Improved Estimates of Molecular Weight of Plant Virus RNA by Agarose Gel Electrophoresis and Electron Microscopy after Denaturation with Glyoxal

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(Accepted 5 December 1980)

SUMMARY

The RNA molecules of 13 plant viruses were denatured by heating at 50 °C in 1 M-glyoxal, 50% dimethyl sulphoxide, and their mol. wt. (expressed as the sodium salts) estimated both by electrophoresis in 0.75% agarose gel, and by measuring their lengths in the electron microscope. The two methods gave similar results. Compared with previous estimates obtained by electrophoresis at 60 °C in 2% polyacrylamide gels containing 8 M-urea, the new estimates were similar for molecules of mol. wt. up to about \(1.7 \times 10^6\) (about 5000 nucleotides) but became increasingly larger as the mol. wt. exceeded this value. The mol. wt. of the RNA-1 molecules of a range of nepoviruses were all found to be about \(2.7 \times 10^6\) to \(2.8 \times 10^6\) (about 7800 to 8100 nucleotides) by the glyoxal/agarose method compared with about \(2.1 \times 10^6\) to \(2.2 \times 10^6\) (about 6100 to 6400 nucleotides) by the hot urea/polyacrylamide method. The estimated mol. wt. of RNA-1 (B-RNA) of cowpea mosaic virus (CPMV) was \(2.39 \times 10^6\) (6900 nucleotides), about 20% greater than the commonly used value, those of the single RNA species of apple chlorotic leafspot and heracleum latent viruses were \(2.48 \times 10^6\) (7170 nucleotides) and \(2.52 \times 10^6\) (7260 nucleotides) respectively and that of parsnip yellow fleck virus was \(3.34 \times 10^6\) (9650 nucleotides). Values of \(2.37 \times 10^6\) (6840 nucleotides) and \(0.54 \times 10^6\) (1550 nucleotides) were found respectively for RNA-1 and RNA-2 of the CAM strain of tobacco rattle virus (TRV). For the nepoviruses, and for CPMV and TRV, the new mol. wt. estimates were in good agreement with those predicted from the morphology or sedimentation behaviour of the virus particles. Electrophoresis of glyoxylated RNA in agarose gels is preferred to other electrophoretic methods for determining the mol. wt. of RNA molecules larger than about \(1.7 \times 10^6\) (about 5000 nucleotides), and appears reliable for mol. wt. up to at least about \(3.5 \times 10^6\) (10000 nucleotides).

INTRODUCTION

Electrophoresis of undenatured RNA in polyacrylamide gels, although commonly used to estimate mol. wt., may give incorrect values because the RNA molecules do not have hydrodynamically equivalent conformations. Several methods have been described for determining RNA mol. wt. by electrophoresis under denaturing conditions. Denaturation with formaldehyde (Boedtker, 1971) may not eliminate all secondary structure (Grivell et al., 1971; Wellauer & Dawid, 1973). Electrophoresis in polyacrylamide gels in the presence of 98 or 99% formamide (Boedtker et al., 1973; Pinder et al., 1974) has been widely adopted but

0022-1317/81/0000-4456 $02.00 © 1981 SGM
RNA may retain some secondary structure in this reagent at room temperature; to avoid this, Reijnders et al. (1973) performed electrophoresis at 30 °C and Spohr et al. (1976) used temperatures of 45 to 55 °C. Reijnders et al. (1973) electrophoresed RNA in polyacrylamide gels in the presence of 8 M-urea at 60 °C. Methyl-mercury(II) hydroxide (Bailey & Davidson, 1976) and glyoxal (McMaster & Carmichael, 1977) have also been used as denaturants.

Reijnders et al. (1974) estimated the mol. wt. of the RNA molecules of several plant viruses by the hot urea/polyacrylamide method. Murant & Taylor (1978) used this technique to study the RNA species of a range of nepoviruses but considered that, even in 2% polyacrylamide, the mol. wt. estimates for molecules larger than about 2 \( \times 10^6 \) mol. wt. were unreasonably low, although the estimates for molecules smaller than this were probably accurate. There were three reasons for believing this.

