VISIBLE AND ULTRAVIOLET LIGHT SCATTERING BY TOBACCO MOSAIC VIRUS NUCLEIC ACID

by

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INTRODUCTION

In a previous study, NORTHROP AND SINSHEIMER[1] demonstrated that the particle weight of the ribose nucleic acid (RNA) prepared from tobacco mosaic virus (TMV) by brief heating, and maintained under conditions which minimize degradation, was approximately $2.0 \cdot 10^6$. A more detailed study has now been made of the influence of the preparative conditions upon the particle weight obtained. In addition, studies of the light scattering at two wavelengths (4358A and 3131A) by the RNA particle, when dissolved in solvents of varying ionic strength, have permitted a determination of its size and shape.

THEORY

The theory of the interpretation of light scattering data is adequately given in several review articles (OSTER[2], ZIMM, STEIN AND DOTY[3], and DOTY AND EDSALL[4]). A correction to the determination of molecular weight for the case of small anisotropic particles is given by NORTHROP AND SINSHEIMER[1]. It can be shown that this correction will also be exact for a large perfectly random coil made from an anisotropic fiber. HORN AND BENOIT[5,6], have carried out the exact calculation for a large anisotropic rigid rod. In both cases the correction is given in terms of the depolarization ratio ρ_v for vertically polarized incident light.

Numerical values of the molecular form factor $P(\theta)$ have been given by DOTY AND STEINER[7] for several models of interest. It has been shown (OSTER[2]) that $P(\theta)$ for a given model can be uniquely determined from the ratio of the scattered intensities at any two scattering angles symmetrical about 90°. These are usually chosen to be 45° and 135°; the ratio of the scattering at 45° to that at 135° is called the dissymmetry Z.

MATERIALS AND METHODS

Apparatus. The light-scattering equipment used was essentially similar to that previously employed[1], with appropriate modification for ultraviolet work. The primary source of light was a CH-3 mercury arc. The 4358 A and 3131 A lines of the mercury spectrum were isolated with a quartz prism monochromator, the exit slit of which served as a secondary source for the scattering apparatus. A simple lens of fused quartz was used to illuminate the scattering cell.

A 1P28 photomultiplier tube was used to measure the scattered radiation. For molecular weight

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measurement, the output of the phototube was read directly with a sensitive galvanometer, while for depolarization measurements, a d.c. amplifier was employed prior to the galvanometer.

The instrument was calibrated at both wavelengths with Ludox* following the method described by NORTHROP AND SINSHEIMER[1]. The turbidity of a Ludox solution as a function of wavelength followed the $1/\lambda^4$ scattering law, indicating no absorption at 3131 A.

It was necessary to correct the scattering at 3131 A for the absorption of the glass scattering cell. The 2 mm thick partition between the sample and hemi-cylindrical compartments of the cell had an absorption coefficient of 0.465 at 3131 A. The scattered rays passed through the partition obliquely at scattering angles other than 90°. Since the absorption at 90° was included in the calibration, it was only necessary to correct the scattering at other angles to that of the 90° absorption. Thus, the scattered intensity was multiplied by $e^{0.465(\csc\theta-1)}$. Since this correction is symmetrical about $\theta = 90°$, it does not affect the dissymmetry measurements.

The optical density at 3131 A for a 1 cm path through a 0.3 mg/ml solution of RNA was 0.016. The average path of light through the solution was 2.2 cm. An approximate correction for the absorption was made by multiplying the scattering at all angles by $10^{2.2 \cdot 0.016}$.

The polarizer, inserted between the lens and scattering cell for depolarization measurements, was a Foucault prism of calcite with a 2.5 cm square aperture. The analyzer, mounted between the cell and photomultiplier was a Glan-Thompson calcite prism with a 9 mm aperture and ratio of width to length of 1:3. Glycerine rather than Canada balsam was used between the halves. The analyzer could be rotated to detect either the horizontally or vertically polarized component of the scattered radiation. The extinction for crossed polarized and analyzer was $5 \cdot 10^{-4}$.

Correction for the variation in sensitivity of the photomultiplier to the horizontal and vertical components of radiation was made in the depolarization measurements. Such corrections were negligible in the molecular weight calculations.

An instrument similar to that of BRICE AND HALWER[8] and SCHULZ, BOCHMANN AND CANTOW[9], employing photoelectric detection and a movable slit was used to make the refractive increment measurements with 3131 A radiation. The instrument was calibrated using $CoSO_4$** solutions and data given by ROBERTS[10].

