SOME PHYSICAL PROPERTIES OF INFECTIVE RIBOSE NUCLEIC ACID ISOLATED FROM TOBACCO MOSAIC VIRUS

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SUMMARY

Ribose nucleic acid (RNA) prepared from essentially monodisperse tobacco mosaic virus (TMV) by heating dilute salt solutions of the virus to 90° for 1 to 3 minutes, was found to be infective. The RNA displayed a quite narrow distribution of sedimentation constants, 50 % of the molecules sedimenting between 27 and 30 S, even though a slower sedimenting component (~ 20 S) was usually observed. The molecular weight determined from both light-scattering and sedimentation-viscosity measurements was 1.94 ± 0.16 million and the radius of gyration (6°, ionic strength of 0.06) was 316 Å. The latter was extremely sensitive to temperature and ionic strength, indicating that the polynucleotide chains of the RNA molecules were flexible, and not at all rigid or rodlike in character. Prolonged standing, even at 5°, invariably resulted in a loss of infectivity, a broadening of the distribution of sedimentation constants, and a molecular weight decay. As the latter was quite irreproducible quantitatively, even under identical experimental conditions, it would appear that the instability was due to an impurity, possibly leaf ribonuclease, carried along in the preparation, rather than an inherent property of the RNA molecule.

INTRODUCTION

Highly purified solutions of ribose nucleic acid (RNA) prepared from tobacco mosaic virus (TMV) were shown to be infective by GIERER AND SCHRAMM[1] and by FRAENKEL-CONRAT[2] some two years ago, and their results were later confirmed by COMMONER[3]. The fact that it was possible to isolate RNA which is biologically active made possible a study of the correlation between this activity and the physical properties of the molecule. In addition to the intrinsic interest of this problem, it was important because, unlike desoxyribose nucleic acid (DNA), RNA obtained from a wide variety of sources appeared to be a quite structureless molecule of variant molecular weight. Prior to this there was no way of deciding whether the absence of a unique three-dimensional structure was characteristic of native or biologically active RNA, or whether the essential native structure had been destroyed during the isolation process in all samples thus far examined. Moreover, in view of the increasing number of inter-

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esting three-dimensional structures which have been proposed for synthetic polynucleotides[4], the question of whether or not naturally occurring biologically active RNA has a secondary structure, in the absence of protein, seems particularly pertinent.

Although concurrence has been reached on the main aspects of the infectivity of TMV RNA, little is known about the physical nature of the infective molecule. Since the molecular weight of TMV rods 3000 Å long is 39 million[5], the maximum possible molecular weight of RNA is 2.15 million (assuming 5.5 % by weight of the TMV is RNA*). Hence it is possible, in principle, to determine the number of RNA molecules per virus particle by measuring the molecular weight of RNA prepared from monodisperse TMV. The results published to date, however, give molecular weights ranging from 200,000 to about 2,000,000, there being a considerable controversy as to whether there are one or many RNA molecules per virus particle[6-9].

From the start we were aware that RNA is a particularly elusive molecule and that some of the discrepancies in the published results could be attributed to the irreproducibility of the measurements made on RNA. Not only infectivity studies but also physical chemical results obtained on the infective molecule were sufficiently diverse to be suspect. For example, SCHUSTER, SCHRAMM AND ZILLIG[8] quote sedimentation constants between 13.5 and 18.5 S, and reduced specific viscosities between 0.8 and 1.4 (100 ml/g), while FRAENKEL-CONRAT[9] reports that his preparations had sedimentation constants from 6 to 10 S. In both of these cases the range of values given is so much greater than the experimental error of the measurement that the explanation must lie elsewhere. The only reproducible set of data yet presented on TMV RNA is that of SINSHEIMER AND HOPKINS[7], who examined 13 different preparations by light scattering and obtained a result of 1.70 $\pm$ 0.07 million. Unfortunately infectivity measurements were not made on these samples.

There appear to be three possible explanations for the irreproducibility of the data on RNA. First of all, most of the RNA has been prepared from TMV of unspecified uniformity, and one cannot expect RNA to be more uniform or constant in its molecular properties than the TMV from which it is prepared. Second, the isolation methods all involve steps which could damage the RNA molecules. In each of these methods, the TMV protein is separated from the RNA by denaturing it. The difference between the methods is in the denaturing agent used, heat, detergent (sodium dodecyl sulfate), or phenol. The critical step lies in the prompt separation of the RNA from the denaturing agent after the protein has been denatured. Unless this is done, a degraded inactive product is obtained[8]. It is in the difficulty in performing this feat reproducibly that the large errors arise. Finally, RNA preparations, even if the same initially, may be quite different a few hours after they have been prepared because they are not all equally stable.

