

カブモザイクウイルスRNAの諸性質 (1)

分子量

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Properties of Ribonucleic Acid Isolated from Turnip Mosaic Virus

1. Determination of Molecular Weight

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崔 璋京*・松山宣明*・脇本 哲*: カブモザイクウイルス RNA の諸性質 1. 分子量

Abstract

Nucleic acid of turnip mosaic virus (TuMV-RNA) prepared by ammonium carbonate method was infectious, sensitive to ribonuclease and tolerant to deoxyribonuclease. The sedimentation coefficient of TuMV-RNA was determined as 38.6 ± 0.7 S with linear-log sucrose density gradient centrifugation. TuMV-RNA denatured with formaldehyde had sedimentation coefficient of 24.3 ± 0.5 S, showing 37 % reduction as compared with native RNA. The molecular weights of TuMV-RNA calculated from sedimentation coefficient of native RNA and formaldehyde-treated RNA were 3.00×10^6 and 3.05×10^6 , respectively. The value, 3.1×10^6 , almost similar to the above, was also obtained by polyacrylamide gel electrophoresis. (Received April 2, 1979)

Introduction

The molecular weight of turnip mosaic virus ribonucleic acid (TuMV-RNA) has been calculated with different methods by several workers. Hill and Shepherd⁷⁾ estimated as 3.5×10^6 daltons with polyacrylamide gel electrophoresis, while Hill and Benner⁸⁾ determined as 3.07×10^6 with sedimentation analysis or as 3.31×10^6 with polyacrylamide gel electrophoresis. Boedtker²⁾ described that molecular weight of RNA could be accurately estimated by sedimentation analysis after the treatment with formaldehyde, because formaldehyde breaks the hydrogen bonding in secondary structure of RNA and eliminates their effect on molecular weight calculation. This formylation was successfully applied for several potato virus Y (PVY) group viruses^{8,11,13)} and 3.16×10^6 daltons was given as molecular weight of TuMV-RNA⁸⁾.

To ascertain the molecular weight of TuMV-RNA, the following three different methods were applied ; (1) sedimentation analysis of native TuMV-RNA, (2) sedimentation analysis of formaldehyde-treated RNA and (3) polyacrylamide gel electrophoresis.

Materials and Methods

Virus isolate and its purification. TuMV isolate, host plants for virus propagation, and method for virus purification were previously described⁵⁾. Freshly harvested leaves were used for virus purification throughout this experiment.

Preparation of TuMV-RNA. The method reported by Brakke and Van Pelt³⁾ was applied for preparing TuMV-RNA. One volume of the purified virus suspension was added with an equal volume of 0.2 M ammonium carbonate buffer, pH 9.0, containing 0.002 M ethylenediaminetetraacetic acid (disodium salt) (Na_2 EDTA) and 2 % sodium dodecyl sulfate (SDS). EDTA-treated bentonite⁶⁾ was also added to

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make concentration of 200-400 $\mu\text{g/ml}$. The mixture was incubated at 4 C for 12-16 hr and was layered on a linear-log sucrose density gradient. The buffer (SSC) containing 0.15 M sodium chloride (NaCl) and 0.015 M sodium citrate, pH 7.0, was used as gradient solvent. The gradient was allowed to stand overnight at 4 C to form linear-log type⁴⁾. Before centrifugation, 1 ml of the gradient from the top was replaced with 1 ml of the mixture containing TuMV-RNA. Centrifugation was run at 22,000 rpm at 14 C for 16 hr in a Spinco SW 25.1 rotor of Beckman Model L 2 ultracentrifuge. The gradients were fractionated and analysed with a Hitachi Model 100-60 spectrophotometer. The fraction showing the highest UV absorption at 260 nm was used as purified TuMV-RNA in the following experiments.

Nuclease treatment. TuMV-RNA obtained by density gradient centrifugation was mixed with 5 μg of pancreatic ribonuclease (RNase) (P-L Biochemicals Inc.) or 20 μg of deoxyribonuclease (DNase) (Boeinger Mannheim) and incubated at 37 C for 30 min. The preparation was then centrifuged on a linear-log sucrose density gradient column. After fractionation of the column, spectrophotometric analysis was carried out to make clear the nuclease effect.