Material. The tobacco mosaic virus was the same preparation described by NORTHROP AND SINSHEIMER[1]. The virus was stored at 2° C in 0.033 *M* phosphate buffer, pH 7.2. A particle weight for the TMV of $4.0 \cdot 10^7$ was obtained by the light scattering technique. The virus infectivity was tested on *N. glutinosa* and was in agreement with that given by previous workers for the crystalline virus[11].

The basic procedure for denaturing the virus protein by heating is described by COHEN AND STANLEY[12] and by KNIGHT[13]. In the work reported here, the stock virus suspension in 0.033 *M* phosphate buffer pH 7.2 was centrifuged at 40,000 *g* for one hour. The sedimented pellet was washed several times and then resuspended in the buffer in which the heating was to be done. The primary buffer for this purpose was 0.1 molar in sodium acetate pH 5.7 and 0.2 molar in NaCl. This will be referred to henceforth as the scattering buffer. The suspension, initially at 2° C and in a Pyrex test tube (2 mm wall thickness), was heated for the desired time by immersing the tube in boiling water with constant stirring. "Normal" cooling was accomplished by immersing the tube in ice water immediately after heating. The RNA was kept below 5° C at all times after preparation to avoid spontaneous degradation.

The time required to cool an RNA preparation from 100° C to 10° C by immersion in ice water was about 1 minute, 15 seconds. In the preparations discussed later, fast cooling was accomplished by pouring the hot preparation over pieces of copper that had been precooled to −80° C by "dry" ice. The amount of copper was such that the final equilibrium temperature was about 10° C. The time required to reach 10° C was about 15 seconds.

Slower than "normal" cooling was achieved by immersing the tube in ice water for 7 seconds, removing to air for 15 seconds, re-immersing, *etc.* The time required to reach 10° C by this method was approximately twice that with continuous ice water cooling.

All preparations were cleaned for scattering measurements by centrifugation at 700 *g* to remove the coagulated protein, followed by filtration through ultrafine porosity sintered glass filters until all visible dirt was removed. In some cases (see Table I) the preparations were centrifuged at 40,000 *g* before filtration.

The ultraviolet absorption was used to determine the RNA concentrations. The constant used to relate the optical density at 2570 A (D_{2570}) for a 1 cm path length to concentration (c) was that given by NORTHROP AND SINSHEIMER[1] for pH 5.7:

$$c(\text{mg/ml}) = 0.0396 \times D_{2570\text{A}}.$$

The optical density of a solution was found to be independent of salt concentration at zero, 0.3 *M* NaCl, and 0.5 *M* NaCl.

* A colloidal silica suspension kindly furnished by E. I. du Pont De Nemours & Company.

** Anhydrous $CoSO_4$ was kindly furnished by Dr. H. H. DIEHL, Iowa State College, Ames, Iowa.

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RESULTS

Refractive increment. The refractive increment (dn/dc) of the RNA was measured as 0.254 ± 0.006 ml/gm with 3131A radiation. The value with 4358 A radiation of 0.194 ml/gm given by NORTHROP AND SINSHEIMER[1] was verified for these preparations.

TABLE I

MOLECULAR WEIGHTS OF RNA (4358 A)