In addition to the uncertainty about the size of the infective molecule, the shape of the RNA molecule in solution was not well defined. Extensive X-ray work on intact TMV by FRANKLIN[10] and CASPAR[11] showed the RNA to be imbedded within the TMV protein at a radius of 40 Å. One possible arrangement was that the polynucleotide chain be a single-stranded helix with a pitch of 23 Å. There is certainly some doubt as to whether such a helix would be stable once the protein had been removed, but electron micrographs obtained by HART[12] on partially denatured TMV

* The value of 5.5 % was used here because this is consistent with there being 3 phosphorus atoms per TMV protein subunit.

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seemed to indicate that it was. SINSHEIMER and SCHRAMM both originally assumed that RNA was a rod in solution, although the former did acknowledge that it ceased to be a rod in the presence of salt. More recently, GIERER suggested that RNA has a shape intermediate between that of a rod and a coil[6]. The configuration of RNA thus remained in doubt.

The purpose of this investigation was to study the molecular weight, size, and shape of the RNA molecule. To do this, we were naturally primarily interested in solutions of RNA which proved to be infective. For this reason infectivity tests were made on many of the samples studied. Although we plan to study the physical properties of RNA prepared by each of the three different methods (heat, detergent and phenol), the present report is restricted to light scattering, sedimentation, and viscosity measurements on RNA samples prepared by heating TMV in dilute salt solutions.

EXPERIMENTAL METHODS

TMV samples

RNA was only prepared from TMV samples which had a very narrow size distribution. The preparative method, a modification of the differential centrifugation commonly used, has been described previously[5]. Two TMV samples examined in detail had a molecular weight of 39.0 $\pm$ 1.2 million based on light-scattering and sedimentation-viscosity measurements, and an electron micrograph length of 3020 Å[5]. The uniformity of other TMV samples was checked by measuring the flow birefringence length and the sedimentation constant.

Preparation of RNA

RNA was prepared by a slight modification of the original COHEN AND STANLEY method[13]. 5 to 20 ml of TMV (approximately 1 g/100 ml) in dilute neutral salt solutions were heated by immersing a pyrex test tube in a water bath kept at temperatures ranging from 85 to 95° for time intervals of 1 to 6 min. From the standpoint of yield, reproducibility, and stability, the following conditions appeared necessary: the volume should not exceed 10 ml, the concentration of TMV should be less than 1.2 g/100 ml, the ionic strength greater than 0.02, the pH between 7 and 8.5, and the solutions placed in a bath at 90° $\pm$ 1° for 2 min. Under these conditions, the RNA was kept at 90° for less than 30 sec after the protein has coagulated. After removal from the high temperature bath, the suspension was rapidly cooled in an ice bath. In a cold room (4°), the RNA was separated from the denatured TMV protein by filtering through a medium sintered glass filter using a pad of Johns-Manville Hyflo Super-Cel. If the filtrate did not appear absolutely clear, it was refiltered through a fresh filter, but this was seldom necessary. The filtrate was then used immediately without further purification, or frozen and stored at —20°. Yields of about 4–5 % by weight were obtained. This method made it possible to measure the viscosity and infectivity 15 min after the TMV protein had been denatured, and the sedimentation and light scattering within an hour.

Handling of RNA

RNA solutions were never exposed to temperatures greater than 5° unless the effect of temperature was being studied. Sterile techniques were used whenever

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possible after early experiments revealed that RNA solutions were very susceptible to mold and bacteria. Except in viscosity measurements, $CHCl_3$ was added as an extra precaution. Concentrations were kept as high as possible until just before measurements were made since we found that RNA solutions were much less stable when dilute. For example, an 0.044 g/100 ml solution kept at 25° for 30 min, cooled to 4°, diluted and centrifuged at 4°, had the same sedimentation constant (26.1 S) and distribution as the control (stored at 4° throughout). If, however, the solution was diluted to 0.0024 g/100 ml in the cold, allowed to come to room temperature (21°) and then centrifuged there, the sedimentation constant fell to 11.4 S. Although we have no simple explanation for this effect, it may explain some of the anomalies in the results obtained by others. On the other hand we observed less of a solvent effect than has been reported[3,9]. Sedimentation and infectivity results indicated no difference in the stability of RNA over an ionic strength range from 0.020 to 0.20 (phosphate buffer) nor did adding a chelating agent (versene, pH 7.5) enhance the stability. For this reason we cannot conclude that RNA is stabilized at higher concentrations by trace amounts of metal ions, even though our results are too incomplete to rule out this possibility.