(i) Some nepoviruses, such as arabis mosaic virus (AMV), raspberry ringspot virus (RRV) and tobacco ringspot virus (TobRV), have two types of particle sedimenting at 125 to 130S, one type containing a single molecule of the larger species of genome RNA (RNA-1) and the other containing two molecules of the smaller species (RNA-2). The mol. wt. of RRV RNA-1 should therefore be about twice that of RNA-2 but the hot urea/polyacrylamide technique yielded values of 2.1 \( \times 10^6 \) and 1.4 \( \times 10^6 \) respectively, a ratio of only 1.5.

(ii) The two genome RNA species of tobacco rattle virus (TRV) are packaged separately in rod-shaped particles of different lengths and the ratio of the RNA mol. wt. should be similar to that of the particle lengths. With the CAM strain the expected ratio is about 4 but that found with the hot urea/polyacrylamide technique was only 3.2.

(iii) Estimates of the mol. wt. of the large RNA molecules of viruses from several different plant virus groups were all in the range 2.0 \( \times 10^6 \) to 2.2 \( \times 10^6 \); this seems unlikely to be correct and suggests that the assumed linear relationship between mobility and log mol. wt. does not hold in 2% polyacrylamide gels for RNA molecules as large as this.

We now report experiments in which RNA molecules were denatured with glyoxal (McMaster & Carmichael, 1977) and their mol. wt. estimated by electrophoresis in 0.75% agarose gels and by electron microscopy. These estimates appear reliable for RNA molecules with mol. wt. up to at least 3.5 \( \times 10^6 \). A preliminary report has already appeared (Murant et al., 1979).

**METHODS**

*Virus isolates.* Isolates of the following viruses were those used by Murant & Taylor (1978): arabis mosaic virus (AMV), cherry leaf roll virus (CLRV), myrobalan latent ringspot virus (MLRV), raspberry ringspot virus (RRV), strawberry latent ringspot virus (SLRV), tobacco mosaic virus (TMV), tobacco ringspot virus (TobRV), tomato black ring virus (TBRV) and tomato ringspot virus (TomRV). Other virus isolates used were: apple chlorotic leafspot virus (ACLSV), isolate C-8 of Lister et al. (1965); cowpea mosaic virus (CPMV), an isolate of the yellow strain supplied by A. van Kammen; heracleum latent virus (HLV; Bem & Murant, 1979); parsnip yellow fleck virus (PYFV), isolate P-121 of Murant & Goold (1968); and tobacco rattle virus (TRV), isolate CAM of Harrison & Woods (1966). CLRV, MLRV, TobRV, TomRV, CPMV and TRV were cultured under licence from the Department of Agriculture and Fisheries for Scotland.

*Virus purification.* AMV, CLRV, TRV, TobRV and TBRV were propagated in *Nicotiana clevelandii*; ACLSV, HLV, MLRV, RRV and SLRV in *Chenopodium quinoa*; TMV in *Nicotiana tabacum* cv. Samsun; PYFV in *Spinacia oleracea*; TomRV in *Cucumis sativus* and CPMV in *Vigna unguiculata* ssp. *unguiculata*.

AMV, MLRV, RRV, SLRV, TobRV and TBRV were purified as previously described for RRV (Murant et al., 1972). The other viruses were purified as described by the following authors: CLRV and TomRV, Stace-Smith (1966); CPMV, van Kammen (1967); TMV,
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Gooding & Hebert (1967); PYFV, Murant & Goold (1968); TRV, Cooper & Mayo (1972); ACLSV, Lister & Hadidi (1971); and HLV, Bem & Murant (1979).

**Preparation of RNA samples.** Virus RNA and *Escherichia coli* rRNA were prepared by phenol/cresol extraction as described by Murant & Taylor (1978).

**Denaturation of RNA.** Glyoxal, technical grade, 40% (w/v) solution in water (BDH) was deionized immediately before use by adding mixed-bed resin (Amberlite MB-1; BDH), 1 g resin/2 to 3 ml glyoxal, and stirring for about 30 min. RNA samples were precipitated from ethanol and resuspended in 1 M-glyoxal, 50% (w/v) dimethyl sulphoxide, 10 mM-sodium phosphate buffer pH 7 and incubated for 1 h at 50 °C, as described by McMaster & Carmichael (1977). The samples were then cooled to room temperature and used immediately for electron microscopy or electrophoresis.