Preparation No.	*Preparation procedure (virus conc., buffer*, heating time, centrifugation**, etc.)*	*Conc. of RNA*** (mg/ml)*	*Dissymmetry*	*Intercept* $zc/R(0^\circ)$	*Particle wt* $\times 10^{-6}$
1	20 mg/ml, 70″, 40,000 *g*, 1 h	0.309	1.40	0.765	2.15
2	20 mg/ml, 70″, 40,000 *g*, 1 h	0.290	1.42	0.630	2.59
3	20 mg/ml, 90″	0.435	1.48	0.750	2.17
4	20 mg/ml, 90″, 40,000 *g*, 1 h, 15 min	0.237	1.33	0.565	2.88
5	20 mg/ml, 60″	0.360	1.39	0.652	2.50
6	10 mg/ml, 90″	0.212	1.31	0.940	1.73
7	10 mg/ml, 0.001 *M* phos., 60″	0.184	1.32	0.92	1.77
8 (a)	20 mg/ml, 0.001 *M* phos., 60″	0.221	1.36	0.92	1.77
(b)	dialyze out phosphate	0.154	1.40	0.925	1.76
9	10 mg/ml, 0.01 *M* phos., 70″	0.186	1.41	0.98	1.66
10	10 mg/ml, 0.0005 *M* phos., 90″	0.158	1.37	0.995	1.64
11	10 mg/ml, 0.001 *M* phos., 60″	0.190	1.31	0.96	1.70
12	6.4 mg/ml, (TMV buffer changed to scattering buffer by dialysis) 70″, 40,000 *g*, 1 h	0.090	1.34	0.88	1.86
13	6.4 mg/ml, (TMV buffer changed to scattering buffer by dialysis) 70″	0.120	1.38	0.94	1.73
14	6.4 mg/ml, (TMV buffer changed to scattering buffer plus 0.001 *M* phos. by dialysis), 70″	0.101	1.24	1.10	1.49
15	10 mg/ml, 60″	0.174	1.29	0.94	1.74
16	5 mg/ml, 60″, 0.001 *M* phos.	0.206	1.30	0.98	1.66
17	5 mg/ml, 60″	0.225	1.28	0.98	1.66
18	10 mg/ml, 0.001 *M* phos., 70″ (scattering curves obtained in water and 0.075 *M* NaCl)	0.212	1.30	0.985	1.65
19	10 mg/ml, 0.001 *M* phos., 70″ fast cooling	0.123	1.51	0.50	3.26
20	10 mg/ml, 0.001 *M* phos., 100″ fast cooling	0.187	1.70	0.40	4.08
21	10 mg/ml, 0.001 *M* phos., 100″ slow cooling	0.134	1.22	1.16	1.41
	Average for preparations 6–18		1.33		1.68

* Concentration of salt in addition to scattering buffer.
** Other than the centrifugation at 700 *g*.
*** As sodium ribonucleate.

Particle weight. An investigation of the procedure for the preparation of the TMV RNA by heating revealed at least three factors which affected the particle weight of the resulting RNA. These are (1) the concentration of TMV at the time of heating, (2) the presence of phosphate*, and (3) the rate of cooling after heating. Table I summarizes the effects of variation of these parameters on the resulting RNA. The first five preparations, with a 20 mg/ml concentration of TMV, did not yield consistent values for the RNA particle weight. Two of the values (preparations 1 and 3) are in the range previously

* KNIGHT[13] reported that the presence of 0.001 *M* phosphate aided protein coagulation.

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found[1]. In some of these preparations, considerable difficulty in filtration was encountered and it was necessary to centrifuge the RNA at 40,000 g to remove large particles before filtration. Evidently at this virus concentration, small variations in experimental procedure can result in wide fluctuations in the particulate nature of the RNA obtained.

Preparations 6–18 indicate that over a considerable range of preparative variables, a constant RNA particle weight of $1.7 \cdot 10^6$ can be obtained. This weight is obtained when the TMV is heated in scattering buffer at concentrations of 5 and of 10 mg/ml. Increase of the virus concentration to 20 mg/ml produces the larger but variable particle weights indicated in preparations 1–5. However, after addition of 0.001 M phosphate buffer to the scattering buffer, before heating, the same RNA particle weight, $1.7 \cdot 10^6$, is obtained for all TMV concentrations from 5–20 mg/ml. At a virus concentration of 10 mg/ml, the particle weight of the RNA is independent of the phosphate concentration employed from zero to 0.1 M.

Preparations 8a and b indicate that removal of the phosphate (by dialysis at 2° C) after preparation of the RNA had no effect on the particle weight. Similarly, addition of phosphate to a RNA preparation of higher particle weight (prepared by heating in the absence of phosphate at a virus concentration of 20 mg/ml) had no effect on the particle weight. The phosphate must be present during the time of heating of the virus.

In these preparations about 2.7% of the virus weight remained in solution as RNA after removal of the coagulated protein by centrifugation.

All of these preparations 1–18 were cooled, after the heating period, by the "normal" procedure. Preparations 19–21 indicate possible effects of variation in the rate of cooling. A lower particle weight ($1.4 \cdot 10^6$) is obtained with slower cooling (preparation 21) indicating the possibility of RNA degradation even during the "normal" cooling period.

