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Molecular Weight Determination of Sendai RNA by Dimethyl Sulfoxide Gradient Sedimentation

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The molecular weight of the large RNA of Sendai virus has been determined by sedimentation analysis in sucrose gradients containing 99% dimethyl sulfoxide (DMSO) to be 2.3×10^6. Sendai RNA recovered from 99% DMSO was found to cosediment with nondenatured Sendai RNA at 46 to 48*s* in ordinary sucrose gradients. The molecular weight value of 2.3×10^6 is considerably smaller than the estimates of 6×10^6 to 7×10^6 determined under nondenaturing conditions, suggesting a unique structure for Sendai RNA.

Sendai virions contain a large single-stranded RNA (3, 4, 18) which is encapsulated in a nucleocapsid structure morphologically similar to tobacco mosaic virus (TMV) but approximately three times as long (14, 15). The molecular weight of parainfluenza viral RNA (Sendai, Newcastle disease virus [NDV], simian virus 5 [SV5]) has been estimated by sucrose gradients under nondenaturing conditions to be approximately 6×10^6 to 7×10^6 (3, 9, 11). We have recently measured the size of this RNA under completely denaturing conditions, 99% dimethyl sulfoxide (DMSO) (28), and have found the molecular weight to be 2.3×10^6, a value considerably smaller than those obtained from sedimentation analyses under nondenaturing conditions. Since Sendai RNA, which was recovered from a DMSO gradient by ethanol precipitation, was found to cosediment in ordinary sucrose gradients with nondenatured Sendai RNA, it is unlikely that this lower-molecular-weight determination was due to fragmentation of the Sendai genome (see Discussion). The implications of this difference are discussed.

MATERIALS AND METHODS

Viral RNA. ^{32}P-Sendai virus (Harris strain, obtained from R. D. Barry, Cambridge, England) and ^{32}P-NDV (California strain, obtained from B. Colby, Storrs, Conn.) were grown in 9-day-old embryonated chicken eggs labeled with 0.5 mCi of $^{32}P_i$ per egg (at time of infection) for 72 h at 34 C and 48 h at 39 C, respectively. ^{3}H-Sendai virus was labeled by preinjecting the eggs one day before infection with 0.3 mCi of ^{3}H-uridine (20 Ci/mmol). Virus was purified as previously described (19), except that the virus was banded once in 25 to 65% sucrose gradients (4 h at 26,000 rpm, 7 C, in the Spinco SW27 rotor) instead of banding in tartrate gradients.

RNA was isolated from the virions as follows. Virus suspensions, at approximately 5 mg/ml in TNE buffer (19), were made 0.2% in sodium dodecyl sulfate (SDS) and 100 μg/ml in Proteinase K (Merck) and incubated for 20 min at 25 C. The solution was then twice extracted with an equal volume of phenol (saturated with TNE) at 25 C, and the water phase was made 0.2 M in NaOAc, pH 5.3. The RNA was precipitated with two volumes of ethanol and left overnight at −20 C. The RNA was then collected by centrifugation, dissolved in distilled water, and centrifuged in sucrose gradients as described below. Fractions containing the fast sedimenting RNA peak (cf. Fig. 1A) were pooled, and the RNA was recovered from the gradient by ethanol precipitation. ^{32}P-labeled Sendai and NDV RNA, on isolation, contained approximately 20,000 counts per min per μg, ^{3}H-labeled Sendai RNA, 3,300 counts per min per μg.

Other RNAs. Radioactive cellular RNAs were obtained by labeling nonconfluent uninfected cultures of mouse kidney cells with 4 μCi of ^{14}C-uridine (60 mCi/mmol) per ml or chicken embryo fibroblast cultures with 25 μCi of ^{3}H-uridine (20 Ci/mmol) per ml for 27 h. RNA was extracted as described by Acheson et al. (1). ^{14}C-RNA contained 10,000 counts per min per μg, and ^{3}H-RNA, 150,000 counts per min per μg.