**Electrophoresis of denatured RNA.** Electrophoresis was in 0-75% agarose (electrophoresis grade, BDH) gels containing 10 mM-sodium phosphate buffer pH 7. Gels 7 cm long were prepared in glass tubes of 6 mm internal diam. Each gel was trimmed at the top with a razor blade and supported by a single layer of muslin fixed over the bottom of the tube. About 2 to 10 μg glyoxal-denatured RNA (depending on the number of RNA species) was placed on each gel. Current was applied for 15 min at 0.5 mA/gel and then for about 90 min at 1 to 2 mA/gel, until the bromophenol blue marker dye reached the bottom of the tube. After electrophoresis the gels were stained for 1 h in 0.05% toluidine blue in 0.06 M-phosphate buffer pH 7, then destained overnight in the same buffer.

**Electron microscopy of denatured RNA.** RNA samples were prepared for electron microscopy by a modification of the formamide spreading technique of Davis *et al.* (1971). The trough containing the hypophase and the bars used to wipe the liquid surface were made of Teflon. These and all glassware used during the spreading procedures were acid-washed. Double glass-distilled water was used to rinse the apparatus and to dilute all reagents. Glyoxylated RNA samples were diluted 10- to 20-fold with 1 M-tris, 0-1 M-EDTA pH 8.5 to remove excess glyoxal which sometimes interfered with spreading. The samples, now containing about 20 μg/ml RNA, were diluted 50-fold into 50% (w/v) formamide, 20 mM-tris, 2 mM-EDTA pH 8.5, 40 μg/ml cytochrome c (Sigma type VI, horse muscle). By gradual application to a low-angled glass slide, 20 μl of these dilutions were spread on to a hypophase containing 20% (v/v) formamide, 1 mM-tris, 0-1 mM-EDTA pH 8.5. After about 1 min the spreads were picked up on pyroxylin films which had been freshly mounted on 200-mesh copper/rhodium grids (Graticules Ltd., Tonbridge, Kent, U.K.). The grids were agitated for about 30 s in 0-05 mM-uranyl acetate (freshly made by diluting a stock solution of 0-05 mM-uranyl acetate, 0-05 mM-HCl with 90% ethanol), then rinsed in 90% ethanol, touched dry on filter paper and rotary shadowed with platinum at an angle of 8°. The grids were examined in a Philips EM301 electron microscope operating at 80 kV and photographed at a magnification of 7500; to obtain accurately reproducible magnifications the specimen height was adjusted so that focus was achieved at a standardized objective lens current. Control spreads of TMV RNA and *E. coli* rRNA were photographed on the same occasion as test samples. Plates were projected × 25 and the RNA molecules traced and measured with a 1D-TT-20 digitizer (Summagraphics Corp., Fairfield, Conn., U.S.A.) linked to a 4051 graphics system (Tektronix Inc., Beaverton, Oregon, U.S.A.). A maximum likelihood computer programme (Ross, 1975) was used to fit a normal or double normal distribution curve to the data for each RNA preparation and to provide estimates of the mean length of each RNA species.

**Estimation of RNA mol. wt.** The following RNA species were used as standards (mol. wt. values calculated for the sodium salts): TMV RNA, 6340 nucleotides, mol. wt. 2.19 × 10⁶ (Caspar, 1963); *E. coli* 23S rRNA, 2904 nucleotides, mol. wt. 1.009 × 10⁶ (Brosius *et al.* 1980) and *E. coli* 16S rRNA, 1541 nucleotides, mol. wt. 0.534 × 10⁶ (Brosius *et al.* 1978;
Table 1. Estimates of mol. wt. of plant virus RNA species by electrophoresis under denaturing conditions

