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Association of three RNA molecules with potato mop-top virus

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Summary

Potato mop-top virus particles, purified from systemically infected *Nicotiana benthamiana* plants and then disrupted by heating with sodium dodecyl sulphate and 2-mercaptoethanol, contained only a single polypeptide of M_r 19 100 detectable by polyacrylamide gel electrophoresis. Single-stranded RNA preparations from virus particles, when subjected to electrophoresis in an agarose gel containing methylmercuric hydroxide as a denaturant, were shown to contain approximately equal proportions of three RNAs of 6.5, 3.2 and 2.5 kb. Double-stranded RNA preparations extracted from systemically infected *N. benthamiana* leaves or from locally infected *N. debneyi* leaves, was shown by polyacrylamide gel electrophoresis to contain a major species of 3.2 kbp and two minor species of 6.5 and 2.4 kbp.

Additional keywords: Furoviruses, genomic RNA, dsRNAs, M_r, polypeptide.

Potato mop-top virus (PMTV), which is transmitted by the potato powdery scab fungus, (*Spongospora subterranea*), causes a significant decrease in the yield and quality of tubers of susceptible potato cultivars. The virus occurs in Northern and Central Europe, the Andean region of South America, Africa, Israel and Japan, and probably also in other countries where *S. subterranea* is found (reviewed by Jones, 1988).

PMTV has rigid, rod-shaped particles 18-20 wide and of two predominant lengths (125 nm and 290 nm) (Roberts and Harrison, 1979). On the basis of its fungal transmission and particle morphology, PMTV has been recognized as a member of the newly established *Furovirus* group by the International Committee on Taxonomy of Viruses (Brown, 1989). However, the nature, number and size of the nucleic acid components of PMTV have not been reported previously. We report here that purified PMTV particles contain three segments of single-stranded RNA and compare their sizes with those of three double-stranded RNA segments isolated from PMTV-infected plants.

Isolate T of PMTV (Harrison and Jones, 1970), kindly provided by the late Dr P.R. Massalski (Scottish Crop Research Institute, Invergowrie, Dundee, Scotland) was usually propagated in *Nicotiana benthamiana* although occasionally also in *N. debneyi*. Seedlings were grown to the four to six leaf stage, kept for 24 hours in the dark and, using celite as an abrasive, then mechanically inoculated with partially purified virus or infective *N. benthamiana* leaf extracts (2ml sodium phosphate buffer, pH 7.0 per gram of leaf tissue). Plants were maintained in a glasshouse at 16-22 °C, natural light being

supplemented to an 18 hour photoperiod with mercury vapour lamps. After two to three weeks, systemically-infected *N. benthamiana* leaves had chlorotic patches and were distorted. Virus was purified from such leaves by the method, slightly modified, used by Putz and Kuszala (1978) for purifying beet necrotic yellow vein virus. Infected leaves (100 g) were homogenized in 0.1 M tris-HCl pH 9.0 (200 ml) and carbon tetrachloride (100 ml). The homogenate was stirred at 20 °C for 15 min and then centrifuged at 5000 *g* for 15 min. The upper, aqueous phase was collected and polyethylene glycol (PEG) 6000 and NaCl were added to 5% (w/v) and 0.2 M respectively. The mixture was stirred for 30 min, incubated for 90 min at 4 °C and then centrifuged at 12 500 *g* for 20 min. The pellets were resuspended in 0.01 M tris-HCl pH 9.0 (100 ml), incubated overnight at 4° C and centrifuged at 5000 *g* for 10 min. The supernatant was collected, the virus reprecipitated with PEG and resuspended in 20 ml of dilute tris-HCl buffer. Resuspended virus was layered over a cushion of 20% (w/v) sucrose in 0.01 M tris-HCl pH 9.0 (12 ml) and centrifuged at 85 500 *g* for 20 min in a Beckman SW 28 rotor. The supernatant was decanted into a fresh SW 28 tube, centrifuged at 126 000 *g* for 2 h and the pelleted virus was resuspended in 0.01 M tris-HCl pH 9.0 (0.5 ml).

The purity of the virus preparation was assessed by polyacrylamide gel electrophoresis. Virus particles were dissociated by heating at 100 °C for 5 min in 65 mM tris-HCl pH 6.5, containing 1% (w/v) sodium dodecyl sulphate (SDS) and 2.5% (w/v) 2-mercaptoethanol, and the protein was analysed by discontinuous polyacrylamide gel electrophoresis as described by Laemmli (1970). After staining gels with Coomassie Brilliant Blue R250, only a single polypeptide species was detected (not shown), indicating that the preparations contained less than 1% of contaminating host proteins. The M_r of the polypeptide was estimated to be 19 100; this value is in good agreement with previous estimates of 18 500 to 20 000 for the M_r of PMTV capsid polypeptide (Kassanis et al., 1972; Randles et al., 1976).