Determination of molecular weight. Molecular weight of TuMV-RNA was determined by means of sedimentation of native and formaldehyde-treated RNAs in linear-log sucrose density gradient centrifugation and polyacrylamide gel electrophoresis. Formaldehyde treatment was conducted as follows: Nine volumes of TuMV-RNA in SSC were mixed with 1 volume of formaldehyde solution (4 volumes of 37 % reagent grade formaldehyde plus 1 volume of 5 times concentrated SSC). The mixture was heated at 63 C for 15 min, immediately chilled with ice⁸⁾, and analysed with density gradient centrifugation.

To determine sedimentation coefficient of the native and formylated TuMV-RNA, 23 S (MW : 1.1×10^6) and 16 S (MW : 5.6×10^5) ribosomal RNAs of *E. coli*¹⁾ were used as makers. To calculate the molecular weight of RNA from S value, the formulas $M = 1557 S^{2.07}$ for native RNA¹⁰⁾ and $S = 0.083 M^{0.38}$ for formylated RNA³⁾ were used.

Polyacrylamide gel electrophoresis was carried out as follows: TuMV-RNA was suspended in E buffer (0.12 M Tris-HCl, 0.06 M sodium acetate and 0.003 M Na₂ EDTA, pH 7.2) and electrophoresed at 6 mA per gel for 3 hr in 2.4 % polyacrylamide gels prepared by the method of Bishop *et al.*¹⁾. Gels were scanned for absorbance at 260 nm in a Gilford Model 2400 spectrophotometer.

Results

Isolation of TuMV-RNA and its infectivity

RNA prepared from freshly purified TuMV and suspended in SSC had UV absorption maximum at 258 nm and minimum at 234 nm (Fig. 1). The ratios of OD 258 / OD 234 varied from 1.81 to 2.18 and OD 258 / OD 280 from 1.89 to 2.04, indicating minimal contamination of protein. Yields of RNA were 20-30 $\mu\text{g} / \text{mg}$ of virus (estimated from $E_{1\text{cm}}^{0.1\%} 258=24$).

The RNA preparations (concentration : 50 - 100 $\mu\text{g} / \text{ml}$) were infectious when inoculated to turnip (*Brassica rapa* L.) leaves with bentonite (100 - 200 $\mu\text{g/ml}$). Infectivity of the RNA preparation was lost by treatment with pancreatic RNase (5 $\mu\text{g} / \text{ml}$) at 37 C for 30 min.

Sensitivity to nucleases

Native TuMV-RNA was sedimented as a single band at about 6.0 - 6.4 cm from the meniscus on sucrose density gradient centrifugation (Fig. 2 a), while no sedimen-

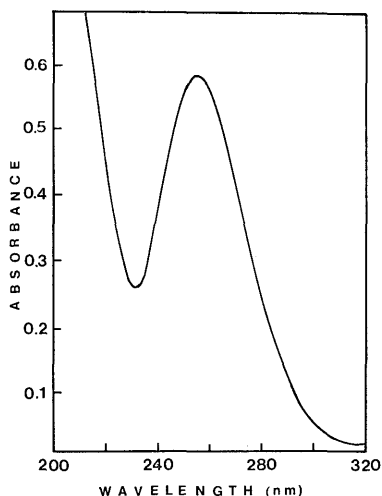


Fig. 1. UV absorption spectrum of TuMV-RNA in SSC prepared by ammonium carbonate method.

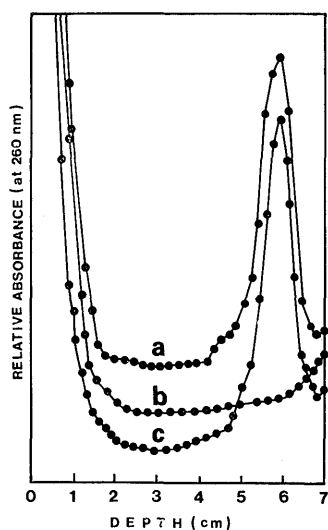


Fig. 2. UV absorbance scanning profiles of TuMV-RNA preparation centrifuged in linear-log sucrose density gradient at 22,000 rpm for 16 hr in SW 25.1 rotor at 14 C. Abbreviations are : (a) native TuMV-RNA, (b) TuMV-RNA treated by RNase, (c) TuMV-RNA treated by DNase.