Storage of RNA

No change in infectivity or in sedimentation distribution could be detected on solutions which were frozen and stored at —20° providing they were only frozen and thawed once. Sedimentation was measured initially and after seven days at —20°. Usually aliquots of not greater than 10 ml were frozen in a round bottom flask.

Infectivity tests

Assays were performed using *Nicotina glutinosa* and the half-leaf method. Samples were mixed with 600 grit carborundum and applied to the leaves with brushes commonly used to spread butter. Under optimal conditions our TMV samples produced 200–300 lesions per μg of TMV while our most infective TMV RNA preparations assayed 3–5 lesions per μg of RNA.

Several facts about our RNA infectivity should be emphasized. Firstly, the infectivity of heat-prepared TMV RNA is quantitatively similar to that reported by GIERER AND SCHRAMM for TMV RNA prepared by phenol treatment[1]. Our best preparations give between 3–5 lesions/half leaf/μg RNA. Secondly, most infectivity is destroyed by brief contact with very small amounts of RNAse (1/50 μg for 10 min at 20°). Some residual infectivity (1 to 25 % of the RNA infectivity) is usually observed following RNAse treatment. Centrifugation experiments indicate that this is most likely due to small amounts of undenatured TMV particles. Two of our RNA preparations were centrifuged in a swinging bucket rotor and a comparison made of the infectivity of the pellet and supernatant fractions. In both experiments the vast majority of the RNAse-resistant infectivity was pelleted by a single high speed centrifugation which did not appreciably sediment the RNA.

Concentration determinations

Concentrations were determined by measuring the optical density of the solutions at 258 mμ using 1-cm cells in a Beckman Model DU spectrophotometer. Measurements were made at room temperature (~ 20°) unless otherwise noted. The extinction

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coefficient in g/100 ml is 225 for dilute salt solutions, and 258 in water[13]. SINSHEIMER reported 252 in both water and salt solutions[7], and GIERER obtained 258 in water[15], indicating that the value is at least confirmed for aqueous solutions. The optical density was measured as a function of temperature by circulating thermostated water through the compartment around the cell housing. A blank cell was used to measure the temperature of the solution.

Intrinsic viscosity

Ostwald-Fenske viscometers requiring 4 ml and having a water flow time of about 240 sec at 25° and 430 sec at 5° were used to measure the viscosity of RNA solutions. When possible, several concentrations were measured simultaneously, and changes could then be followed for several days.

Sedimentation constant

Sedimentation velocity studies of RNA solutions (about $3 \cdot 10^{-5}$ g/ml) were carried out in a Spinco Model E Ultracentrifuge equipped with ultraviolet absorption optics, and during the later series of runs, with temperature control. The chamber, rotor, and centrifuge cells were always precooled to 4° and sedimentation runs made at temperatures between 3 and 6° unless otherwise noted. A Spinco Analytrol was used to read the films and the arithmetic mean sedimentation constant determined by measuring the rate at which half the molecules sedimented. The sedimentation constant was then corrected to that for water at 20° as described previously[16].

Light scattering

Light-scattering measurements were made in a slightly modified Brice-Speiser photometer using a thermostated cell in which the temperature could be maintained between 5 and 6° for several days[16]. Water was prevented from condensing on the exposed cell walls by keeping a large supply of fresh silica gel in the photometer housing and by dehumidifying the room in which the photometer was kept. It was assumed that dn/dc was 0.188 at 4360 Å, a value obtained for desoxyribose nucleic acid (DNA)[17] and in fair agreement with the value of 0.194 obtained by SINSHEIMER AND NORTHROP[18].

