Some Properties of Isometric Virus Particles which Contain the Satellite RNA of Tobacco Mosaic Virus

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SUMMARY

A ssRNA, which has a satellite-like relationship with tobacco mosaic virus (TMV), had an estimated mol. wt. of $0.38 \times 10^6$ and was found to be encapsidated in isometric particles with a diameter of 17 nm. The capsid protein had a mol. wt. of $18 \times 10^3$. This satellite virus of TMV (STMV) is highly immunogenic and no serological relatedness was detected between it and either TMV or satellite tobacco necrosis virus (STNV) or satellite panicum mosaic virus (SPMV) in immunodiffusion tests with antibodies specific for STMV, TMV, STNV and SPMV. Randomly primed STMV cDNA hybridized with STMV-specific dsRNA and ssRNA but did not hybridize to ssRNA of TMV, SPMV, panicum mosaic or cucumber mosaic viruses. The dsRNAs of different isolates of STMV differed in their electrophoretic mobilities, but they all shared nucleotide sequence homology. Particles of STMV tended to aggregate and crystallize upon purification. Replication of STMV in tobacco plants was supported by TMV but not by cucumber mosaic virus. This is the first report of a satellite virus of a rod-shaped plant virus.

INTRODUCTION

Satellite viruses are known to be associated with tobacco necrosis virus (TNV) (Kassanis, 1981), panicum mosaic virus (PMV) (Buzen et al., 1984) and maize white line mosaic virus (WLMV) (Gingery & Louie, 1985). All three satellite viruses are independently encapsidated in isometric particles, 17 nm in diameter, and are not serologically related to their helpers. Helper viruses for these satellites have 30 nm diameter isometric particles and monopartite ssRNA genomes.

We have reported that a satellite dsRNA (mol. wt. $0.6 \times 10^6$) and a ssRNA of approximately half that size are associated with some field isolates of tobacco mosaic virus strain U5 (TMV-U5) (Valverde & Dodds, 1986). Reasons for proposing a satellite nature for the RNAs include the fact that satellite RNA was dependent upon tobamoviruses (TMV-U5 or TMV-U1) for its replication. In this paper we present results which indicate that the satellite ssRNA is encapsidated in a protein distinct from that of TMV and describe some properties of this satellite virus, which we have called satellite tobacco mosaic virus (STMV).

METHODS

Virus isolates. Isolates of TMV-U5 (TMV-U5/S+, an isolate which had the satellite, and TMV-U5/S−, the same isolate but without the satellite) and cucumber mosaic virus (CMV), strain S were those used by Valverde & Dodds (1986). Six other field isolates (isolates 1 to 6) of TMV-U5/S+ were also used in this study.

Virus purification. CMV-S was purified as described by Dodds et al. (1985) and further purified by sucrose density gradient centrifugation in 5 mm-EDTA, pH 7.8. TMV-U5 and STMV were purified from infected Nicotiana tabacum cv. Turkish tissue extracts buffered in 0.2 m-K$_2$HPO$_4$, pH 7.2 by precipitation with 10% polyethylene glycol, followed by alternate low (8000 g for 15 min) and high (90000 g for 120 min) speed...
centrifugations and two cycles of sucrose density centrifugation in 10 to 40% linear gradients of sucrose prepared in distilled H₂O in a Beckman SW27 rotor at 25000 r.p.m. for 180 min. The absorbance of centrifuged sucrose density gradients was recorded at 254 nm, and fractions corresponding to absorbance peaks of slowly sedimenting STMV were collected. Purified preparations of TMV-U5/S⁺ and STMV were negatively stained with 20% phosphotungstic acid pH 7-0 (adjusted with 1 M-NaOH) and viewed with an electron microscope.

Analytical ultracentrifugation. The sedimentation coefficient of STMV was determined by centrifuging purified STMV (at three different concentrations: 0.5, 0.35 and 1.0 mg/ml) in distilled H₂O at 44000 r.p.m. in the An-D rotor of a Beckman analytical ultracentrifuge with Schlieren optics. The temperature of all runs was 20 °C and the sedimenting boundary was photographed at 4 min intervals. The S value was calculated by the graphical method of Markham (1960). Purified preparations of bean pod mottle virus [three components with S values of 54 (T), 91 (M) and 112 (B)] at a concentration of 3 mg/ml in 0.01 M-phosphate buffer pH 7.2 were run simultaneously for comparison.