<table>
<thead>
<tr>
<th>Virus</th>
<th>RNA-1</th>
<th>RNA-2</th>
<th>RNA-3</th>
<th>RNA-1 (satellite RNA)</th>
<th>No. of determinations</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMV</td>
<td>2.08</td>
<td>1.42</td>
<td>-</td>
<td>2.78 ± 0.015</td>
<td></td>
</tr>
<tr>
<td>TobRV</td>
<td>2.08</td>
<td>1.46</td>
<td>-</td>
<td>2.73 ± 0.044</td>
<td></td>
</tr>
<tr>
<td>RRV</td>
<td>2.14</td>
<td>1.45</td>
<td>-</td>
<td>2.76 ± 0.018</td>
<td></td>
</tr>
<tr>
<td>TBRV</td>
<td>2.08</td>
<td>1.63</td>
<td>0.48</td>
<td>2.69 ± 0.043</td>
<td>0.51 ± 0.011</td>
</tr>
<tr>
<td>MLRV</td>
<td>2.3</td>
<td>1.90</td>
<td>0.51</td>
<td>2.81 ± 0.033</td>
<td>0.48 ± 0.009</td>
</tr>
<tr>
<td>CLRV</td>
<td>2.19</td>
<td>2.00</td>
<td>-</td>
<td>2.82 ± 0.011</td>
<td></td>
</tr>
<tr>
<td>TomRV</td>
<td>2.17</td>
<td>2.05</td>
<td>-</td>
<td>2.80 ± 0.011</td>
<td></td>
</tr>
<tr>
<td>SLRV</td>
<td>2.10</td>
<td>1.45</td>
<td>0.39</td>
<td>2.87 ± 0.025</td>
<td></td>
</tr>
<tr>
<td>CPMV</td>
<td>2.07‡</td>
<td>1.37‡</td>
<td>-</td>
<td>2.39 ± 0.034</td>
<td></td>
</tr>
<tr>
<td>TRV</td>
<td>2.04</td>
<td>0.64</td>
<td>-</td>
<td>2.37 ± 0.016</td>
<td></td>
</tr>
<tr>
<td>ACLSV</td>
<td>2.15‡</td>
<td>-</td>
<td>-</td>
<td>2.48 ± 0.010</td>
<td></td>
</tr>
<tr>
<td>HLV</td>
<td>2.15‡</td>
<td>-</td>
<td>-</td>
<td>2.52 ± 0.035</td>
<td></td>
</tr>
<tr>
<td>PYFV</td>
<td>2.23§</td>
<td>-</td>
<td>-</td>
<td>3.34 ± 0.075</td>
<td></td>
</tr>
</tbody>
</table>

* Previous estimate from electrophoresis at 60 °C in polyacrylamide gels containing 8 M-urea.

† Reijnders et al. (1974).
‡ Bern & Murant (1979).

Carbon et al., 1978). Mol. wt. of test RNA species were obtained by inverse estimation from the regression of either (i) electrophoretic mobility on log mol. wt. of the standards, or (ii) molecular length on mol. wt. of the standards.

RESULTS AND DISCUSSION

Electrophoresis of glyoxylated RNA in agarose gels

When glyoxylated RNA preparations were electrophoresed in 0.75% agarose gels, a good straight-line relationship (Fig. 1) was found between the mobilities of the RNA standards and log mol. wt. Table 1 gives estimates of RNA mol. wt. obtained for 13 plant viruses with this
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technique, and includes for comparison previous estimates obtained by electrophoresis at 60 °C in polyacrylamide gels containing 8 M-urea. Values for the number of nucleotides in each RNA species can be derived from these data by dividing by the average nucleotide mol. wt. (sodium salt) of the three RNA standards (346.5); data obtained in this way are essentially the same as those given directly by inverse estimation from the regression of electrophoretic mobility on log_{10} nucleotide number of the RNA standards.