THE MOLECULAR WEIGHT, SIZE, AND SHAPE OF TMV RNA

Since the tobacco mosaic virus used in this investigation was essentially monodispersed with a molecular weight of 39.0 ± 1.2 million, we are able to set the maximum possible molecular weight of TMV RNA at 2.15 million*. The number of RNA molecules per virus particle can therefore be obtained by measuring the molecular weight and molecular weight distribution of RNA. And although infectivity measurements were not carried out on every RNA preparation studied, enough tests were made to enable us to associate the biological activity of our samples with certain physical properties. These are discussed below.

Sedimentation

The ultraviolet absorption sedimentation diagrams of 18 different freshly prepared samples of RNA were measured in phosphate buffer at several different ionic

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strengths at about 5° and the results of sixteen of these are summarized in Table I.

The other two had sedimentation constants below 20 S, and exhibited no infectivity, and were therefore excluded.

TABLE I

"MEAN" SEDIMENTATION CONSTANTS OF RNA SOLUTIONS

Number of samples measured	*Ionic strength*	*Average Sw,20°*
3	0.020	24.4 ± 0.3
11	0.04–0.06	27.1 ± 1.5
2	0.20	29.0 ± 0.5

More impressive than the high values of the sedimentation constants was the relatively sharp distribution obtained. A typical distribution of sedimentation constants is shown in Fig. 1. We find that 50 % of the solute species sediment over a very narrow range, between 27 and 30 S. It is important to point out that while the sedimentation constant was found to be somewhat dependent on ionic strength (Table I), the distribution was not.

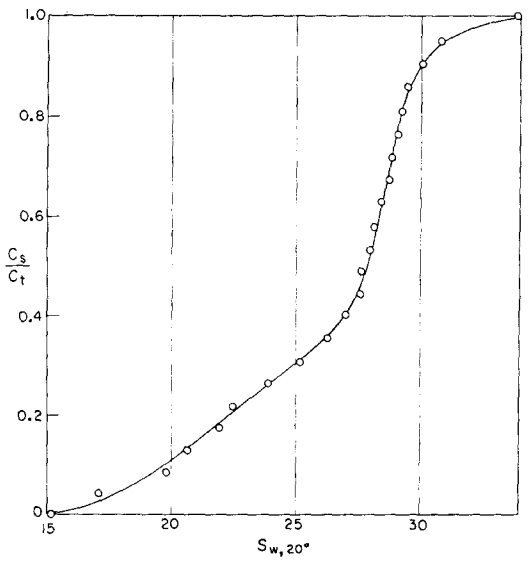

Fig. 1. Distribution of sedimentation constants: RNA 16, phosphate buffer, pH 8.5, $\Gamma/2 = 0.06$, $c = 1.50 \cdot 10^{-5}$ g/ml, after 42 min at 39,460 rev./min.

Although the distribution of sedimentation constants shown in Fig. 1 is quite sharp, there nevertheless seem to be two distinct components present, one sedimenting with an average $S = 28.6$, and the other with $S = 21.3$. The relative amount and value of the slower component varies somewhat from preparation to preparation, but we have not yet succeeded in making a preparation in which it is totally absent. It is highly probable that this low S component is the result of damage to the RNA molecules during the preparation. By way of comparison, it should be pointed out,

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however, that the distribution shown in Fig. 1 is very much sharper than that obtained for the best DNA samples measured to date.

The change in sedimentation constant with time was measured on four different RNA preparations, all of which were stored at 4°. In one case, a 40% drop was observed in four days (from 24.5 to 17.6 S) while in another case the sedimentation constant changed only 8% in ten days (from 27.2 to 25.1 S). The distribution of sedimentation constants always became broader, however, by a factor of two or more, resulting in an appreciable drop in the fraction of molecules having sedimentation constants between 27 and 30. This fraction may be a far more critical number than the mean sedimentation constant.

Viscosity

Results obtained for the reduced specific viscosity of seven different samples of RNA measured at 5° are shown in Fig. 2 and summarized in Table II. It is evident

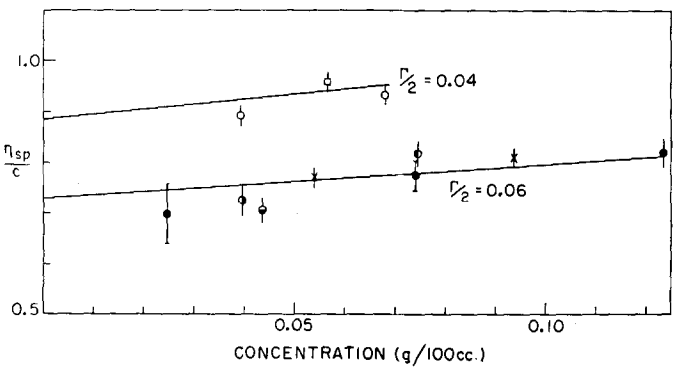

Fig. 2. Viscosity of RNA solutions: × RNA 4, ● RNA 5, ○ RNA 7, □ RNA 8, ◐ RNA 14, ◑ RNA 16, ◒ RNA 17.