Protein analysis and serology. Caspide protein subunits of TMV-U5 and STMV were analysed by SDS-PAGE (Laemmli, 1970) in 10% polyacrylamide gels (40:1 acrylamide: bisacrylamide). Antiserum to TMV-U5 and STMV were obtained from rabbits following four weekly subcutaneous injections containing 2.5 mg of purified virus in 0.5 ml distilled H₂O emulsified in 0.5 ml of Freund's incomplete adjuvant. Antigen and antisera (titre 1:1024) to TMV monomers and a faster sedimenting smaller one that corresponded to aggregated virions were collected. Purified preparations of TMV-U5/S⁻ and STMV were negatively stained with 20% phosphotungstic acid pH 7-0 (adjusted with 1 M-NaOH) and viewed with an electron microscope.

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Satellite virus of TMV

Fig. 1. Electron micrograph of a purified preparation of TMV-U5/S+ stained with 2% phosphotungstic acid pH 7.0, showing a full-length rod-shaped virion of TMV surrounded by spherical virions of STMV. Bar marker represents 50 nm.

Fig. 2. Absorbance profiles (254 nm) of sucrose density gradients containing purified virions of TMV-U5/S− (a), and TMV-U5/S+ (b) following rate-zonal centrifugation. ▼, TMV peak; ▽, STMV peak.

(2) An extra, slowly sedimenting, major component was always associated with purified preparations of TMV-U5/S+ (Fig. 2b) and 17 nm diameter isometric particles were obtained from fractions that contained this component. Particles of STMV aggregated very readily and even formed crystals when gradient-purified virus was stored in buffers such as 0·01 M-potassium phosphate and 0·01 M-sodium citrate (pH from 6·5 to 7·5). This problem was minimized by using distilled H2O. Yields of STMV and TMV-U5 were approximately 30 mg and 20 mg respectively from 10 g of infected tissue.

Analytical ultracentrifugation

STMV sedimented as a single component with a sedimentation coefficient of approximately 42 ± 1 (Fig. 3). This value was the average of six analyses of three different concentrations of STMV.
Fig. 3. Schlieren patterns obtained by analytical ultracentrifugation of purified preparations of STMV (a) and bean pod mottle virus (b). Sedimentation is from left to right.

Fig. 4. Electrophoretic analysis of viral proteins (extracted from purified virions) in SDS-polyacrylamide gels (10%): (1) mol. wt. markers ($\times 10^{-3}$), (2) STMV, (3) TMV-U5/S-.

Protein analysis and serology

A single polypeptide with mol. wt. of $18 \times 10^3$ which is assumed to be the coat protein was detected by electrophoresis of SDS-soluble protein of sucrose density gradient-purified STMV virions (Fig. 4). It consistently migrated with a slower mobility than that of TMV-U5 capsid
protein. Antisera to STMV and TMV-U5/S− had titres of 1/1024 and 1/64 respectively and using these antisera no serological relationship was found between STMV and TMV-U5. Only homologous reactions were obtained in tests with STMV, SPMV and STNV and their antisera.

**Nucleic acid and hybridization**

Nucleic acid purified from STMV was digested by RNase but not by DNase, which indicates that it is ssRNA. Linear molecules (Fig. 5) of STMV dsRNA were observed when purified preparations were viewed with the electron microscope. Gels used to electrophorese ssRNAs of STMV, TMV-U5/S+, TMV-U5/S− and CMV-S under denaturing conditions are shown in Fig. 6(a). Those showing the ssRNAs of PMV, SPMV and TMV-U5/S+ are shown in Fig. 7(a). Mol. wt. determinations indicated that STMV RNA had a mol. wt. of 0·38 × 10^6. No detectable nucleotide sequence homology between STMV cDNA and the ssRNAs of TMV-U5, CMV, PMV and SPMV was detected by Northern hybridization (Fig. 6b and 7b). Hybridization was detected when cDNA probe to STMV was reacted with STMV ssRNA.