The glyoxal/agarose and hot urea/polyacrylamide electrophoretic methods gave similar estimates of mol. wt. for RNA molecules up to about 1.7 \times 10^6 (about 5000 nucleotides) but as the mol. wt. increased above this value the estimates for glyoxylated RNA in agarose gels increasingly exceeded those found in the hot urea/polyacrylamide gels. Thus, with the glyoxal/agarose method the mol. wt. estimates for the RNA-1 molecules of a range of nepoviruses were about 2.7 \times 10^6 to 2.8 \times 10^6 (about 7800 to 8100 nucleotides) compared with about 2.1 \times 10^6 to 2.2 \times 10^6 (about 6100 to 6400 nucleotides) by the hot urea/polyacrylamide method, an increase of nearly 30%. The estimate for the RNA-1 (B-RNA) of CPMV (2.39 \times 10^6; 6900 nucleotides) was nearly 20% larger than the hot urea/polyacrylamide value and that for PYFV RNA (3.34 \times 10^6; 9650 nucleotides) was 50% larger; the mol. wt. estimates for RNA-1 of TRV and for the single RNA molecules of ACLSV and HLV were also greatly increased. Even the TMV RNA standard behaved differently in the two methods because, to obtain a satisfactory linear relationship between mobility and log mol. wt. for the TMV RNA and E. coli 16S and 23S rRNA standards, Caspar's (1963) value of 2.19 \times 10^6 was assumed for the mol. wt. of TMV RNA in the glyoxal/agarose experiments, whereas Boedtker's (1960) value of 2.0 \times 10^6 was used in the previous experiments with the hot urea/polyacrylamide method (Murant & Taylor, 1978). Lehrach et al. (1977) also found that TMV RNA behaved in agarose gels as if it had a mol. wt. of 2.2 \times 10^6.

Electron microscopy of glyoxylated RNA

The relative sizes of the RNA standards and of some of the other RNA species in Table 1 were estimated by comparing the lengths of glyoxylated RNA molecules by electron microscopy. The molecules showed no evidence of secondary structure (Fig. 2) and frequency diagrams of their lengths showed prominent modal peaks (Fig. 3). Table 2 gives computed mean molecular lengths of the different RNA species, together with their mol. wt. obtained by inverse estimation from the regression of length on mol. wt. for the RNA standards, the line being constrained to pass through the origin. The mol. wt. estimates have wide 95% confidence limits because of the small number of points used in the regression, but they agree well with those obtained from electrophoresis of glyoxylated RNA in agarose gels (Table 1).

A better way of comparing the data in Tables 1 and 2 is given in Fig. 4, which shows a close correlation (r = 0.998, P > 0.001) between mean molecular length and the mol. wt. values determined by electrophoresis of glyoxylated RNA in agarose gels (or, for the RNA standards, the values assumed from the literature). The regression line of estimated mol. wt. on length for the seven test RNA species passes almost exactly through the origin, and very close to the three RNA standards. This suggests that the mol. wt. of 2.19 \times 10^6 assumed for TMV RNA in the electrophoresis experiments is close to the true value. Finally, Fig. 4 shows that (providing that accurate values are used for the mol. wt. of the RNA standards) electrophoresis of glyoxylated RNA in 0.75% agarose gels gives reliable estimates of RNA mol. wt. up to at least 3.5 \times 10^6.

The average internucleotide distance given by the slope of the regression line of length on nucleotide number was 0.273 ± 0.002 nm, or 0.264 ± 0.004 nm if the line was constrained to pass through the origin; these values compare with that found for glyoxylated molecules of
Fig. 2. Electron micrograph of glyoxylated RRV RNA prepared as described in Methods and rotary shadowed with platinum at an angle of 8°. Note the complete absence of secondary structure and the presence of molecules of two predominant lengths, those marked with long arrows (RNA-1 molecules) being about twice as long as those marked with arrowheads (RNA-2 molecules).

Fig. 3. Frequency diagrams of the lengths of glyoxylated molecules of *E. coli* 16S and 23S rRNA, TMV RNA, RRV RNA-1 and RRV RNA-2.
Fig. 4. Relation between the mean lengths of glyoxylated RNA molecules and their mol. wt. estimated by electrophoresis in 0.75% agarose gels or, for the RNA standards, the mol. wt. values assumed from the literature. The fitted line is the regression of estimated mol. wt. on length for the seven test RNA species, excluding the TMV RNA and \textit{E. coli} rRNA standards. $\triangle$, RRV RNA; $\blacksquare$, PYFV RNA; $\square$, TRV-CAM RNA; $\blacktriangle$, TMV RNA standard; $\blacklozenge$, \textit{E. coli} 16S and 23S rRNA standards. The equation of the fitted regression is $y = 1.320 (\pm 0.0382) x + 0.0083 (\pm 0.0688)$.