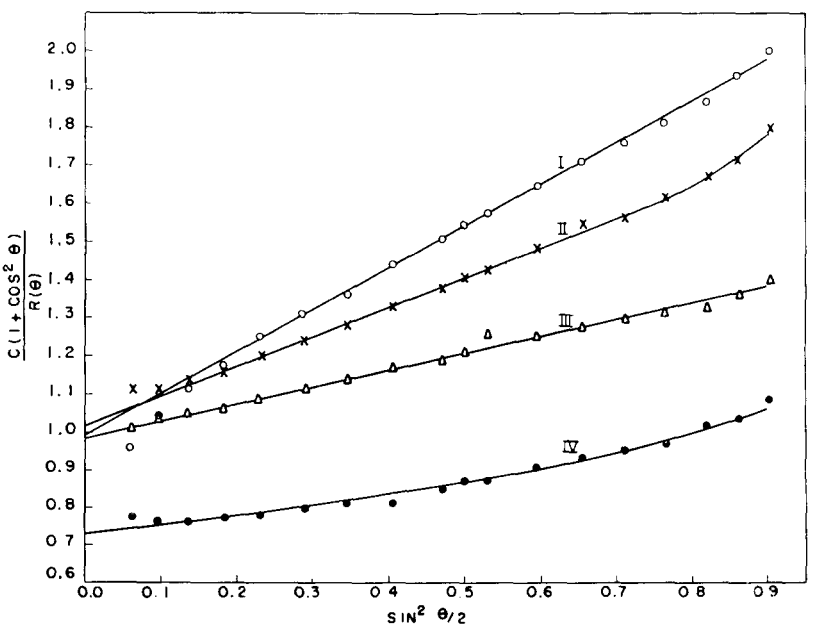

Fig. 1. Scattering curves (4358 A). ● 0.5 M NaCl; △ 0.3 M scattering buffer; × 0.075 M NaCl; ○ water.

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More rapid cooling (preparations 19 and 20) results in variable weights apparently greater than the maximum possible RNA content of the virus.

A typical scattering curve with 4358 A radiation, from which the particle weights were determined, is shown in Fig. 1, curve III. The particle weights of several preparations were also calculated from scattering data with 3131 A radiation, and are shown in Table II. A typical scattering curve at this wavelength is shown in Fig. 2, curve III. The 3131 A curves are corrected for cell absorption, but not for the absorption of the RNA solution. The particle weights calculated from the light scattering with 3131 A radiation are considered to be within reasonable agreement of those calculated from 4358 A scattering in view of the several absorption corrections.

TABLE II

PARTICLE WEIGHTS OF RNA (3131 A)

*Prep. No.**	*RNA conc. mg/ml*	*Dissymmetry***	*Intercept* $\left\{\frac{Kc}{R(0^\circ)}\right\}$	*Particle* $wM \times 10^{-6}$***
3	0.348	1.89	0.122	1.98
11	0.190	1.64	0.154	1.57
15	0.174	1.66	0.150	1.61
16	0.206	1.67	0.128	1.88
18	0.212	1.70	0.165	1.46
		Average§ 1.67		Average§ 1.63

* See Table I for details of preparation.
** In 0.3 *M* salt.
*** Corrected for depolarization and RNA absorption.
§ Excluding preparation 3.

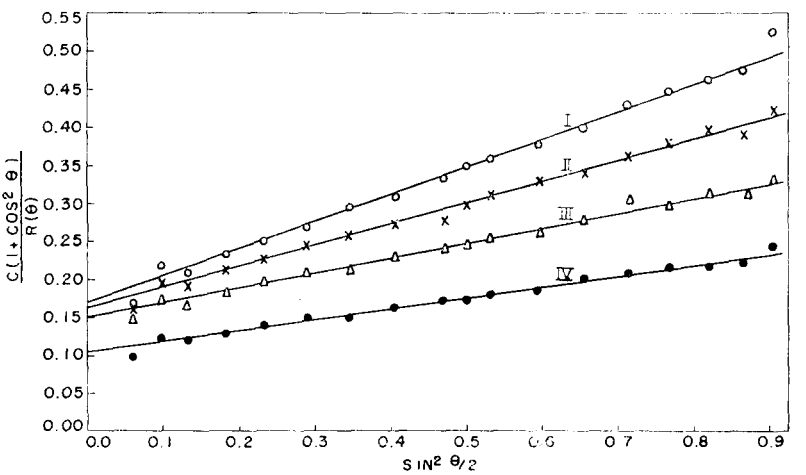

Fig. 2. Scattering curves (3131 A). ● 0.5 *M* NaCl; △ 0.3 *M* scattering buffer; × 0.075 *M* NaCl; ○ water.