To extract the nucleic acid PMTV particles were incubated for 30 min at 37 °C with 1% (w/v) SDS, 100 μg/ml proteinase K, 10 mM tris-HCl pH 8.0 and 1.0 mM EDTA and then extracted with phenol. After precipitation with ethanol, the pellet was washed twice with 70% (v/v) ethanol, dried under vacuum and resuspended in a small volume of sterile of distilled water. The nucleic acid was analysed under denaturing conditions in 1% agarose gels containing 10 mM methylmercuric hydroxide, as described by Bailey and Davidson (1976), by co-electrophoresis with tobacco mosaic virus RNA (6.4 kb) and red clover necrotic mosaic virus RNA 1 (4.0 kb) and RNA 2 (1.4 kb). After staining with ethidium bromide, three clear bands were seen with mobilities corresponding in size to 6.5 kb, 3.2 kb and 2.5 kb (Fig. 1). When the nucleic acid was incubated with ribonuclease A (1 μg/ml) in 10 mM tris-HCl pH 8.0 for 30 min at 37 °C prior to gel electrophoresis, the three bands could no longer be detected (not shown), showing that they are all single-stranded RNA.

To obtain double-stranded (ds) RNA, total RNA was extracted from systemically infected *N. benthamiana* leaves by the method of Taylor and Powell (1982). ssRNA was removed by precipitation with 2M LiCl (Baltimore, 1966) and dsRNA was precipitated from the supernatant by incubation at −70 °C for 1 h after the addition of 3 M sodium acetate (0.1 vol), isopropanol (0.1 vol) and tRNA (10 μg/ml). The dsRNA was pelleted by centrifugation, dried under vacuum and resuspended in sterile distilled water. Analysis in a 5% polyacrylamide gel (Bozarth and Harley, 1976) by co-electrophoresis with dsRNA size standards from *Penicillium stoloniferum* viruses (PsV)

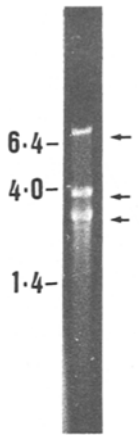

Fig. 1. Denaturing agarose gel of PMTV ssRNA. Arrows on the right of the gel indicate PMTV ssRNA components. Numbers on the left of the gel refer to the size of standards in kb.

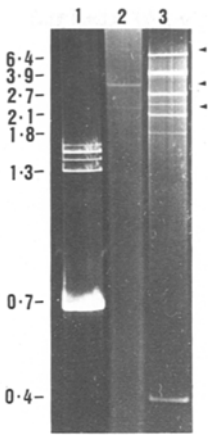

Fig. 2. 5% polyacrylamide gel of dsRNA extracted from *N. benthamiana* tissue infected with PMTV. Lane 1, PsV dsRNA; lane 2, PMTV dsRNA; lane 3, AfV dsRNA. Arrows on the right of the gel indicate PMTV dsRNA components in lane 2. Numbers on the left of the gel refer to the size of standards in kbp (lanes 1 and 3).

(Bozarth & Harley, 1976) and *Aspergillus foetidus* viruses (AfV) (Buck and Ratti, 1977) revealed three dsRNAs of approximately 6.5, 3.2 and 2.4 kbp (Fig. 2). Their double-stranded nature was confirmed by their resistance to DNase I, their resistance to ribonuclease A in 2 × SSC buffer (SSC = 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0) and susceptibility to ribonuclease A in 0.1 × SSC buffer (Buck et al., 1971) (not shown). Double-stranded RNAs of the same size were also extracted from locally infected *N. debneyi* leaves, but no dsRNAs were extracted from healthy *N. benthamiana* leaves. The three dsRNAs correspond in size to the three viral ssRNA components; however, whereas the three ssRNAs were present in preparations in roughly equal proportions, the 3.2 kbp dsRNA (RNA 2) was always present in a much higher proportion than the other two dsRNAs. Other very faint bands were not reproducibly detected in all preparations.

Furoviruses have a genome of two RNA components (Brunt, 1988; Brunt and Richards, 1989). The two large RNAs of PMTV are similar in size to the two genomic RNAs (6.5 - 6.9 kb and 3.5 kb) of soil-borne wheat mosaic virus (SBWMV), the type member of the *Furovirus* group. Some isolates of SBWMV contain an additional RNA component (2.1 to 2.4 kb) derived by deletion in the smaller genomic RNA (Shirako and Brakke, 1984). The presence of three RNAs in PMTV is therefore not inconsistent with its being a member of the *Furovirus* group. However, further studies will be required to ascertain if the smallest PMTV RNA is also generated by deletion in one of the larger RNAs and to determine the minimum number of RNA components needed for infectivity. It is noteworthy that beet necrotic yellow vein virus has four RNA components, but it is only considered a possible member of the *Furovirus* group (Brown,

1989) and some of its properties set it apart from the furoviruses (Brunt and Richards, 1989).

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