^{3}H-labeled 45*s* rRNA precursor (220 counts per min per μg), the kind gift of M.-E. Mirault and K. Scherrer, was isolated from Hela nucleoli labeled for 15 min with methionine-*methyl*-^3H CT_3, as previously described (22).

Sucrose gradient sedimentation. Linear sucrose gradients (5-23%) contained 0.1 M LiCl, 10 mM Tris-hydrochloride (pH 7.4), 4 mM EDTA, and 0.1%

SDS. LiCl was used in place of NaCl to aid the solubility of SDS at lower temperatures. Centrifugations were carried out for 105 min at 50,000 rpm, at 7 C, in the Spinco SW56 rotor. Gradients were fractionated by puncturing the bottom of the tube, and drops were collected on paper discs. The paper discs were then dried, washed first in 6% trichloroacetic acid and then in ethanol, dried, and counted by liquid scintillation.

DMSO gradient sedimentation. DMSO gradients were prepared, and sample application was carried out as described by Acheson et al. (1). Because the absolute sedimentation rate of RNA was found to vary considerably in different preparations of the DMSO solutions (presumably due to the hygroscopic nature of the solvent), DMSO solutions were prepared in batch quantities, divided into equal parts, and kept sealed in a desiccator to ensure reproducibility from run to run. Gradients reported here were centrifuged from 3 to 4.5 h at 48,000 rpm, 26 C, in the Spinco SW56 rotor. Gradients were fractionated and counted as described above.

RESULTS

In sucrose gradients containing 0.1 M NaCl, the sedimentation value of Sendai RNA has been measured as 57*s* relative to TMV RNA (3), and 50*s* relative to 28*s* and 18*s* rRNA (18). The RNA of two other type II myxoviruses, NDV and SV5, have both been measured as 50*s* (9, 16). Under the conditions we have used (0.1 M LiCl, see Materials and Methods), the large RNA of four different preparations of Sendai virus was found to sediment as a sharp peak at 46 to 48*s* relative to 28*s* and 18*s* rRNA (Fig. 1A). However, when the same RNA was run in gradients containing 99% DMSO, Sendai RNA was found to sediment just ahead of 28*s* rRNA, at 32*s*. (All sedimentation values in DMSO reported here are relative to the ribosomal RNAs taken as 18*s* and 28*s*, in DMSO). Because of this unexpected finding, the DMSO gradients were calibrated with an RNA marker expected to sediment faster than Sendai RNA in DMSO, namely, the 45*s* ribosomal RNA precursor (29). As can be seen in Fig. 2, the 45*s* rRNA precursor (46*s* under our conditions on sucrose) was found to sediment at 43*s* in DMSO. The molecular weight of Sendai RNA using the abovementioned markers (and R17

