction by isopycnic centrifugation in CsCl (0-43 g/ml) in 10 mM-
sodium acetate pH 6.0. The common strain of tobacco mosaic virus (TMV), squash mosaic virus
(SqMV), PVY and watermelon mosaic virus 2 (WMV 2) were propagated and purified as
previously described (Gooding & Hebert, 1967; Hiebert & Puricifull, 1981; Yang et al., 1983).
Sindbis virus (strain AR 339) RNA was provided by R. E. Johnston, Dept. of Microbiology,
NCSU.
Fig. 1. Electrophoresis of SPFMV capsid protein in 10% polyacrylamide gel. SPFMV, WMV 2 and PVY capsid proteins were electrophoresed in lanes 1, 2 and 3 respectively. Molecular weight markers were bovine serum albumin ($6.6 \times 10^4$), egg albumin ($4.5 \times 10^4$), trypsinogen ($2.4 \times 10^4$), $\beta$-lactoglobulin ($1.84 \times 10^4$) and lysozyme ($1.43 \times 10^4$).

Electrophoresis of dissociated SPFMV capsid protein in 10% polyacrylamide gels using a discontinuous buffer system (Laemmli, 1970) revealed a major polypeptide with a molecular weight of $3.8 \pm 0.1 \times 10^4$ and one to three minor, faster migrating polypeptides (Fig. 1). The molecular weight estimates for PVY and WMV 2 capsid proteins, $3.5 \times 10^4$ and $3.65 \times 10^4$, respectively, obtained from these studies are slightly higher than, but within the range of the molecular weights reported for the capsid proteins of potyviruses (Hollings & Brunt, 1981; Matthews, 1979; Purcifull & Hiebert, 1979). The same molecular weight estimates were obtained following electrophoresis in 7.5%, 10% and 12% polyacrylamide gels. For immunoblotting experiments, proteins from purified SPFMV and from healthy and SPFMV-infected *I. nil* plants were electrophoresed in 10% gel, transferred to nitrocellulose (Bio-Rad Transblot System) and probed with SPFMV antisera (following the manufacturer’s instructions). These experiments showed that the three minor polypeptides reacted with SPFMV antiserum and that they were present in infected tissue but not in healthy tissue (data not shown). Therefore, these slightly faster migrating polypeptides are probably degradation products of virus coat protein; coat proteins from other potyviruses are known to be susceptible to partial degradation (Moghal & Francki, 1976).

Viral nucleic acid was isolated by incubating purified virions in an equal volume of dissociation buffer [200 mm-ammonium carbonate pH 9-0, containing 2% SDS, 2mm-sodium EDTA, 0.05% (w/v) proteinase K] for 20 min at room temperature with gentle agitation and then centrifuging the solution in linear-log sucrose density gradients ($g_a$, 150000) in 150 mm-NaCl, 15 mm-sodium citrate, pH 7-0 (SSC) for 4 h at 16 °C. Modifications to this method were that TMV was kept in dissociation buffer for 2 h and that 200 mm-NaCl was added to the dissociation buffer used to extract BMV particles. Gradient fractions were made to 300 mm-sodium acetate, mixed with 2-5 vol. ethanol and kept overnight at $-20$ °C. RNA was recovered
Fig. 2. Electrophoresis of SPFMV RNA in 1.2% agarose containing 10 mM-methylmercuric hydroxide. The gel was stained with ethidium bromide (1 µg ml). Lane 1, BMV RNA; lane 2, BMV RNA 3; lane 3, BMV RNAs 1 and 2; lane 4, TMV RNA; lane 5, PVY RNA; lane 6, SPFMV RNA; lane 7, Sindbis virus RNA; lane 8, a mixture of RNA from lanes 1 to 6.

Fig. 3. Electrophoretic analysis of poly(A)+ and poly(A)- fractions obtained from oligo(dT) cellulose column. Lane 1, unfractionated SPFMV RNA; lane 2, SPFMV poly(A)+ RNA; lane 3, SPFMV poly(A)+ RNA; lane 4, SqMV poly(A)+ RNA; lane 5, SqMV poly(A)+ RNA; lane 6, TMV poly(A)+ RNA; lane 7, TMV poly(A)+ RNA. Electrophoresis was as in Fig. 2 except that methylmercuric hydroxide was omitted.

by centrifugation at 12,000 g for 30 min, resuspended in sterile distilled H2O and stored at -20°C. For infectivity assay, nucleic acid solutions at 100 or 200 µg RNA/ml in 35 mM-potassium phosphate, 50 mM-glycine, pH 9.2 containing 0.05% (w/v) bentonite and 1% (w/v) Celite, were inoculated to Chenopodium amaranticolor and I. nil.