(average 24.3 ± 0.5 S) as compared with native RNA was resulted. Substitution of 24.3 for S value of the formula of Brakke and Van Pelt³⁾ gave a molecular weight of 3.05×10^6 daltons.

tation pattern was evident when RNA preparation had been treated with RNase indicating complete decomposition (Fig 2 b). Similar treatment with DNase had no effect on the sedimentation pattern (Fig. 2 c).

Determination of molecular weight of TuMV-RNA by sedimentation coefficient

TuMV-RNA was centrifuged with marker RNAs to estimate sedimentation coefficient. In six repetitions of the experiments, TuMV-RNA showed an average sedimentation coefficient of 38.6 ± 0.7 S (Fig. 3). With formula of Hull *et al.*¹⁰⁾, molecular weight of viral RNA was calculated as 3.00×10^6 daltons.

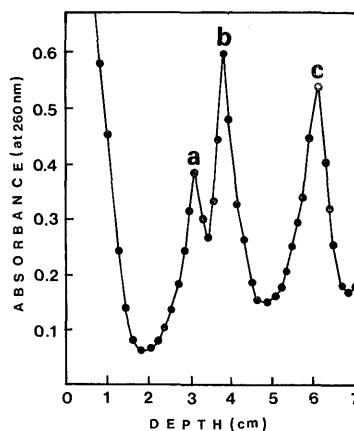


Fig. 3. UV absorbance scanning profiles of TuMV-RNA and markers centrifuged in linear-log sucrose density gradient at 22,000 rpm for 16 hr in SW 25.1 rotor at 14 C. Abbreviations are : (a) and (b), 16 and 23 S RNAs of *E. coli* ribosome, (c) TuMV-RNA.

TuMV-RNA treated with formaldehyde also gave a single band on density gradient centrifugation (Fig. 4), however, about 37 % reduction in S value

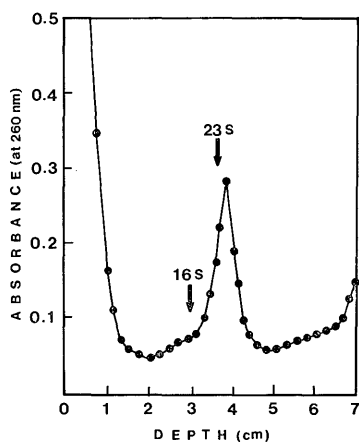


Fig. 4. UV absorbance scanning profiles of formaldehyde-treated TuMV-RNA centrifuged in linear-log sucrose density gradient at 22,000 rpm for 16 hr in SW 25.1 rotor at 14 C.

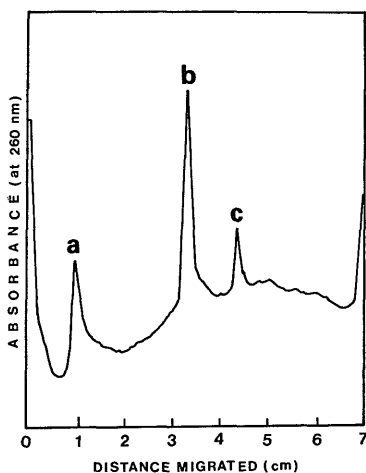


Fig. 5. Absorbance scanning of TuMV-RNA and markers after polyacrylamide gel (2.4 %) electrophoresis at 6 mA per gel for 3 hr. Abbreviations are : (a) TuMV-RNA, (b) and (c), 23 and 16 S RNAs of *E. coli* ribosome, respectively.

guanidine-HCl were not. The properties of TuMV-RNA such as sensitivity to RNase, tolerance to DNase, reduction of S value with formaldehyde treatment, sedimentation in the density gradient column and immigration in polyacrylamide gel electrophoresis elucidated in this experiment indicate that TuMV-RNA is a single-stranded RNA composed of a single component.