Depolarization. Only the depolarization for vertically polarized incident light was measured, since this quantity may be used for both molecular weight and size correction. Typical plots of $\varrho_v(\theta)$ *vs* $\sin^2 \theta/2$ at 4358 A and 3131 A are shown in Fig. 3. The average values of ϱ_v (0°) and ϱ_v (90°) at the two wavelengths based on eight measurements are shown in Table III.

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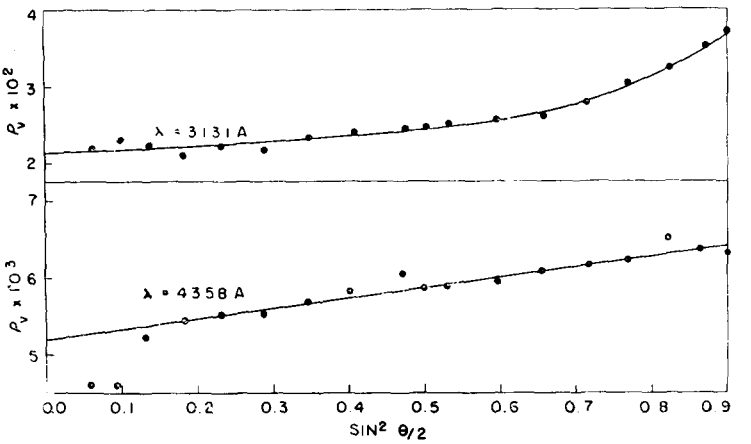

Fig. 3. Depolarization curves.

The values at 4358 A are a factor of ten lower than those previously reported[1]. The difference seems larger than would be expected from the slight reduction in apparent particle weight.

The particle weights calculated from 3131 A scattering data in Table II are corrected for the observed depolarization, but this correction is not included in the scattering curves of Fig. 2.

TABLE III

DEPOLARIZATION DATA

	$\lambda = 4358\,A$	$\lambda = 3131\,A$
Distilled water		
ϱ_v (0°)	0.0055	0.035
ϱ_v(90°)	0.0063	0.043
0.3 M salt		
ϱ_v (0°)	0.0039	0.018
ϱ_v(90°)	0.0045	0.020

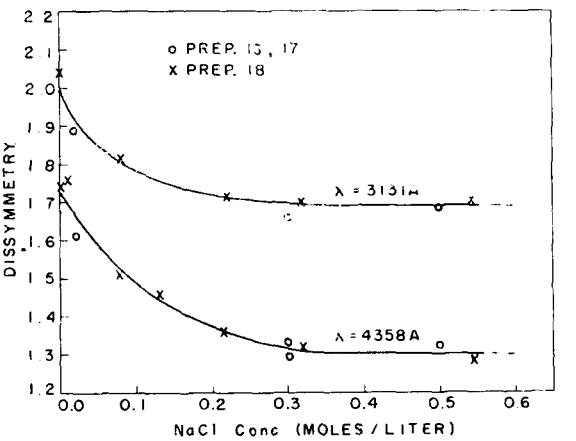

Fig. 4. Effect of ionic strength on dissymmetry.

Effect of solvent salt concentration. The RNA scattering curve has an average dissymmetry of 1.33 with 4358 A radiation and 1.69 with 3131 A radiation in a solvent of equivalent salt concentration of 0.3 M. ROWEN[14] has reported that the dissymmetry of the scattering curve from sodium deoxyribonucleate preparations (and hence the molecular shape) varied with the salt concentration of the solvent. A similar effect is observed for these RNA preparations (particle weight = $1.7 \cdot 10^6$) and is shown for both wavelengths in Fig. 4. The limiting dissymmetry at zero salt concentration is 1.74 with 4358 A radiation and 1.98 with 3131 A radiation. Constant values of 1.33 with 4358 A radiation and 1.69 with 3131 A radiation are obtained for salt concentrations of 0.3 M or higher.

Scattering envelopes were obtained at several of the different salt concentrations

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and are shown in Figs. 1 and 2. It will be noted that the ordinate intercept is approximately constant for salt concentrations from zero to 0.3 M, but decreases as the salt concentration is increased from 0.3 M to 0.5 M. This decrease in intercept corresponds to a particle weight increase from $1.7 \cdot 10^6$ to an average of $2.1 \cdot 10^6$.