The nucleic acid from SPFMV particles was infective and thus we suppose plus-sense. It was RNA because it reacted with orcinol but not diphenylamine (Shatkin, 1969) and was digested by ribonuclease type 1A (0.0076 units RNase/50 µg nucleic acid in 10 mM-KCl) but not by deoxyribonuclease DN-CL (0.6 units DNase/50 µg nucleic acid in 1 mM-MgCl2) (Sigma). The RNase digestion was at 37°C for 30 min and the DNase digestion was at 25°C for 2 h. Calf thymus DNA, TMV RNA and sterile distilled water were used as controls. The thermal denaturation profile (A260) of SPFMV RNA in SSC was measured using a Beckman DUR spectrophotometer interfaced with a Gilford Model 2527 thermostreamer. The absorbance of SPFMV RNA increased gradually with increasing temperature; the hyperchromicity was 29% and the Tm was 51°C.
The molecular weight of SPFMV RNA was determined by electrophoresis in denaturing agarose gels with, as markers, Sindbis virus RNA (mol. wt. 4.2 × 10^6), PVY RNA (mol. wt. 3.2 × 10^6), TMV RNA (mol. wt. 2.05 × 10^6), BMV RNA species 1 to 4 (mol. wt. 1.19, 1.07, 0.8, 0.275, all × 10^6, respectively). Electrophoresis was in 1.2% agarose in 100 mM-boric acid, 10 mM-sodium borate and 1 mM-disodium EDTA, pH 8.1 containing 10 mM-methylmercuric hydroxide. SPFMV RNA migrated slower than PVY RNA in denaturing gel electrophoresis and sedimented more rapidly than PVY RNA in linear-log sucrose density gradients. The estimated mol. wt. was 3.65 × 10^6 (Fig. 2). This value is about 10% greater than the mol. wt. estimated for other potyviruses, but is in proportion to the length of SPFMV particles assuming the same ratio of RNA weight to virion length as for other potyviruses.

The analysis of SPFMV RNA for polyadenylated [poly(A)^+] regions was conducted as previously described (Dougherty, 1983) by column chromatography using oligo(dT)-cellulose (Type III, Collaborative Research, Waltham, Mass., U.S.A.). RNAs used in the analysis were isolated from virions as described above and further purified by chloroform–phenol extraction. Some SPFMV RNA bound to oligo(dT)-cellulose in 0.01 M-Tris-HCl pH 7.6, 0.5 M-NaCl, 0.001 M-EDTA and is referred to as poly(A)^+ (Fig. 3). The proportions of SPFMV RNA with polyadenylated sequences in RNA from five virus preparations were 54.2%, 61.2%, 50.7%, 44.4%, and 50.6%. Most of the poly(A)^+ SPFMV RNA was of genome length (Fig. 3). The poly(A)^− fraction contained genome length and fragmented SPFMV RNA (Fig. 3). Similarly, 35 to 50% of tobacco etch virus RNA (Hari et al., 1979) and 71% of WMV 2 RNA (data not shown) bound to similar columns. RNA from SqMV and TMV, used as controls in these experiments, contained 60 to 80% and no detectable poly(A)^+ RNA, respectively.

The biochemical properties of the SPFMV virion described here together with the biological properties of the virus (Cali & Moyer, 1981; McLean, 1959; Lawson et al., 1971; Moyer & Kennedy, 1978) confirm that SPFMV is a potyvirus. However, the virion length, and RNA molecular weight are larger than those of other well characterized potyviruses. The relatively long virion length reported for SPFMV (Cali & Moyer, 1981; Campbell et al., 1974; Moyer & Kennedy, 1978; Nome et al., 1974) reflects a correspondingly larger RNA genome, and is not an artefact caused by virions elongating following reaction with divalent cations.

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REFERENCES


Short communication


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