Properties of Ribonucleic Acid and Coat Protein of Peanut Chlorotic Ring Mottle Virus

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Key words : peanut chlorotic ring mottle virus, potyvirus, RNA glyoxalation, coat protein.

Peanut chlorotic ring mottle virus (PCRMV) which belongs to the potyvirus group occurs very widely on peanut in the South East Asian countries. PCRMV shows a very distant serological relationship with peanut mottle virus (PnMV), and also a positive reaction with blackeye cowpea mosaic virus (BICMV), bean common mosaic virus (BCMV) and soybean mosaic virus (SMV)¹⁾. This paper describes some properties of the nucleic acid and coat protein of PCRMV, several potyviruses pathogenic to legumes as well as of turnip mosaic virus (TuMV).

PCRMV was purified as described in a previous report¹⁾. Isolation of PCRMV-RNA was performed by the method of Brakke and Van Pelt²⁾ with a few modification. PCRMV-RNA was prepared by disrupting the virions in 10 mM sodium phosphate buffer (Na-P. B.), pH 7.0, containing 1% sodium dodecyl sulfate (SDS) and EDTA-treated bentonite, followed by sucrose density gradient centrifugation. The fractions which were associated with the infectivity on *Chenopodium amaranticolor* were concentrated by precipitation with ethanol. The pellet was resuspended in 10 mM Na-P. B., pH 7.0, and stored at -70 C until use. RNA concentration was determined spectrophotometrically by using an extinction coefficient of 24 cm² mg⁻¹ at 260 nm.

Nuclease treatment was carried out by mixing the solution with 1 µg/ml of pancreatic ribonuclease (RNase) (Sigma) or 5 µg/ml deoxyribonuclease (DNase) (P-L Biochemical Inc.) in the presence of 5 mM MgCl₂ and incubating at 35 C for 30 min. The preparation was then centrifuged on a linear sucrose density gradient column for 3 hr at 216, 400×g in a Hitachi RPS65T rotor at 4 C. The gradients were scanned at 254 nm by an absorbance monitor (ISCO model UA-5) equipped with a density gradient fractionator (ISCO model 640). Reaction of nucleic acid with formaldehyde was analysed by the method of Miura *et al.*³⁾.

Molecular weight (MW) of viral RNA was determined by analysing the sedimentation of RNA using linear sucrose density gradient centrifugation, and the electrophoretic mobility relative to the standard markers by agarose submarine gel electrophoresis.

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To determine the sedimentation coefficient of RNA, 23S (MW : 1.009×10^6) and 16S (MW : 0.534×10^6) ribosomal RNAs of *Escherichia coli*^{4,5)} were used as markers. The preparations of RNA and SDS-disrupted virus were centrifuged as described previously. To calculate the MW of RNA from the S values, the formula $M = 1557 S^{2.07}$ was used⁶⁾.

Agarose submarine gel electrophoresis was carried out as follows: the preparations of non-denatured RNA and SDS-disrupted virus electrophoresed at 80 V for 3 hr in 1% agarose gel (13.5 × 13.5 cm, 4 mm thick) in 10 mM Na-P. B., pH 7.0. The gel was stained with ethidium bromide (1 µg/ml), placed on an illuminator (260 nm wavelength), and photographed. The denatured RNAs were prepared in 1 M glyoxal, 50% (v/v) dimethyl sulfoxide, 10 mM Na-P. B., pH 7.0, as described by MacMaster and Carmichael⁷⁾. These preparations were electrophoresed at 80 V for 4 hr in 0.75% agarose gel. The gel was stained with 0.05% Toluidine Blue O and destained with 60 mM Na-P. B., pH 7.0, as described by Murant *et al.*⁸⁾.

The following RNA species were used as standards for the estimation of RNA MW: TMV-RNA, MW 2.19×10^6 ⁹⁾; tobacco ringspot virus (TobRSV) RNA 1 and RNA 2, MW 2.4×10^6 , 1.4×10^6 (non-denaturing condition), or 2.73×10^6 , 1.34×10^6 (glyoxalated denaturing condition)⁹⁾; 23S and 16S ribosomal RNA as described above.

Polyacrylamide gel electrophoresis (PAGE) of viral proteins was performed according to the method of Laemmli¹⁰⁾ using a 10% acrylamide slab gel. MW markers included bovine serum albumin (MW 68,000), ovalbumin (45,000), chymotrypsinogen A (25,000) (Boehringer Mannheim), TobRSV protein subunits (54,000) and southern bean mosaic virus protein subunits (29,000).

PCRMV-RNA showed a maximum UV absorption at 258 nm and minimum at 238 nm. The ratios of OD_{258}/OD_{238} and OD_{258}/OD_{280} were 1.86 and 2.10, respectively. Native PCRMV-RNA was sedimented as a single band by sucrose density gradient centrifugation, and the RNA (5 µg/ml) caused local lesions (average number 74 per one leaf) on the inoculated leaves of *C. amaranticolor*. On the other hand, when PCRMV-RNA (500 µg/ml) had been treated with RNase, the sedimenting component disappeared, and its infectivity was lost completely. Similar treatment with DNase in the presence of 5 mM $MgCl_2$ had no effect on the sedimentation pattern and infectivity. After 24 hr incubation with formaldehyde, PCRMV-RNA exhibited a hyperchromicity of 29% and shift of 2–4 nm of longer wavelength in the ultraviolet absorption spectrum. These results indicate that the nucleic acid of PCRMV is a single-stranded RNA.