Table 2. \textit{Estimates of mol. wt. of RNA species from their mean lengths obtained by electron microscopy of glyoxylated RNA molecules}

<table>
<thead>
<tr>
<th>RNA species</th>
<th>Mean length of RNA molecules ($\mu$m $\pm$ s.e.)</th>
<th>Estimated RNA mol. wt. ($\times 10^{-6}$) with 95% confidence limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standards</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TMV</td>
<td>1.69 $\pm$ 0.072</td>
<td></td>
</tr>
<tr>
<td>\textit{E. coli} 23S</td>
<td>0.74 $\pm$ 0.014</td>
<td></td>
</tr>
<tr>
<td>\textit{E. coli} 16S</td>
<td>0.38 $\pm$ 0.010</td>
<td></td>
</tr>
<tr>
<td>Test species</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RRV RNA-1</td>
<td>2.11 $\pm$ 0.019</td>
<td>2.77 (2.52-3.02)</td>
</tr>
<tr>
<td>RRV RNA-2</td>
<td>1.00 $\pm$ 0.009</td>
<td>1.32 (1.13-1.50)</td>
</tr>
<tr>
<td>CLRV RNA-1</td>
<td>2.16 $\pm$ 0.012</td>
<td>2.83 (2.57-3.08)</td>
</tr>
<tr>
<td>CLRV RNA-2</td>
<td>1.72 $\pm$ 0.025</td>
<td>2.26 (2.03-2.48)</td>
</tr>
<tr>
<td>TRV RNA-1</td>
<td>1.70 $\pm$ 0.033</td>
<td>2.23 (2.01-2.46)</td>
</tr>
<tr>
<td>TRV RNA-2</td>
<td>0.45 $\pm$ 0.007</td>
<td>0.60 (0.42-0.77)</td>
</tr>
<tr>
<td>PYFV</td>
<td>2.54 $\pm$ 0.332</td>
<td>3.33 (3.05-3.61)</td>
</tr>
</tbody>
</table>

\textit{E. coli} 23S rRNA by Hsu \textit{et al.} (1973). Their value was 0.256 nm but can be recalculated from accurate nucleotide composition data as 0.275 nm.

\textit{Comparison of RNA mol. wt. estimates with values expected from the properties of virus particles}

The estimates of RNA mol. wt. obtained from electrophoresis of glyoxylated RNA in 0.75% agarose gels seem reliable not only because they were similar to those found by electron microscopy but also because they agree with those expected from the particle properties mentioned in Introduction, i.e. (i) with AMV, RRV and TobRV the estimated mol.
wt. of RNA-1 was about twice that of RNA-2, and (ii) with TRV-CAM the estimated mol. wt. of RNA-1 was about four times that of RNA-2.

With isometric plant viruses that have several sedimenting components of the same diameter but different RNA content, it is possible to make a further assessment of the accuracy of RNA mol. wt. estimates by substituting them into the Svedberg equation. Assuming that the virus particles consist only of protein and RNA, and making the approximation that the mol. wt. and partial specific volumes of these constituents are the same as for free protein and RNA in solution (not strictly true because, whereas in solution the RNA is an alkali metal salt, in the virus particle a proportion of the phosphate residues will bind to the protein), this equation may be written:

$$s_{20,w} = \frac{D_{20,w}}{RT} \left[ M_p \left( 1 - \bar{\nu}_p \rho_{20} \right) + M_N \left( 1 - \bar{\nu}_N \rho_{20} \right) \right]$$

where $s_{20,w}$ = sedimentation coefficient in water at 20 °C; $D_{20,w}$ = diffusion coefficient in water at 20 °C; $R$ = gas constant ($8.31 \times 10^7$ ergs deg$^{-1}$ mol$^{-1}$); $T$ = absolute temperature (293-2 K); $M_p$ and $M_N$ are the total mol. wt. of protein and RNA in the particle respectively; $\bar{\nu}_p$ and $\bar{\nu}_N$ are the partial specific volumes of the protein and RNA respectively; and $\rho_{20}$ = density of water at 20 °C (0.9982 g/ml).
Table 3. Data used in the preparation of Fig. 5, together with diffusion coefficients calculated from the slopes of the lines