TABLE II

VISCOSITY OF RNA SOLUTIONS AT 5°

$\Gamma/2$	*Solvent*	*pH*	*c (g/100 ml)*	η_{sp}/c	$[\eta]^*$	$[\eta]_{av.}$
0.20	Phosphate	7.0	0.027	0.82	0.814	0.81
0.08	Phosphate		0.0935	0.81	0.786	
	and versene	7.5	0.0537	0.77	0.757	0.77
0.06	Phosphate	8.5	0.0745	0.82	0.802	
			0.0435	0.705	0.694	0.74
			0.0396	0.725	0.715	
0.06	Versene	7.5	0.1235	0.82	0.79	
			0.0740	0.775	0.757	0.75
			0.0247	0.70	0.694	
0.04	Phosphate	7.0	0.068	0.935	0.918	
			0.0565	0.96	0.946	0.92
			0.0392	0.895	0.886	
0.02	Phosphate	7.0	0.067	1.10	1.08	
			0.032	1.19	1.18	1.13
0	Water	6.0	0.023	1.60	1.59	1.59

* Estimated using k' 0.5 in the Huggins equation.

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that at ionic strengths below 0.06, the reduced specific viscosity is extremely sensitive to the salt concentration. It should be noted that the same preparation (RNA 4) was used in making the measurement in water and phosphate buffer at ionic strengths of 0.020, 0.080 and 0.20, and hence the difference reported above cannot be attributed to differences in samples. Moreover, at ionic strengths greater than 0.04, we find a fairly reproducible value of 0.756 $\pm$ 0.036 for the estimated intrinsic viscosity.

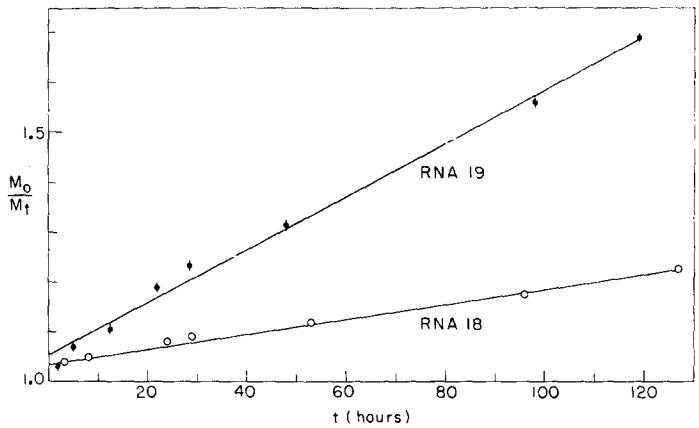

Fig. 3. "Spontaneous" degradation of RNA in phosphate buffer, pH 8.5, at 6°: ○ RNA 18, ● RNA 19.

If we compare our result with the intrinsic viscosities reported by other investigators for RNA samples measured at comparable ionic strengths, we find that our result is appreciably lower. The values range from 1.1 to 1.6[6,8,19,20] although an intrinsic viscosity as low as 0.80 has been reported recently[15]. It would seem that the intrinsic viscosity of infective RNA can vary a good deal. Nevertheless the lower value is in better agreement with the light-scattering dimensions obtained in this investigation (see below) and those reported by SINSHEIMER AND HOPKINS[7].

On the whole we found the reduced specific viscosity an insensitive measure of either the molecular weight or the true stability of an RNA sample. In several cases there was no detectable change in the viscosity with time although the sedimentation constant had decreased by 10% or more. In one RNA preparation, which had no biological activity, and which had a light-scattering molecular weight of 1.1 million and a sedimentation constant of 19 S, the reduced specific viscosity of a 0.049 g/100 ml solution was 0.785. Until these anomalies in the viscosity of RNA solutions can be explained, it seems unwise to use this property to characterize RNA preparations.