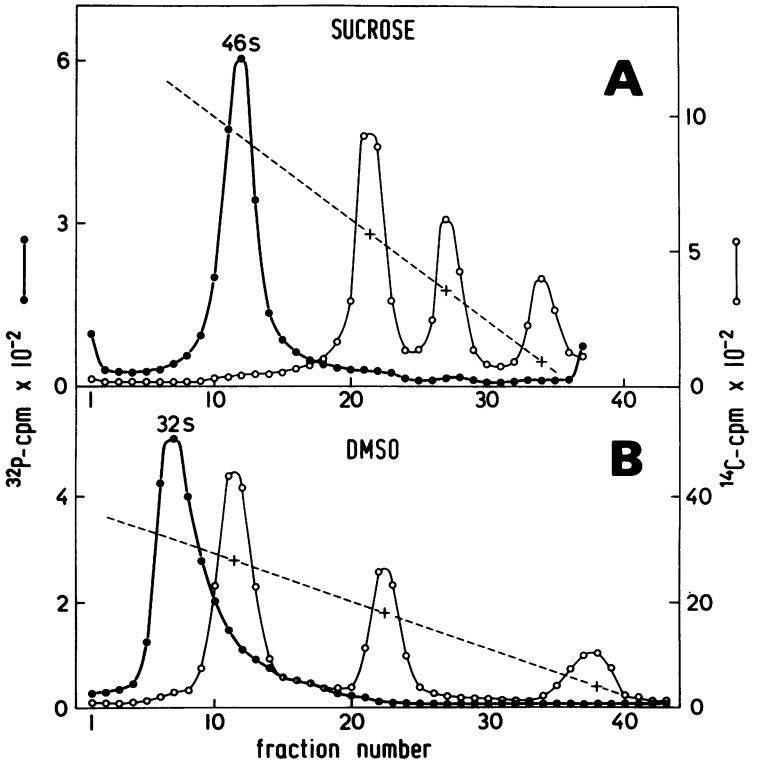

FIG. 1. *Comparison of ^{32}P-Sendai RNA and ^{14}C-cellular RNA (4s, 18s, and 28s RNA) on ordinary sucrose (A) and DMSO gradients (B). Gradient (A) contained 0.6 μg of ^{32}P-Sendai RNA (3,200 counts/min) and 0.5 μg of ^{14}C-cellular RNA. Gradient (B) contained 0.6 μg of ^{32}P-Sendai RNA and 2.0 μg of ^{14}C-cellular RNA. Conditions of centrifugation and counting are described in Materials and Methods.*

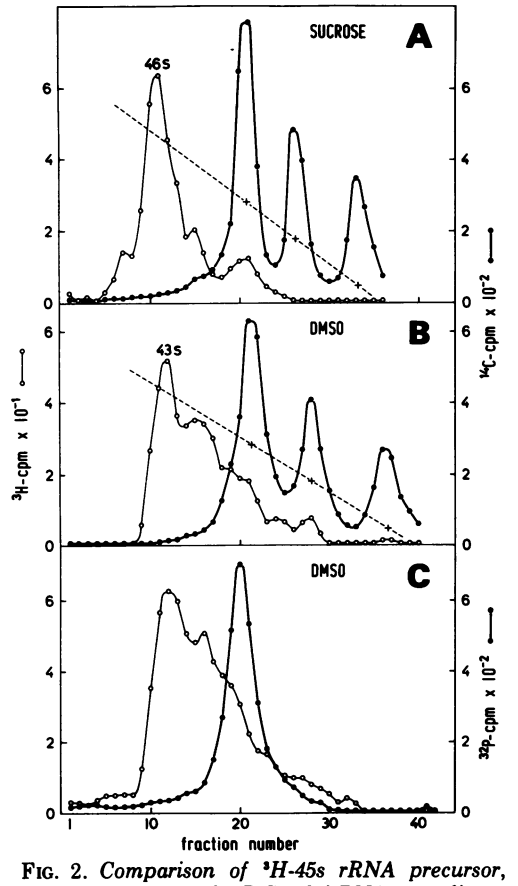

FIG. 2. *Comparison of ^{3}H-45s rRNA precursor, ^{14}C-cellular RNA, and ^{32}P-Sendai RNA on ordinary sucrose (A) and DMSO gradients (B) and (C). Gradient (A) contained 2.9 μg of ^{3}H-45s rRNA and 2.0 μg of ^{14}C-cellular RNA. Gradient (B) contained 2.9 μg of ^{3}H-45s rRNA and 2.0 μg of ^{14}C-cellular RNA. Gradient (C) contained 2.9 μg of ^{3}H-45s rRNA and 2.0 μg of ^{32}P-Sendai RNA (4,100 counts/min).*

RNA, data not shown) was estimated to be 2.3 $\times$ 10^6 (Fig. 3).