<table>
<thead>
<tr>
<th>Virus</th>
<th>Coat polypeptide mol. wt. (× 10⁻⁹) and reference</th>
<th>Calculated mol. wt. of protein shell* (× 10⁻⁹)</th>
<th>Partial specific volume (ml/g)†</th>
<th>Sedimentation coefficient, s₂₀,₅ (svedbergs) and reference</th>
<th>Calculated diffusion coefficient, D₂₀,₅⁺ (× 10⁻⁷ cm²/s ± S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMV</td>
<td>54 Mayo et al. (1971)</td>
<td>3.24</td>
<td>0.74</td>
<td>0.55</td>
<td>53, 93, 126 R. Stace-Smith (see Murant, 1970a)</td>
</tr>
<tr>
<td>RRV</td>
<td>54</td>
<td>3.24</td>
<td>0.74</td>
<td>0.55</td>
<td>52, 92, 130 Murant et al. (1972)</td>
</tr>
<tr>
<td>TobRV</td>
<td>57</td>
<td>3.42</td>
<td>0.74</td>
<td>0.55</td>
<td>53, 91, 126 Schneider &amp; Dienert (1966)</td>
</tr>
<tr>
<td>TBRV</td>
<td>57</td>
<td>3.42</td>
<td>0.74</td>
<td>0.55</td>
<td>55, 97, 121 Murant (1970b)</td>
</tr>
<tr>
<td>MLRV</td>
<td>53</td>
<td>3.18</td>
<td>0.74</td>
<td>0.55</td>
<td>50, 109, 126 G. P. Martelli, personal communication</td>
</tr>
<tr>
<td>CLRV</td>
<td>54</td>
<td>3.24</td>
<td>0.74</td>
<td>0.55</td>
<td>52, 115, 128 Jones &amp; Mayo (1972), Walkey et al. (1973)</td>
</tr>
<tr>
<td>TomRV</td>
<td>58</td>
<td>4.14</td>
<td>0.74</td>
<td>0.55</td>
<td>53, 119, 127 Schneider et al. (1974)</td>
</tr>
<tr>
<td>SLRV</td>
<td>44 + 29 Mayo et al. (1974)</td>
<td>4.38</td>
<td>0.74</td>
<td>0.55</td>
<td>58, 94, 126 Mayo et al. (1974), G. P. Martelli, personal communication</td>
</tr>
<tr>
<td>CPM~*</td>
<td>44 + 25 Geelen et al. (1972)</td>
<td>4.14</td>
<td>0.730</td>
<td>0.55</td>
<td>58, 95, 115 Van Kammen (1967)</td>
</tr>
</tbody>
</table>

* Assuming 60 molecules of each polypeptide/particle.
† Assumed values (Markham, 1962) except that values for proteins of TobRV, CLRV, TomRV and CPMV were calculated (Cohn & Edsall, 1943) from the amino acid compositions given respectively by Stace Smith et al. (1965), Walkey et al. (1973), Tremaine & Stace-Smith (1968) and Wu & Bruening (1971).
‡ Calculated as described in the text.
If the assumptions are correct, a graph of $s_{20,w}$ against $M_p (1 - \bar{v_p} \rho_{20}) + M_N (1 - \bar{v_N} \rho_{20})$ for the different sedimenting components should give a straight line of slope $D_{20,w}/RT$ passing through the origin. Fig. 5 shows plots obtained in this way using RNA mol. wt. values obtained from electrophoresis of glyoxylated RNA in agarose gels (Table 1) and other data from Table 3. Considering that the data are from a variety of sources and are therefore not uniformly accurate, the points form remarkably good straight lines; this suggests that the estimates of RNA mol. wt. from agarose gels are close to the real values. The slopes of the lines ($= 0.0041 \times D_{20,w}$) passing through the origin and fitted to the experimentally determined points were used to obtain the approximate estimates of $D_{20,w}$ given in the last column of Table 3. From the relation