Light scattering

Molecular weights and sizes were measured on four different RNA samples, all in phosphate buffer (pH 8.5, ionic strength 0.06). Because of the instability of the solutions it was only feasible to measure the scattering of a single concentration, and to correct to zero concentration by assuming $B = 1 \cdot 10^{-4}$. As this is only a 5% correction, it is the same order of magnitude as errors due to concentration and dn/dc measurements, and hence not too serious a matter. The results are given in Table III.

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Although these molecular weights are comparable to those obtained by SINSHEIMER[7] the reproducibility is not good. Part of the explanation for this is that these samples had quite different distribution of sedimentation constants even though the mean sedimentation constant was almost identical. Furthermore, the stability of the solutions was quite different.

TABLE III

LIGHT SCATTERING OF RNA SOLUTIONS

Sample	$Kc/R_\theta \cdot 10^7$	c (g/ml $\cdot 10^4$)	$(Kc/R_\theta)_{c=0} \cdot 10^7$	$M \cdot 10^{-6}$	R_g (Å)
16	5.00	1.24	4.75	2.10	306
18	5.85	0.79	5.70	1.75	316
19	6.54	1.11	6.32	1.58	330
20	5.20	1.25	4.95	2.02	312

The fall in molecular weight was followed for several days on samples 18 and 19. Both of these samples were prepared from the same sample of TMV, and were measured in the same solvent at the same temperature. The molecular weight of RNA 18 fell only 20 % in 100 h while that of RNA 19 fell 60 % over the same time interval. Since the pH, ionic strength, and temperature were the same, the molecular weight decay certainly cannot be attributed to a simple hydrolysis of the phosphodiester bond even though the rate appeared to be first order in both cases (see Fig. 3). The best explanation for these data appears to be that both our preparations contained a contaminant which catalyzes the hydrolysis, and that the concentration of the latter varies from sample to sample. RNAse is a logical candidate but thus far we have not been able to obtain any direct evidence to support this suggestion.

The light-scattering radius of gyration was apparently more reproducible than the molecular weight. The average of the 4 values listed in Table III is 316 $\pm$ 7 Å, and this precision is as good as could be expected. The root-mean-square end-to-end distance $(r^2)^{1/2}$ calculated from the radius of gyration is 770 Å, while that obtained from the viscosity measurements using the FLORY-FOX equation[21] is 900 Å. It is surprising to find the viscosity giving a higher value than the light-scattering measurement and we interpret this result in one of two ways. Either the measured viscosity is too high, or the light-scattering dimension is too low because of unusually large deviations from ideality. The latter is the more probable explanation because the radius of gyration was measured at a finite concentration, rather than on data extrapolated to infinite dilution. This point will be clarified once RNA solutions can be stabilized sufficiently to permit light-scattering measurements at several concentrations.

Since the viscosity and sedimentation constant of sample 16 are known, the molecular weight can be calculated from the FLORY-MANDELKERN equation[21] and the result compared to that obtained from light-scattering data, if the assumption of a randomly coiled configuration is made. When we do this we obtain a sedimentation-viscosity molecular weight of 1.78 million from $S = 27.9$ and $[\eta] = 0.715$, using a value of 0.55 for the partial specific volume and $2.3 \cdot 10^6$ for $\Phi^{1/3}P^{-1}$. The agreement is as good as should be expected considering the uncertainties in the value of the partial specific volume and of $\Phi^{1/3}P^{-1}$, in determining the sedimentation-viscosity molecular

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weight, and in dn/dc and c and in determining the light-scattering molecular weight. For example, if we had made no non-ideality correction and had used Sinsheimer's value for dn/dc (0.194), the light-scattering molecular weight would be reduced to 1.87 million for sample 16. Or if we had used a value of $2.1 \cdot 10^6$ for $\Phi^{1/3}P^{-1}$, the sedimentation-viscosity molecular weight would have been raised to 2.04 million. We conclude, therefore, that the molecular weight of RNA 16 is 1.94 ± 0.16 million.

By using the FLORY-MANDELKERN equation to calculate the molecular weight, we have assumed that RNA is a random coil rather than a rigid rod or ellipsoid. The agreement in molecular weight which we obtained tends to justify our assumption. Better proof for the model is found, however, in comparing our results with those obtained on RNA from calf liver microsomes. Here we find that our values for S, $[\eta]$ and M fit very well on the log–log plots of S *vs.* M and $[\eta]$ *vs.* M obtained for the microsomal RNA[22]. Such a result implies that both RNA samples consist of homologous molecules, and since microsomal RNA was shown to exist in a random coil configuration, TMV RNA should have the same configuration. Moreover the ionic strength dependence of the viscosity, as well as of the light-scattering dimension reported by SINSHEIMER, already showed RNA to be a flexible molecule. The temperature dependence of the viscosity and light-scattering dimension corroborated this.