The possibility that, as for Rous sarcoma virus (RSV) RNA (10, 12, 23), the large difference in sedimentation rate of Sendai RNA in DMSO and sucrose gradients might be due to disaggregation of a segmented genome in DMSO was next examined. ^{32}P-labeled Sendai RNA was prepared by first sedimenting it on a DMSO gradient, and the RNA sedimenting at 32s_{DMSO} was recovered from the gradient by ethanol precipitation after the profile of the gradient had been determined by Cerenkov counting (cf. Fig. 1B). This Sendai RNA was then mixed with nondenatured, ^{3}H-labeled Sendai RNA and cell RNA markers and resedimented in an ordinary sucrose gradient. The results, shown in Fig. 4, demonstrate that Sendai RNA sediments at the same position, 46 to 48*s*, even after recovery from the DMSO gradient. Thus, unlike RSV RNA, the sedimentation behavior of Sendai RNA in sucrose gradients is unchanged by DMSO treatment, and 46 to 48*s* Sendai RNA appears to be a single, continuous RNA chain of 2.3 $\times$ 10^6 (see Discussion).

Although the molecular weight of Sendai RNA has not previously been measured under denaturing conditions, Duesberg (10) has measured the molecular weight of the large RNA of NDV, another type II myxovirus, in DMSO gradients as 6.3 $\times$ 10^6 daltons. We have therefore compared the large RNAs of Sendai and NDV on both ordinary sucrose and DMSO gradients. We find that Sendai and NDV RNA are indistinguishable in sedimentation rate in both ordinary sucrose (reference 4, Fig. 5A) and DMSO gradients (Fig. 5B), and therefore conclude that the large RNA of NDV, like that of Sendai virus, has a molecular weight of 2.3 $\times$ 10^6.

DISCUSSION

As previously mentioned, Duesberg (10) has reported the molecular weight of NDV RNA in

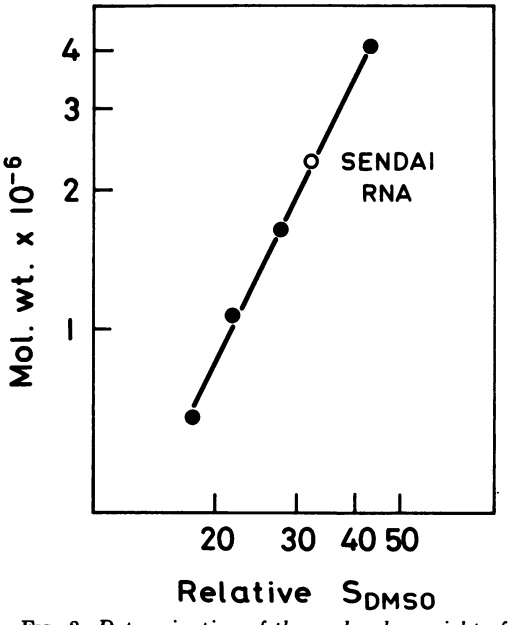

FIG. 3. *Determination of the molecular weight of Sendai RNA by sedimentation in DMSO gradients. RNAs used as reference markers were: 18s rRNA, 0.65 $\times$ 10^6 (20); R17 RNA, 1.05 $\times$ 10^6 (12); 28s rRNA, 1.65 $\times$ 10^6 (20); 45s rRNA precursor, 4.1 $\times$ 10^6 (27). All s_{DMSO} are relative to 28s and 18s rRNA taken as 28s and 18s in DMSO. The molecular weight of Sendai RNA, represented by an open circle, is determined as 2.3 $\times$ 10^6.*

