MOLECULAR WEIGHT OF TOMATO BUSHY STUNT VIRUS-RNA

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1. Introduction

The molecular weight of tomato bushy stunt virus-RNA (TBSV-RNA) has never been determined [1], probably because it is diffucult to extract the RNA of this virus by the classical RNA-extraction methods. With regard to the recent reinvestigation of the structure of TBSV [2] and the finding of two types of protein subunits [3], a knowledge of TBSV-RNA molecular weight would be useful to correlate the physico-chemical data and the structure of TBSV. This paper reports an isolation method for TBSV-RNA and the determination of its molecular weight by ultracentrifugation analysis after reaction with formaldehyde and by polyacrylamide gel electrophoresis.

2. Materials and methods

TBSV was prepared as previously described [4]. TBSV at 6 mg per ml of 0.1 M Na borate buffer, pH 9.0, was dissociated by a 12 hr incubation at 0° with mersalyl, 2×10^{-2} M, and bentonite (half weight of virus). The RNA was extracted from the reaction mixture by the phenol method in the presence of 0.5% of SDS. The RNA was precipited three times by 75% ethanol and dissolved in 0.02 M Na acetate buffer, pH 4.8. The absence of residual mersalyl in the RNA solutions was checked by dithyzone. The average 258 mμ/232 mμ absorbance ratio of TBSV-RNA is 2.4, and its infectivity determined with *Nicotiana glutinosa* is about 2% of that of an equal amount of encapsulated RNA. TBSV-RNA solutions in 0.05 M Na phosphate buffer, pH 7.0, examined in the ultracentrifuge with ultraviolet optics, contain a large component with an S_{20w} = 27.1 S which accounts for 40–60% of the total absorbance.

TMV [5], turnip yellow mosaic virus (TYMV) [6], and Alfalfa mosaic virus-RNA (AMV-RNA) [7] were isolated from purified virus solutions by the classical phenol method. *Escherichia coli* ribosomal RNA were isolated from *E. coli* MRE 300 cultures by phenol extraction [8].

The molecular weight of TBSV-RNA was determined according to the method of Boedtker [9]. 35% reagent grade formaldehyde (from Merck) was used as indicated by Boedtker. Sedimentation rates were measured with a spinco model E ultracentrifuge equipped with ultraviolet optics at 20–22°. The experimental sedimentation coefficients are only converted to standard temperature (20°).

Polyacrylamide gel electrophoresis was performed according to Loening [8]. Gels were prepared in 10.0 × 1.0 cm plastic tubes containing 2.4% acrylamide

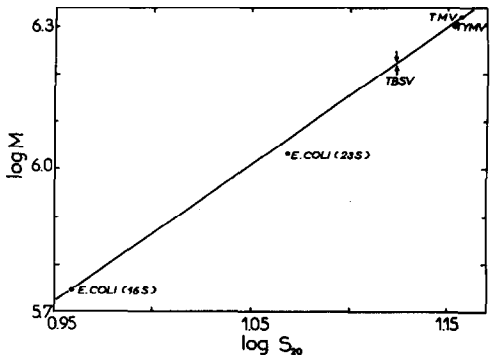

Fig. 1. Dependence of the sedimentation coefficient on molecular weight for various RNA samples after reaction with formaldehyde.

Table 1

Sedimentation coefficients of RNA samples after reaction with formaldehyde, and electrophoretic migrations in polyacrylamide gel. The experimental sedimentation coefficients measured at temperature between 20 and 22° were only converted to standard temperature of 20°. The corresponding S_{20} values obtained by Boedtker are given without correction for density and viscosity of the solvent.

RNA	(M.W.) X 10^{-6}	Ref.	S_{20} observed	S_{20} (Boedtker)	Electrophoretic migration (cm)
TMV	2.1	5	14.38	14.31	1.33
TYMV	2.0	6	14.25		
TBSV	1.65		13.29		1.95
AMV (25 S)	1.32	7			2.53
AMV (20 S)	0.99				3.29
E. coli (23 S)	1.08	8	11.70	11.63	3.04
E. coli (16 S)	0.56		9.11	9.12	

and 0.12% methylene bisacrylamide. The electrophoretic buffer contained 40 mM tris, 20 mM Na acetate, and 2 mM EDTA, pH 7.8. 20 to 50 μg of RNA dissolved in electrophoretic buffer containing 10% glycerol were layered on the gel after a pre-electrophoresis (45 min at 5 mA/gel). Electrophoresis was continued for 4 hr at 8 mA/gel at 4°. Gels were then gently extruded and scanned at 270 mμ using a Beckman DU spectrophotometer with scanning attachment.

