A Comparison of Molecular Weights of Foot-and-Mouth Disease Virus RNA Fragments Determined from Lengths and s-Rates

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(Accepted 17 November 1975)

SUMMARY

A comparison was made of the calculated mol. wt. of RNA fragments from foot-and-mouth disease virus (FMDV) types A12 strain 119, C3 Resende and O1 Brugge. The mol. wt. were calculated by two methods: from measurements of strand lengths in the electron micrographs and from the observed sedimentation rates (s-rates). RNA extracted from virus by dialysis against water usually had three to four prominent strands of different lengths. Mol. wt. calculated from s-rates (and converted to strand lengths) generally agreed with those measured on electron micrographs. Differences which occurred appeared to be attributable to further breakage during processing for electron microscopy. Major fragment strand lengths range from 0.65 to 2.45 μm. The modal lengths of RNA fragments were preparation-dependent, ranging from 1.25 to 1.95 μm for A12 119, 1.05 to 1.75 μm for C3 Resende, and 1.65 to 2.45 μm for O1 Brugge. There was one fragment length 1.95 μm common to all three types of FMDV RNA and several others which appear in at least two types. Calculations using the molar ratios of nucleotide residues in FMDV RNA, a mol. wt. of FMDV RNA of 2.65 × 10^6 and an internucleotide spacing of 3.17 Å indicate that intact FMDV RNA should be 2.62 μm long and therefore would contain approx. 8270 nucleotides. The derived mathematical expression for the relationship between mol. wt. (M) and s-rate (S) giving the best fit for all data was M = 1725 S^2.07, a result close to that derived by Spirin (1963) for other single-stranded RNAs.

INTRODUCTION

The electron microscope has been used to determine the strand length and mol. wt. of RNA from several viruses (Granboulan & Girard, 1969; Granboulan & Scherrer, 1969; Weber et al. 1974; Jacobson & Bromley, 1975). The analytical ultracentrifugation and degradation of RNA have been discussed by Huppert & Pelmont (1962). An extensive analysis of the physical properties and chemical characteristics of RNA from FMDV has been published by Newman (1969). From this work and that of others (Arlinghaus, Kaczmarczyk & Polatnick, 1969), it appears that FMDV RNA has considerably less structural stability than the RNAs referenced above and breaks down readily into several fragments. In the present study, FMDV RNA fragments obtained by dialysis against water of three immunological types of purified FMDV were examined for similarities or differences in length or mol. wt. Analyses of the fragments were carried out by electron microscopy and analytical ultracentrifugation. Absorption-temperature profiles show that FMDV degrades...
spontaneously in water or at low ionic strength (0.001 M-Na⁺) into protein and RNA (Bachrach, 1964). The RNA produced in this manner has low infectivity and as shown below is highly fragmented.

METHODS

Virus and RNA fragments. Purified FMDV types A₁₂ strain 119, C₃ Resende and O₁ Brugge were prepared by methods described previously (Bachrach, Trautman & Breese, 1964). The purified viruses at concentrations ranging from 2 to 5 mg/ml were stored at 4 °C in 0.2 M-KCl in 0.5 M-potassium phosphate buffer at pH 7.5. Some viruses had been prepared recently, while some type A₁₂ preparations had been stored for as long as 5 or 6 years. The suspensions were checked by electron microscopy to ascertain that they contained principally whole virus particles; no empty capsids or previously-released RNA fragments were observed. To degrade the viruses and release their RNAs, 0.5 to 1.0 ml of virus was dialysed against 2 to 31 of demineralized distilled water. Dialysis proceeded for 18 to 24 h at 4 °C on a slow shaker. The resulting suspension was recovered and stored at 4 °C in stoppered plastic test tubes without further treatment.

Electron microscope determination of fragment lengths and molecular weights. The dialysed samples were placed directly by platinum loop onto carbon-coated formvar substrates on copper electron microscope grids that had been pre-treated with a loop drop of 0.1% bovine plasma albumin in distilled water. In preliminary experiments, rotary shadowing at a low angle with uranium was used to reveal the strands but in most experiments the dried strands were stained with 0.2% phosphotungstic acid brought to pH 7.4 with potassium hydroxide. The grids were examined in the electron microscope, and 20 or more random areas were photographed at a constant magnification. Enlargements of the micrographs were measured with a rolling-wheel device used to determine distances on nautical charts. It was calibrated by measuring lines of known lengths and converting values to micrometers.