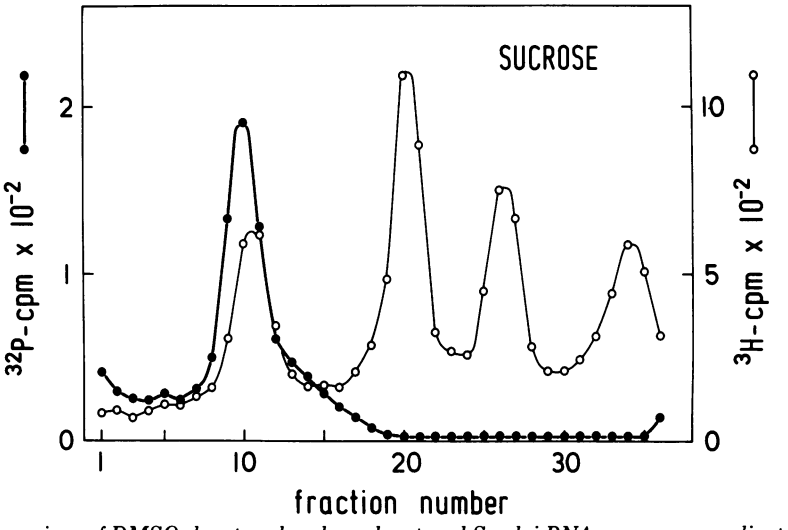

FIG. 4. *Comparison of DMSO-denatured and nondenatured Sendai RNA on sucrose gradients. ^{32}P-Sendai RNA (3.5 μg, 10,400 counts/min) was first sedimented in a DMSO gradient (cf. Fig. 1B), and the profile of the gradient was determined by Cerenkov counting. RNA present in the three peak tubes was recovered from the gradient by ethanol precipitation and dissolved in distilled water. A sample of this material (1,200 counts/min) was mixed with 1.1 μg of nondenatured ^{3}H-Sendai RNA and 0.09 μg of ^{3}H-cellular RNA and then sedimented in an ordinary sucrose gradient.*

DMSO gradients to be 6.3×10^6, whereas we find both NDV and Sendai RNA to be 2.3×10^6, using similar methods. The molecular weight value of 6.3×10^6, however, was obtained by means of the empirical equation determined by Strauss et al. (28) for the absolute sedimentation rate of RNA in the analytical ultracentrifuge and not by extrapolation from the sedimentation rate of RNAs of known molecular weight determined under similar conditions, i.e., sucrose gradients in DMSO. Because of the large effects of sucrose on the viscosity of solutions, it is unlikely that the empirical equation determined by Strauss et al. was applicable in this case.

The large difference in molecular weight between that expected for a 46 to 48*s* RNA (27) and that measured under denaturing conditions can be accounted for if Sendai RNA contained a great deal more secondary structure than the ribosomal RNAs. Sendai RNA has recently been shown to self-anneal, to variable extents (16–60%, references 24, 25) in a concentration-dependent fashion. However, as Portner and Kingsbury (24) have pointed out, the fact that the RNA had been degraded to approximately 10*s* during the annealing reaction did not allow them to distinguish whether this annealing was intra- or intermolecular. We have also found that Sendai RNA self-anneals (15–25%, data not shown), and if this annealing is in fact intramolecular, it could explain the abovementioned discrepancy in sedimentation rate under denaturing and nondenaturing conditions.

There is, however, another possibility to explain this discrepancy. Our conclusions that the 46 to 48*s* RNA is composed of a single chain of 2.3×10^6 molecular weight is based on the unaltered sedimentation rate of Sendai RNA on sucrose gradients after DMSO treatment (cf. Fig. 4). However, if Sendai RNA were capable of rapid and quantitative reassociation (e.g., by annealing of complementary ends) due to the increase in concentration when the RNA is removed from the denaturing solvent by ethanol precipitation and subsequent solution in water, then the 46 to 48*s* RNA could be composed of more than one RNA chain of 2.3×10^6 molecular weight. Although this latter possibility seems unlikely, there is some evidence in its favor. In the virion, parainfluenza virus RNA occurs in the form of a nucleocapsid which is remarkably similar in structure to TMV (6), but approximately 3.3 times as long (6–8, 14, 15, 17). These nucleocapsids have been shown to contain from 3.7 to 4.1% RNA (7, 14), only slightly less than the 5.1% RNA determined for TMV (19), whose RNA has a molecular weight of 2.1×10^6 (5). These nucleocapsids should therefore contain 5.0×10^6 to 5.6×10^6 daltons of RNA. Since the molecular weight of Sendai RNA as determined by DMSO gradient sedimentation is