Effect of temperature

After preliminary measurements indicated that the viscosity of RNA solutions increased when the temperature was raised from 5 to 25°, we examined this effect more carefully. The light-scattering result obtained when a solution was measured first at 6°, then at 25°, and finally at 6° again is shown in Fig. 4. Except for a small decrease in molecular weight, the latter remained unchanged while the radius of gyration changed considerably. The results are summarized in Table IV together with the viscosity results obtained on the same sample. The 50 % expansion in the molecular volume was only partially reversible, indicating that the original structure cannot be reformed completely in a short time. The possibility that the RNA molecules were becoming more asymmetric due to an uncoiling and denaturing similar to that observed for globular proteins was naturally considered. To test this, the infectivity and sedimentation constant were measured and we found that there was no loss in infectivity and no change in sedimentation after 30 min at 25°. This indicates that the expansion of the RNA molecule between 5 and 25° does not involve an irreversible denaturation of a native structure.

Some additional insight into the effect of temperature on RNA solutions was obtained by measuring the optical density as a function of temperature and ionic

TABLE IV

EXPANSION OF RNA MOLECULES WITH TEMPERATURE

Temperature	*Molecular weight** $\times 10^{-6}$	*Radius of gyration (Å)*	$(\eta_{sp}/c)_{c=0.04\%}$	$\frac{[\eta]M}{r^3} \times 10^3$
Initial, 6°	2.00	306	0.725	3.5
25°	1.90	375	1.10	3.8
Returned to 6°	1.92	326	0.82	3.2

* Not corrected to zero concentration.

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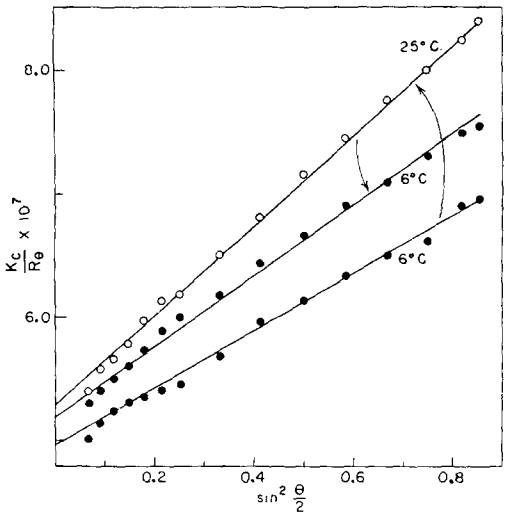

Fig. 4. Light scattering, showing the reversible expansion of RNA molecules: RNA 16, $c = 1.24 \cdot 10^{-4}$ g/ml, phosphate buffer, pH 8.5, $\Gamma/2 = 0.06$, ● 6°, ○ 25°.

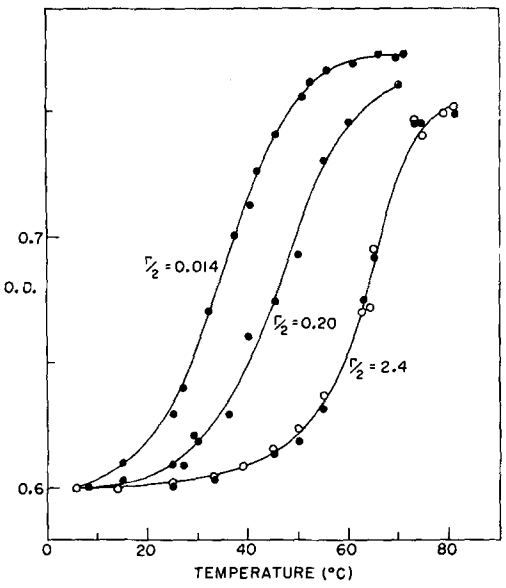

Fig. 5. Optical density of RNA solutions as a function of temperature and ionic strength: RNA 23, phosphate buffer (1:1), ○ preheated to 90° for 10 min.

strength. This experiment was undertaken because of the very interesting results obtained by HALL on RNA prepared from calf liver microsomes[22]. Our results, shown in Fig. 5, show that the optical density, measured at the ambient temperature, increases significantly over a rather narrow temperature interval. The lower the ionic strength, the lower the temperature required to bring about the change. At ionic strengths below 0.20, a measurable rise has occurred at 25°. Since we have found that the RNA molecule expands under just these conditions, it is certainly possible that these two phenomena are related, and might be explained quite simply as the results the breaking of hydrogen bonds between base pairs.