Mol. wt. of the RNA strands were calculated using: (a) the internucleotide spacing in viral RNA (3.17 Å) as determined by Granboulan & Scherrer (1969); (b) the ratios of the weight-average mol. wt. of the four dehydroribonucleotides (A, 26.1 %, 329 mol. wt.; C, 29.1 %, 305 mol. wt.; G, 24.1 %, 345 mol. wt.; U, 20.7 %, 306 mol. wt.; Bachrach et al. 1964; Newman, Rowlands & Brown, 1973); and (c) the weight of the FMDV RNA taken as 2.65 × 10⁶ daltons (Newman et al. 1973; Talbot & Brown, 1972; Vande Woude, Swaney & Bachrach, 1972). The value 2.65 × 10⁶ daltons multiplied by the mole fractions of the dehydroribonucleotides and divided by the mol. wt. of each gave a result of 8270 nucleotides. If they are assumed to be separated by 3.17 Å, then the intact RNA strand length for FMDV RNA would be 2.62 µm. The ratio of this value to the length of the measured fragment length was used to calculate the mol. wt. of the fragment.

Analytical ultracentrifuge determination of molecular weights. A Spinco analytical ultracentrifuge with a Kel-F cell with quartz windows was used to centrifuge the RNA strands. Schlieren optical patterns were measured for calculation of s-rates, and in most experiments the area under the pattern was measured to determine relative concentrations. Measurements of s-rates and calculations of concentrations were made as described by Trautman & Hamilton (1972). The dialysed samples were centrifuged at 20 °C at approx. 40000 rev/min for periods up to 45 min. The pellets were then resuspended in the cell by shaking, and re-centrifuged after the addition of 0.1 ml of RNase at a concentration of 1 mg/ml to confirm that the peaks were composed of single-stranded RNA. The relationship between the mol. wt. (M) of single-stranded RNA and its s-rate as M = 1550 S² (Spirin, 1963) was used to
calculate the mol. wt. of the FMDV RNA fragments. These mol. wt. and those determined by electron microscopy were then compared as to coincidences and differences among the virus types.

RESULTS

Electron micrographs of loop droplets of dialysed samples showed many thin strands of varying lengths, some completely isolated from each other and some intertwined or attached to one another in end-to-end or partially overlapping patterns (Fig. 1). Only clearly distinguishable isolated strands were measured. One hundred or more strands were measured in each sample, and the length distribution was plotted for each virus type. The modal strand length for each type is different and dependent on the preparation. For the most recent preparations (Fig. 2) the modal lengths were 1.95 μm for A12, 1.35 μm for C8 and 1.65 μm for O2. Besides the modal length strands, each sample had three prominent strand lengths that represented 5% or more of the total measured. Histograms prepared from measurements of older samples had somewhat different distribution of lengths.

Schlieren diagrams are shown of an original virus suspension before dialysis in Fig. 3(a); of fragmented RNA in Fig. 3(b); and of the pattern that resulted when 0.1 ml of 1 mg/ml RNase was added to the fragments (Fig. 3c). Measurements of concentration from the areas under the peaks showed that the RNA fragments constituted approx. 33% of the total viral mass. There were three and sometimes four measurable peaks of fragmented RNA in each preparation (Fig. 3b). The s-rates were calculated for the RNA fragment peaks for several centrifuge runs from each type of virus and were converted to equivalent mol. wt. by the Spirin formula.

Comparison of data from two separate methods of measurement of samples which are presumably from a single population but are not identical, requires an evaluation of the measurements. It was assumed that coincidences in mol. wt. calculations from length measurements in the electron micrographs and from the ultracentrifuge peaks were significant. There were 16 classes of s-rates which differed one from the other by 0.5 S or greater measured from all experiments irrespective of virus type or concentration. There were 15 classes of strand lengths from electron micrographs which were separated by 0.1 μm or more, again irrespective of virus type or concentration. When the calculated mol. wt. from the s-rates and the electron micrograph strand lengths were compared, there were 11 instances of coincidence in the two sets of data. Because some of these represented only single s-rate determinations or very few strands measured, the emphasis in comparison between virus types was put on the 3 highest concentrations (Table 1).

The Spirin formula was written as $M = KS^d$ where M and S are mol. wt. and s-rate, respectively, with K as a constant and d as the exponent. The formula may be converted to a linear equation by taking the natural logarithm of both sides so that $\log M = \log K + d \log S$. Electron microscope and sedimentation data were then used in a computer program (Trautman & Hamilton, 1972) for fitting a least squares curve. For different hypothetical pairings of M, derived from the electron microscope measurements, and S, from the measured s-rates, the values of K and d that resulted were very different from the Spirin formula. Such calculations were made to show that the Spirin formula or one with K and d values very close to 1550 and 2.1 gave the best fit for all the data. In fact, when all the data was used the formula applicable to FMDV RNA fragments was $M = 1725 S^{2.07}$.

Table 1 shows the most frequent RNA strand lengths determined by electron microscopy or calculated from s-rates for three preparations of each virus type. Column no. 1 lists the major or modal fragments lengths and columns no. 2 and 3 list the next most frequent
Fig. 1. (a) A typical usable pattern of RNA strands from type A_{18} 119 FMDV. (b) A more twisted pattern of RNA strands from type O_{1} Brugge FMDV. (c) A highly condensed and essentially unusable pattern of RNA strands from type C_{3} Resende FMDV.
Fig. 2. Histograms of the distribution of RNA strands from a single experiment with the most recent preparations. Note the difference in modal length of each type of FMDV RNA. (a) A19, 119 measured; (b) C3 Resende, 134 measured; (c) O1 Brugge, 224 measured.