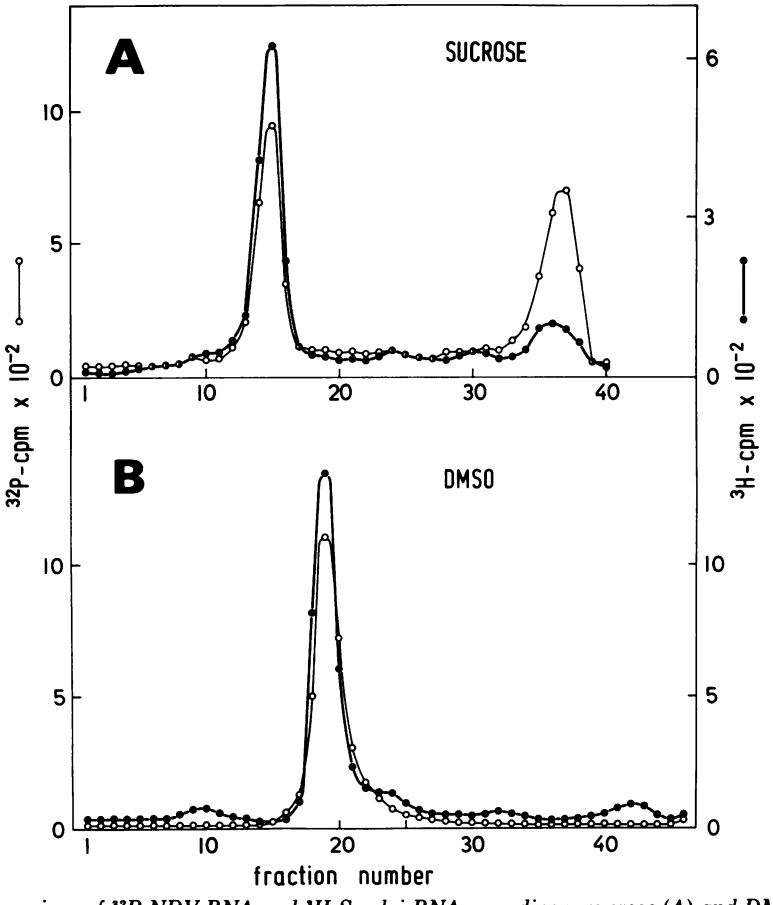

FIG. 5. *Comparison of ^{32}P-NDV RNA and ^{3}H-Sendai RNA on ordinary sucrose (A) and DMSO gradients (B). Gradient (A) contained 38,000 counts/min of ^{32}P-NDV virions and 0.8 μg of ^{3}H-Sendai RNA. The samples were suspended in 75 μliters of water, made 1% in SDS, and then sedimented on an ordinary sucrose gradient. Gradient (B) contained 0.4 μg of ^{32}P-NDV RNA (4,600 counts/min) and 1.6 μg of ^{3}H-Sendai RNA.*

well below this value, Sendai nucleocapsids should therefore contain more than one chain of RNA. It is possible that the structure responsible for these multiple chains of RNA in the nucleocapsid is reassumed on removal of the RNA from DMSO; this reassociation would of course be concentration dependent.

In conclusion, we would like to add a note of caution. This discrepancy between sedimentation value in ordinary sucrose and DMSO gradients is not unique to Sendai RNA. Arif and Faulkner (2) have recently shown that 42*s* Sindbis RNA sediments only slightly faster than 28*s* rRNA in DMSO gradients, and that this RNA, when recovered from a DMSO gradient, coelectrophoreses on polyacrylamide gels with untreated 42*s* RNA. It seems clear that these animal viral RNAs are different from rRNA and bacteriophage RNA in this respect. Even though these RNAs have been purified in a similar manner as rRNA and bacteriophage RNA, the possibility cannot be excluded that they still contain material which can differentially affect their buoyant density in water and DMSO (the inability of Simmons and Strauss [26] to sediment Sindbis viral RNA as a discrete band in DMSO gradients is noteworthy in this respect), thus making molecular weight estimation of these viral RNAs in DMSO gradients unreliable using rRNA and bacteriophage RNA as markers.

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