PCRMV-RNA was centrifuged with marker RNAs to estimate the sedimentation coefficient, and the MW of viral RNA was calculated. In twelve repetitions of the experiments, PCRMV-RNA showed an average sedimentation coefficient of 39.6 ± 1.5 , and MW of viral RNA was calculated as 3.16×10^6 daltons. By the same procedure, MWs of RNA of the other potyviruses were calculated as shown in Table 1.

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When non-denatured PCRMV-RNA was electrophoresed in 1% agarose gel, a rectilinear relationship was observed only among the mobilities of the RNA standards of TMV-RNA and TobRSV-RNAs, while ribosomal RNAs were out of the line (Fig. 1). Based

Fig. 1. Electrophoresis of non-denatured or denatured (glyoxalated) PCRMV-RNA in agarose gel. (A) : non-denaturing condition, (B) : denaturing condition.
 ○: PCRMV-RNA, △: TobRSV-RNA 1, □: TobRSV-RNA 2, ●: TMV-RNA, ×: *E. coli* 23S ribosomal RNA, ■: 16S rRNA. Distance of migration of (A) was calculated by using photographs.

on the former RNA standards, MW of non-denatured PCRMV-RNA was calculated as $3.32 \pm 0.12 \times 10^6$ daltons. MW of RNA in SDS-disrupted PCRMV showed a similar value ($3.35 \pm 0.07 \times 10^6$). On the other hand, when glyoxalated PCRMV-RNA was electrophoresed in 0.75% agarose gel, a rectilinear relationship was observed among the mobilities of all the RNA standards (Fig. 1) and the MW of PCRMV-RNA was thus estimated at $3.49 \pm 0.08 \times 10^6$ daltons. The glyoxalated preparation of the SDS-disrupted virions also gave the same estimate of $3.49 \pm 0.04 \times 10^6$ daltons for MW. The values of the MWs of RNAs from SDS-disrupted virions for the other potyviruses are listed in Table 1.

The values of MWs of RNAs for the potyviruses estimated by sedimentation analysis and agarose gel electrophoresis were different, and the estimates of RNAs by agarose gel electrophoresis under denaturing conditions were slightly larger than those of RNAs under non-denaturing conditions. Murant *et al.*⁸⁾ reported that the electrophoresis of glyoxalated RNA in agarose gels was a reliable method for the determination of MW up to at least about 3.5×10^6 by using 13 plant viruses. This is the first report on the comparison of MW of glyoxalated RNAs of viruses belonging to the potyvirus group.

SDS-PAGE analysis of the coat protein of a freshly purified PCRMV revealed the presence of a main component with a MW of 35,600 and two smaller ones with MWs of 33,400 and 27,800. One to three components were observed in SDS-PAGE for the other potyviruses, and MWs of the main coat protein of PnMV-PN, PnMV-T and TuMV were 30,900, 31,600 and 33,000, respectively. These values are the means of 10-23 de-

Table 1. Molecular weights of viral ribonucleic acid of some potyviruses determined by agarose gel electrophoresis and sucrose density gradient centrifugation under non-denaturing and denaturing conditions

Virus	Agarose gel electrophoresis		Sucrose density gradient centrifugation	
	Non-denaturing condition	Denaturing condition ^{a)}	Non-denaturing condition	
	MW $\times 10^6$	MW $\times 10^6$	Sedimentation coefficient	MW $\times 10^6$
PCRMV	3.32 $\pm$ 0.12 ^{b)}	3.49 $\pm$ 0.08 ^{b)}	39.6 $\pm$ 1.5 ^{c)}	3.16
PnMV-PN	3.34 $\pm$ 0.08	3.48 $\pm$ 0.16	36.9 $\pm$ 0.1	2.73
PnMV-T	3.38 $\pm$ 0.11	3.72 $\pm$ 0.09	37.5 $\pm$ 0.8	2.82
BICMV	3.35 $\pm$ 0.19	3.63 $\pm$ 0.13	38.3 $\pm$ 0.8	2.95
BCMV	3.33 $\pm$ 0.17	3.63 $\pm$ 0.10	39.7 $\pm$ 1.5	3.18
SMV	3.37 $\pm$ 0.07	3.57 $\pm$ 0.10	37.1 $\pm$ 0.9	2.76
TuMV	3.06 $\pm$ 0.12	3.44 $\pm$ 0.12	39.3 $\pm$ 1.4	3.13

a) Denaturation was carried out in 10mM Na-P. B., pH 7.0 containing 1M glyoxal and 50% (v/v) dimethyl sulfoxide.

b, c) The values of b and c are means of 6-13 and 5-14 determinations, respectively.

terminations. It is generally assumed that the smaller components are associated with the degradation of the coat protein subunits during purification and storage.

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和 文 摘 要

福本文良・岩木満朗・土崎常男: Peanut chlorotic ring mottle virus の RNA と外被蛋白の諸性質

Potyvirus group に属する peanut chlorotic ring mottle virus (PCRMV) の純化標品から分離した核酸は、DNase に耐性であるが、RNase に対して感受性であり、ウイルス活性が消失した。また、ホルムアルデヒド処理核酸は、紫外線吸収曲線の吸収極大の位置が長波長に動き、吸収も増大することから、PCRMV 核酸は一本鎖の RNA と考えられた。しょ糖密度勾配遠心法による PCRMV-RNA の S 値は39.6 $\pm$ 1.5であり、この値から分子量は 3.16 $\times 10^6$ と計算された。アガロースゲル電気泳動法による未変性 RNA の分子量は3.32 $\pm$ 0.12 $\times 10^6$ であり、Glyoxal 処理によって変性した場合 3.49 $\pm$ 0.08 $\times 10^6$ が得られた。一方、外被蛋白の分子量はポリアクリルアミドゲル電気泳動法によって35,600であった。

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