The strand lengths show some consistency among the three virus types. From electron microscopy, there was one RNA length 1.95 μm common to all three types of virus, while 1.05, 1.25, 1.45, 1.65, 1.75, and 2.05 μm fragments appear in at least two types. Also, the most frequent RNA fragments from type O1 virus appear generally to be larger than those from virus types A12 and C3. When centrifuge patterns could be measured, the modal strand lengths determined by electron microscopy and s-rate are identical. This identity also occurred for the second most frequent length in one type A12 preparation as well as for both the second and third most frequent lengths in two type C3 preparations. Also, the 2.05 μm fragment was found in one type A12 preparation by both electron microscopy and s-rate measurements, although not with the same frequency. Two lengths, 2.75 and 3.35 μm, are longer than the estimated length of intact FMDV RNA and, therefore, are presumed to be aggregates of smaller units.
Table 1. Lengths of major FMDV RNA fragments and aggregates

<table>
<thead>
<tr>
<th>Virus subtype</th>
<th>Strain</th>
<th>Preparation no.</th>
<th>Electron microscopy</th>
<th>Calculated from s-rates</th>
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<tr>
<td></td>
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<td>1</td>
<td>2</td>
</tr>
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<td>133</td>
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</tr>
<tr>
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<tr>
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<tr>
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<td>Resende</td>
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<td>1.45</td>
</tr>
<tr>
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</tr>
<tr>
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<td>1.25</td>
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</table>

* 1 is modal length, and 2 and 3 are next most frequent lengths.
† Where no figures appear, concentration of RNA fragments were too low to give measurable centrifuge patterns.

DISCUSSION

The data show that the RNA released from FMDV by dialysis against water consists mainly of broken fragments. Some of the most frequently occurring of these have common lengths across two or three of the virus types A12 strain 119, C3 Resende and O1 Brugge, as determined by electron microscopy and s-rate measurements. Arlinghaus et al. (1959) and Newman (1969) have also shown breakdowns in the structure of FMDV RNA, leading to multiple peaks in electrophoretic and ultracentrifuge patterns. The ease with which FMDV RNA is fragmented indicates that there are weak links in its structure. In the present study, the fragment length 1.95 μm (Table 1) appears to be common to RNAs from all three of the virus types A12, C3 and O1, and several other lengths were common to at least two of these virus types.

The variation in values of s-rates obtained by different authors even for the same RNA preparation has been noted by Spirin (1963) and was ascribed to variation in the shape of the molecules and to the presence of residual ions in the solutions. Therefore, variations might be expected among fragments of FMDV RNA. The electron microscope data were more easily obtained since many samples could be made under essentially similar conditions while low concentrations hampered ultracentrifugation. Agreement between measurements apparently depended on the length of time the purified virus had been stored. The histograms of the most recent samples had the best correlation with sedimentation results. The fact that under the most ideal conditions, the C3 Resende virus RNA (Table 1) showed good consistency between electron microscope and sedimentation data suggests that these fragments resulted from breaks at naturally weak linkages. The common fragment lengths from all three virus types might indicate that these lengths contained sequences typical of FMDV as a virus species.

It proved impossible to isolate fragments of common length from the different types of viruses to compare homology such as the 44 to 65 % homology reported by Dietzschold et al. (1971) using the entire FMDV RNA. The significance of the ready fragmentation of FMDV RNA into the strand lengths determined herein and the relationships between common lengths from the different viruses will require more study. The length histograms of vesicular stomatitis virus (VSV) RNA reported by Weber et al. (1974) are different from...
Fig. 3. (a) The sedimentation pattern of an original FMDV suspension before breakdown by dialysis against H₂O. (b) A typical sedimentation pattern of FMDV RNA suspended in distilled water. (c) The sedimentation pattern that results when the material in (b) is resuspended and 0.1 ml of 1 mg/ml RNase is added to the centrifuge cell before re-run.
those of FMDV RNA in that the modal length (3.1 μm) appears to be that of intact negative strands. However, as in FMDV RNA, Weber showed that VSV RNA must also be quite fragile. In contrast to the ready fragmentation of FMDV RNA during isolation from the virus, East et al. (1973) reported that a 28S RNA is the basic subunit of a mouse sarcoma virus RNA even though RNA can only be isolated from virions in several polymeric forms.

I gratefully acknowledge the assistance of S. J. Pyne and J. J. Giovannelli with electron microscopy, and of C. E. Bennett with the ultracentrifuge. Dr H. L. Bachrach and Dr R. Trautman provided valuable comments on the presentation of the data. Mrs J. L. Taylor was very skilled in the typing of the several versions of the text.

REFERENCES


(Received 29 September 1975)