This effect of a high salt concentration is reversible. After preparation in 0.3 M salt, the RNA sample may be first made 0.5 M in salt and then dialyzed to water, or the sample may be dialyzed to water and then the salt concentration increased to 0.5 M, scattering data being taken at the various salt concentrations in between. In both situations, the dependence of dissymmetry and particle weight on salt concentration is the same.

It is noteworthy that the ultraviolet absorption of these RNA preparations does not change with change in salt concentration, unlike the result obtained with deoxyribonucleic acid[15,16].

DISCUSSION

Particle weight. The relation of the separated RNA particle of weight $1.7 \cdot 10^6$ to the RNA in the whole virus is not clear, since phosphorus analyses of the whole virus (0.45%) predict a maximum particle weight of $2.0 \cdot 10^6$ if the RNA were separated in one piece. The predicted value is based upon the assumption of one and only one nucleoside per phosphorus atom, but this assumption would not affect the relative values ($1.7 \cdot 10^6$ and $2.0 \cdot 10^6$) since the computed concentrations (c) of RNA, which are used to interpret the light scattering data are also ultimately based upon this assumption and phosphorus analyses of the scattering solutions. That is

$$1/M \propto c \cdot (\mathrm{d}n/\mathrm{d}c)^2$$

where M = calculated particle weight
c = RNA concentration
$\mathrm{d}n/\mathrm{d}c$ = refractive increment of RNA

so that the calculated M from light scattering will be proportional to the computed concentration c, and thus depend on the same assumption of one nucleoside per phosphorus atom.

As the experiments with slower cooling suggest a possible degradation, it may be that these RNA preparations have already been slightly degraded. Or, as we believe more likely[17], it may be that not all of the phosphorus of the TMV preparation is in the RNA.

The high particle weight obtained by heating the 20 mg/ml virus preparations in the absence of phosphate, and the reduction of the particle weight to $1.7 \cdot 10^6$ by heating in the presence of phosphate, is best interpreted as indicating an aggregation of the virus upon heating in concentrated solution in the absence of phosphate, with resultant production of aggregated RNA. Phosphate is known to prevent virus aggregation[18].

The reversibly-increased particle weight obtained by placing the RNA in 0.5 M NaCl would seem to indicate a side by side aggregation of the RNA particles. An end to end aggregation would have produced a change in the dissymmetry which was not observed.

The very high apparent particle weights obtained with rapid cooling probably indicate a failure to obtain complete coagulation of the protein so that it would not be completely removed by the 700 g centrifugation and subsequent filtration.

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Shape and dimensions of the RNA particle. The scattering data obtained at the two wavelengths permit one to decide, at least for the case when the particles are in distilled water, between the standard scattering models of rigid rod, random (Gaussian) coil, and sphere. The dissymmetry with water as the solvent with 4358 A radiation is 1.74, corresponding to an isotropic rigid rod with $L/\lambda' = 0.495$ or a random coil with $S/\lambda' = 0.335$. L is the length of the rod, S the mean distance between ends of the coil and λ' the wavelength of light in water. Since L and S are independent of wavelength, the ratios L/λ' and S/λ' may be calculated for $\lambda = 3131$ A as 0.734 and 0.496 respectively. From these, the expected dissymmetries with 3131 A radiation would be 2.14 for the rod and 2.51 for the coil. The observed dissymmetry in water with 3131 A radiation was 2.0, and thus the coil as a model may be eliminated. The sphere may be eliminated in a similar manner; the expected dissymmetry with 3131 A radiation would be 3.0. To produce these dissymmetries an isotropic rigid rod would be about 1600 A long.

The observed depolarizations indicate that the RNA is not isotropic. The anisotropy does not affect the previous conclusion eliminating the random coil model as it can be shown that the dissymmetry of a perfectly random coil is independent of an anisotropy in the fiber making up the coil. However, the anisotropy will affect the calculated length L of the rod model. The value of ϱ_v (0°) is 0.0055 for the RNA in distilled water, and using 4358A radiation; this value corresponds to an anisotropy function δ_1 (see HORN AND BENOIT[5,6]) of $\pm$ 0.1. Using $P_v(0°) = 0.035$ with 3131 A radiation gives $\delta_2 = \pm 0.25$. Since curves were not given by HORN AND BENOIT for these values of δ, a graphical interpolation of their curves was made to determine the curves corresponding to $\delta = \pm 0.1$ and ± 0.25. The possible values of L determined in this way are shown in Table IV. $\delta = 0$ corresponds to the isotropic rod.