The molecular weight of TuMV-RNA, $3.00 - 3.1 \times 10^6$, determined independently

Determination of molecular weight of TuMV-RNA by polyacrylamide gel electrophoresis

A single component was also observed when TuMV-RNA was subjected to polyacrylamide gel electrophoresis (Fig. 5). The molecular weight of TuMV-RNA estimated in comparison with marker RNAs was 3.1×10^6 (Fig. 6). This value is almost similar to those obtained by sedimentation analysis before and after formaldehyde treatment.

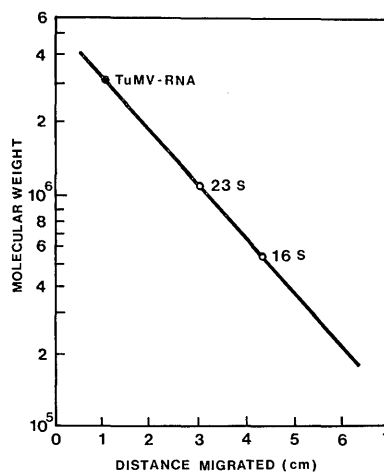


Fig. 6. Relationship between molecular weight and electrophoretic migration of TuMV-RNA and markers in 2.4 % polyacrylamide gel. Marker RNAs are : 23 S RNA (MW : 1.1×10^6) and 16 S RNA (MW : 5.6×10^5) of *E. coli* ribosome.

Discussion

To obtain infectious RNA from purified TuMV, it was made clear in a preliminary experiment that the ammonium carbonate procedure³⁾ was adequate but the methods with phenol and

by sedimentation coefficient and polyacrylamide gel electrophoresis in our experiment showed good coincidence with that reported by Hill and Benner⁸⁾. With regard to the molecular weight of RNAs of PVY group viruses other than TuMV, 3.1×10^6 for PVY-RNA¹¹⁾, 2.94×10^6 for maize dwarf mosaic virus (MDMV) RNA⁸⁾, 3.14×10^6 for tobacco etch virus (TEV) RNA⁸⁾ and 3.2×10^6 for PVY-RNA⁹⁾ were reported up to the present. The former two were determined by sedimentation analysis and the latter two by polyacrylamide gel electrophoresis. These results suggest that PVY group viruses have RNAs almost similar in molecular weight as described by Hill and Benner⁸⁾. Some discordant results showing much lower molecular weight such as 2.7×10^6 for ryegrass mosaic virus RNA¹²⁾ and 2.7×10^6 for MDMV-RNA¹³⁾ were reported, but reconfirmation test seems to be necessary.

Boedtker²⁾ described that an accurate molecular weight of RNA could be determined by eliminating the secondary structure with formaldehyde treatment before sedimentation analysis. In our experiment, however, sedimentation analysis with density gradient centrifugation before and after formaldehyde denaturation gave almost the same molecular weight, coincided with the result reported by Hill and Benner⁸⁾.

Sedimentation analysis of TuMV-RNA denatured with formaldehyde revealed a 37% reduction in S value as compared with native RNA. This value is almost similar to the values, 36 to 39 %, reported for several PVY viruses by Hill and Benner⁸⁾. These results indicate that they possess a similar degree of secondary structure.

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和 文 摘 要

カブモザイクウイルス RNA の諸性質 1. 分子量

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カブモザイクウイルス (TuMV) の純化標品から ammonium carbonate 法によって分離した RNA は 258nm で極大, 234nm で極小の紫外線吸収を示し, A258/234 および A258/280 の比はそれぞれ 1.81~2.18 と 1.89~2.04 で, 純度の高いことを示した。この RNA は宿主植物のカブに感染性を有し, RNase に対しては感受性, DNase に対しては耐性であった。Linear-log ショ糖密度勾配遠心による TuMV-RNA の S 値は, native RNA で 38.6 ± 0.7 , formaldehyde 処理 RNA では 24.3 ± 0.5 であった。これらの S 値から TuMV-RNA の分子量はそれぞれ 3.00×10^6 および 3.05×10^6 と計算された。一方, ポリアクリルアミドゲル電気泳動法によっては分子量 3.1×10^6 が得られた。