The electrophoretic mobilities of the dsRNAs of field isolates of STMV were not identical (Fig. 8a). The dsRNAs of the eight isolates tested showed nucleic acid homology (Fig. 8b). In order to have a more efficient dsRNA transfer, this gel (Fig. 8a) was deliberately overloaded. This resulted in an artefact which consisted of multiple STMV dsRNA bands. Positive hybridization of cDNA probe made from encapsidated STMV ssRNA to blotted STMV dsRNA indicates that the previously reported STMV-specific dsRNA is the replicative form of STMV ssRNA.

A comparison of the type isolate of STMV with that of field isolate no. 6 (which is also shown in Fig. 8) indicated a clear difference in the electrophoretic mobility of the isolate-specific STMV dsRNAs (Fig. 9). Co-inoculation of these two isolates to *N. tabacum* cv. Turkish resulted in the detection of both dsRNA forms in the doubly infected plants. Similar mobility differences (approx. 0·5 × 10^5) were observed when ssRNAs of these two isolates were electrophoresed in agarose gels under denaturing conditions.
Co-inoculation of STMV and TMV-U5/S⁻ to tobacco yielded a virus isolate indistinguishable from TMV-U5/S⁺. Attempts to obtain infection by inoculation of tobacco with STMV alone failed. Co-inoculation of tobacco with STMV and CMV-S resulted in CMV-S infections without detectable STMV.

**Discussion**

Our initial attempts to elucidate the encapsidation of the satellite RNA of TMV were not successful (Valverde & Dodds, 1986). The main reason for this was the use of a procedure for the purification of TMV (Gooding & Hebert, 1967) which was not optimal for the purification of STMV. Modifications that resulted in successful purification of satellite virus particles in addition to TMV particles included the use of 10% polyethylene glycol to precipitate the viruses and distilled H₂O to resuspend virus-containing pellets.
Results presented here confirm the detection of a satellite virus of TMV. It has a serologically distinct isometric virus particle of about 17 nm diameter which encapsidates a unique unsegmented ssRNA. Evidence for this came from the consistent association of this particle with TMV-U5/S+, and not with TMV-U5/S-, and from the conversion of a TMV-U5/S- isolate to a TMV-U5/S+ isolate after addition of STMV virions to the inoculum. Its relatedness to the previously described satellite RNA (Valverde & Dodds, 1986) was confirmed when cDNA probe made to the encapsidated RNA hybridized with the previously characterized dsRNA of the satellite. The ssRNA nature of the encapsidated nucleic acid was established by its sensitivity to enzyme digestion and its ability to serve as a template for cDNA transcription. The linearity of the dsRNA suggests, but does not prove, that the genome is probably linear.

The satellite virus reported here shares some properties with other described satellite viruses, such as STNV, SPMV and SWLMV. Among the similarities are particle shape, a lack of serological relationship with the helper and a lack of nucleic acid homology with the helper. STMV differed from STNV and SWLMV in properties such as sedimentation coefficient, size of capsid protein, host range and host reaction. STMV had a capsid protein size, sedimentation coefficient and RNA size similar to those reported for SPMV (Buzen et al., 1984). These authors
reported a sedimentation coefficient of 42 and a coat protein of $16 \times 10^3$. Nevertheless, serological comparisons using heterologous and homologous antisera, together with differences in ssRNA size, results of nucleic acid hybridization experiments, and host range differences indicate that they are two different satellites. The different electrophoretic forms of STMV dsRNA described here were not unexpected, since similar results have been reported with other satellites (Buizen et al., 1984; Kaper, 1984).

Several properties of this system make it ideal for future work on satellitism. The helper (TMV) is a well studied plant virus, its genome has been cloned and sequenced (Goelet et al., 1982) and a full length clone is available from which infectious RNA can be made (Dawson et al., 1986). Similar work to be done with STMV is in progress. Both helper and satellite have a wide host range, they are very stable and are easily purified in relatively large quantities. The satellite is systemically distributed throughout the plant in high titre along with its helper. This is the first report of a satellite plant virus with a spherical particle which has a helper virus with a rigid rod-shaped particle.

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