TABLE IV

LENGTHS OF THE ANISOTROPIC ROD IN WATER

λ = 4358 A and Z = 1.74		λ = 3131 A and Z = 2.0	
δ_1 = −0.1	L = 1470 A	δ_2 = −0.25	L = 1120 A
δ_1 = 0	1620 A	δ_2 = 0	1420 A
δ_1 = +0.1	1880 A	δ_2 = +0.25	None*

* The "None" indicates that a rod of this anisotropy can not have the observed dissymmetry for any length, including infinity.

Thus the experimental values of $\pm \delta$ do not correspond to a consistent length for the rod at both wavelengths. HORN AND BENOIT[5] have measured ϱ_v for TMV and calculate a value of $\delta = 0.3$. They have also calculated the expected value from index of refraction measurements as $\delta = 0.08$. Assuming the latter to be correct, light scattering gives a value of four times too large. This may also be the case for RNA.

Since the 3131 A wavelength is approaching an absorption band from the long wavelength side, this anisotropy should be larger than that at 4358 A. Referring again to Table IV, the data would be explained if the rod were nearly isotropic to 4358 A radiation and δ_2 approximately equal to + 0.1 with 3131 A radiation. The RNA length would then be 1620 A in water. An estimate of error would be $\pm$ 200 A. This length is considerably less than that of the intact TMV rods (2800 A).

In salt (0.3 M) where the dissymmetries are a minimum, an unambiguous choice between models is not possible. With an analysis similar to that given for water solution, the expected dissymmetries with 3131 A radiation, calculated from the 4358 A radiation

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values, are the same for both the isotropic rigid rod and the random coil, namely 1.62. The observed value of 1.69 does not correspond well to either model. The anisotropy correction to Z for $\delta_1 = \pm 0.08$ with 4358 A radiation is less than the limits of error of the dissymmetry. Assuming a rod, its length would be 1050 ± 100 A. In order for this rigid rod to have a dissymmetry of 1.69 with 3131 A radiation, δ_2 must be -0.1 and not ± 0.17 as the data would indicate. Another possible model would be a bent or kinked rod, but the analysis for this case is uncertain.

SUMMARY

1. The apparent particle weight of TMV RNA prepared by the heat method is sensitive to the conditions of preparation. A consistent particle weight of $1.7 \cdot 10^6$ has been obtained with a range of preparative conditions.

2. The configuration of the RNA particle depends on the solvent salt concentration. It appears to be a rigid rod 1600 A long in water solution. Its length decreases with the addition of salt and reaches a value of 1050 A, based on a rod model. The configuration is uncertain in salt solution.

3. The RNA undergoes a side by side reversible aggregation at salt concentrations greater than 0.3 *M*.

RÉSUMÉ

1. Le poids particulaire apparent de TMV RNA préparé par la méthode calorifique est sensible aux conditions de préparation. Un poids particulaire constant de $1.7 \cdot 10^6$ a été obtenu avec une variété de conditions préparatives.

2. La configuration de la particule RNA dépend de la concentration saline du dissolvant. Elle semble être un bâtonnet rigide d'une longueur de 1600 A en solution aqueuse. Sa longueur diminue avec l'addition de sel et atteint une valeur de 1050 A, basée sur un modèle en forme de bâtonnet. La configuration est indéterminée dans une solution saline.

3. Le RNA subit une aggrégation réversible "côte-à-côte" à des concentrations salines supérieures à 0.3 *M*.

ZUSAMMENFASSUNG

1. Das scheinbare Teilchengewicht von, nach der Wärmemethode hergestellter TMV RNA hängt von den Herstellungsbedingungen ab. Ein konstantes Teilchengewicht von $1.7 \cdot 10^6$ wurde bei einer Reihe von Herstellungsbedingungen gefunden.

2. Die Struktur des RNA-Teilchens hängt von der Salzkonzentration des Lösungsmittels ab. In wässriger Lösung scheint es ein steifes Stäbchen von 1600 A Länge zu sein. Seine Länge nimmt bei Zugabe von Salz ab und erreicht den Wert von 1050 A, auf Grund des Stäbchenmodells. In Salzlösung ist seine Gestalt ungewiss.

3. Übersteigt die Salzkonzentration 0.3 *M*, so lagern sich die RNA-Teilchen reversibel Seite an Seite aneinander.

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