$$D_{20,w} = \frac{kT}{6\pi\eta_{20,w} r}$$

where $k =$ Boltzmann’s constant, $\eta_{20,w} =$ viscosity of water at 20 °C, and $r =$ hydrated radius of the particle, it can be calculated that the hydrodynamic diam. are about 28 to 30 nm for the nepoviruses and about 34 nm for SLRV and CPMV. These values are acceptable, being about 10% larger than the dry diameters found by electron microscopy.

We conclude that electrophoresis of glyoxylated RNA in 0.75% agarose gels (McMaster & Carmichael, 1977) gives reliable estimates of RNA mol. wt. up to at least about $3.5 \times 10^6$ and seems preferable to other common electrophoretic techniques for determining the mol. wt. of RNA molecules larger than about $1.7 \times 10^6$. For molecules smaller than this the hot urea/polyacrylamide method is also suitable, but for larger molecules the assumed linear relationship between log mol. wt. and mobility evidently ceases to hold in this system, even in 2% polyacrylamide, the lowest concentration that can be easily handled. Moreover, in this technique not only the test RNA species but also the standard RNA species should be smaller than about $1.7 \times 10^6$ mol. wt. and thus TMV RNA is an unsatisfactory standard. The fact that it has appeared to plot in line with E. coli rRNA standards in this technique is, we believe, because the mol. wt. value usually assumed (2.0 x $10^6$ or 2.05 x $10^6$) is too low. Taken together, our data from agarose gel electrophoresis and electron microscopy suggest that Caspar’s (1963) value of $2.19 \times 10^6$ is more appropriate for use in conjunction with mol. wt. for the two E. coli rRNA species calculated for the sodium salts from the nucleotide sequences because (i) it is supported by the measurements of the relative lengths of TMV RNA and the two E. coli rRNA species, (ii) it provides estimates for the mol. wt. of RRV RNA-1 and TRV-CAM RNA-1 that agree with those expected from the properties of the virus particles, (iii) it provides mol. wt. estimates for all the RNA molecules tested that are closely correlated with their lengths, and the regression of estimated mol. wt. on length passes closely through the origin (Fig. 4) and (iv) it provides estimates for mol. wt. of the RNA species of multicomponent isometric viruses that fit the Svedberg equation (Fig. 5).

Three further points require emphasis. Firstly, the determination of mol. wt. of the large RNA molecules of plant viruses by electrophoresis requires extrapolation and obviously this is why it is important to use an accurate value for the mol. wt. of TMV RNA. Secondly, the fitting of a regression line to data for only three RNA standards is a process that is subject to considerable error, although this can be compensated for to some extent by adequate replication. However, both these difficulties will be resolved only when sequence data are obtained for more of the readily available RNA species. In the meantime, it seems that the two RNA species of RRV could provide useful standards because our electron microscope observations show that, in accordance with what is expected from the particle properties, RNA-1 of this virus is almost exactly twice as long as RNA-2, and the mol. wt. of RNA-2 can be determined by gel electrophoresis without the need for extrapolation. Finally, in view of the above comments, the new mol. wt. estimates presented in Table 1 are not likely to be
free from error. It should be emphasized that the standard errors presented are merely a measure of the reproducibility of the experiments and not of the intrinsic accuracy of the data. Nevertheless, it seems clear that the new mol. wt. estimates are much more accurate than those obtained previously.

It is a pleasure to acknowledge the excellent technical assistance of Erica Bell. We also thank Pauline Topham for statistical advice, Maureen McMaster for the diagrams, M. A. Mayo and H. Barker for purified preparations of CPMV, TRV-CAM and TobRV, and J. Dunez, R. W. Fulton, R. Stace-Smith and A. van Kammen for supplying virus isolates.

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(Received 6 October 1980)