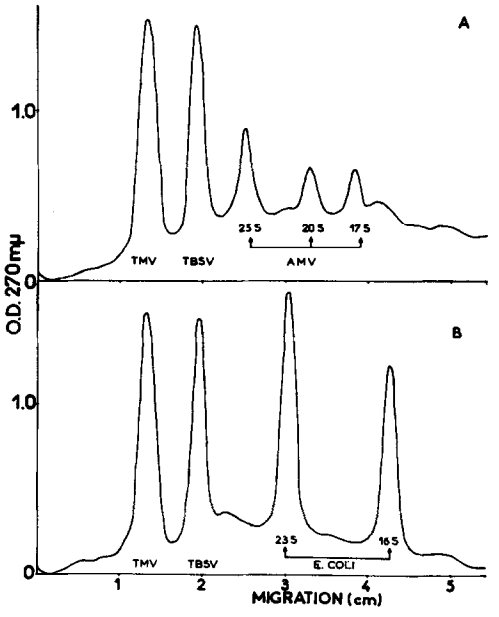

Fig. 2. Scanning at 270 mμ of polyacrylamide gel: (A) TMV-RNA, TBSV-RNA and AMV-RNA; (B) TMV-RNA, TBSV-RNA and E. coli-r RNA. The electrophoresis was performed simultaneously for 4 hr at 8 mA/gel.

3. Results

The molecular weight of TBSV-RNA was determined by two methods:

3.1. *Sedimentation in formaldehyde*

The sedimentation coefficients of TMV, TYMV, TBSV, *E. coli* 23 S and 16 S RNA were determined after reaction with formaldehyde according to the method of Boedtker [9]. Table 1 gives the sedimentation coefficients and corresponding molecular weights of these RNA without correction for viscosity and density; these results are in agreement with those obtained by Boedtker. Fig. 1 shows the calibration curve of the dependence of sedimentation coefficient on RNA molecular weight after reaction with formaldehyde. The TBSV-RNA molecular weight determined from this curve is equal to $1.65 \pm 0.05 \times 10^6$ daltons.

3.2. *Polyacrylamide-gel electrophoresis*

The analysis of TBSV-RNA on polyacrylamide-gel indicates that the preparations contain a single species of high-molecular weight RNA. Fig. 2 shows the separation of a mixture of TMV-, TBSV-, and

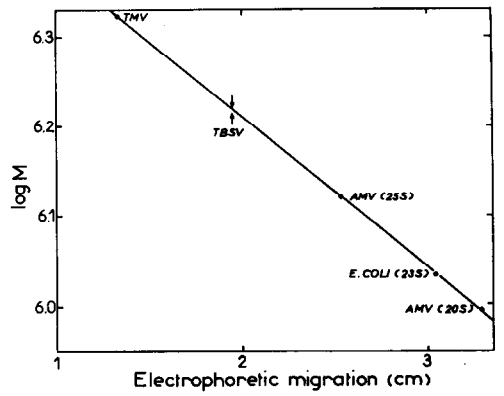

Fig. 3. Semi-log plot of molecular weight against electrophoretic migration of RNA samples in polyacrylamide gel.

AMV- or *E. coli* ribosomal RNA on polyacrylamide gels. The electrophoretic migrations of the RNA samples are given in table 1. Fig. 3 shows the linear relationship of migration to log molecular weight. The migration of TBSV-RNA corresponds to a molecular weight of $1.65 \pm 0.02 \times 10^6$ daltons.

4. Discussion

The molecular weight of TBSV-RNA determined by polyacrylamide gel electrophoresis and by ultracentrifugation analysis after reaction with formaldehyde, has been found to be equal to 1.65×10^6 daltons expressed as the Na salt; thus TBSV contains one RNA molecule of $1.55 \pm 0.02 \times 10^6$ daltons (expressed as the free acid, which is supposed to exist in the virus [10] corresponding to 4790 ± 60 nucleotides of average molecular weight 322.8 (calculated from base the ratio [11]).

The determination of the molecular weight of the major protein component (40,000 ± 2,000) [3, 12, 13] has made consistent the structural model of TBSV proposed by Finch et al. [2]: the virus is composed of 180 major protein subunits rather than 240 as previously proposed as an alternative possibility [14]. Finch et al. have also suggested the existence of a minor protein component within the 12 pentagons of morphological units. This minor protein component of M.W. 28,000 has been recently characterized by Butler [3]. This set of molecular weight data is compatible with the structural model of Finch et al. since the contribution in weight of the two protein components and of one molecule of RNA (1.55×10^6 daltons) gives 9.1×10^6 daltons for the molecular weight of TBSV in agreement with the value of $9.0 \pm 0.2 \times 10^6$ calculated from hydrodynamic measurements of TBSV performed by one of us and other authors [4, 13, 15, 16] (intrinsic viscosity, 3.84 cm^3/g; $S^\circ_{20w} = 133$ S; diffusion coefficient, 1.26×10^{-7} cm^2/sec and partial specific volume, 0.712 cm^3/g).

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