Once again it was important to determine whether or not this change in the optical density was reversible. Therefore in one case ($\Gamma/2 = 2.4$), we made simultaneous measurements on two RNA solutions, one of which had been preheated to 90° for 10 min. Since we were unable to detect any difference, qualitative or quantitative, between the results obtained on the two solutions, we conclude that the optical density change is fully reversible.

DISCUSSION

In this investigation we were primarily interested in answering two questions. What is the molecular weight of RNA when it is isolated from TMV? What is the shape of this molecule in solution?

Our results, like those previously reported by SINSHEIMER, by GIERER, and by SCHRAMM, indicate that most if not all the RNA of a single TMV particle can be isolated as an intact unit. This conclusion follows from a comparison of the molecular weight of the isolated RNA with that reported for the intact virus. We must caution,

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however, that not even our best RNA preparations are completely monodisperse, and some degradation has probably occurred. In spite of this limitation, it seems safe to conclude that at least half of the molecules in an infective solution did have the maximum possible molecular weight. We can examine the results obtained on preparation 16 in terms of the distribution of sedimentation constants obtained (see Fig. 1). This preparation had a weight average molecular weight of 1.94 ± 0.16 million. If we now assume that this reflects the fact that the 70% of the molecules sedimenting with $S = 28.6$ consist of intact RNA molecules, and the 30% with $S = 21.3$ are RNA molecules having half their initial molecular weight, we calculate a weight-average molecular weight of 1.83 million. This agrees nicely with measured value obtained from light scattering and sedimentation-viscosity. While the distribution we have assumed is probably far too simple to adequately represent our solutions, it does serve to illustrate the fact that preparations having average molecular weights somewhat less than the maximum, probably still contain a high proportion of molecules with this molecular weight.

We should further note that in our experiments the biological activity, or infectivity, of TMV RNA is associated with the presence of the 28.6-S component. Of the numerous infectivity measurements made, infectivity was only observed in those preparations containing significant proportion of this component. We are aware, however, that this evidence by itself is insufficient proof that only the large component can initiate infection, and we plan further experiments in this direction.

The shape of the RNA molecule is clearly that of a very tightly coiled and highly flexible polymer chain. The results of the temperature studies, while still preliminary, indicate that the compact RNA molecule is held together at low temperatures and moderate salt concentrations by secondary valence forces. When the local charge density along the polynucleotide chain becomes sufficiently great, the secondary valence bonds are broken and the molecule expands. Raising the ionic strength or lowering the temperature brings about a partial return to the original compact configuration. In those cases in which the expansion and contraction was accomplished without a molecular weight decay, the infectivity did not change.

NOTE ADDED IN PROOF

Recent results[23] indicate that the secondary bonds recognized above may not be randomly arranged, but rather are part of an organized secondary structure. Since it is likely that these bonds are grouped in small regions of the RNA chain which are connected by unbonded disorganized regions, the overall configuration of RNA reflected in its hydrodynamic properties would be indistinguishable from that of a random coil.

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ZONE-SHARPENING IN PAPER ELECTROPHORESIS — A METHOD ALLOWING APPLICATION OF DILUTE PROTEIN SOLUTIONS

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SUMMARY

To obtain good results in ordinary paper electrophoresis it is necessary to apply small volumes (about 0.01 ml) of highly concentrated solutions. Thus, very often the samples to be analysed must be concentrated prior to the electrophoresis. To avoid this preliminary concentration and to obtain a narrow starting zone a method has been developed that allows application of comparatively large volumes (0.1–0.2 ml) of dilute protein solutions (as low as 0.02 %).

INTRODUCTION

Paper electrophoresis is very convenient as a routine method for the characterization of different fractions obtained in protein chromatography. This procedure has been used by, amongst others, the author in a previous work on chromatography of serum proteins[1]. The chromatographic fractions obtained were, however, rather dilute and

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