New Evidence on the Structure of Nepoviruses

(Accepted 20 April 1971)

The nepoviruses (Harrison et al. 1971) are a group of nematode-transmitted plant viruses that have isometric particles with slightly angular outlines and diameters of 28 to 30 nm. Purified preparations of these viruses typically contain particles of three types, differing in RNA content (Stace-Smith, Reichmann & Wright, 1965; Diener & Schneider, 1966). The particle structure of nepoviruses has been little studied but, using evidence obtained by electron microscopy of negatively stained preparations, it was proposed that the protein shells of two nepoviruses, tobacco ringspot (Chambers, Francki & Randles, 1965) and arabis mosaic (Agrawal, 1967), are composed of 42 morphological subunits. This work did not, however, take into account all other possibilities and our results, described below, show that the particles cannot be built of 42 morphological subunits.

Three nepoviruses were used. Raspberry ringspot and tobacco ringspot viruses were purified by making extracts in 0.07M-phosphate buffer, pH 7, of systemically infected Nicotiana clevelandii Gray leaves, clarifying the extracts by adding n-butanol to 8.5% (v/v), and separating the virus from host material by two cycles of high- and low-speed centrifugation followed by passage through an agarose (Sepharose 2B; Pharmacia AB) column and concentrating the material in the virus-containing peak by ultracentrifugation. Arabis mosaic virus was similarly purified from systemically infected leaves of Chenopodium amaranticolor Coste & Reyn.

The three components (T, M and B) found by analytical ultracentrifugation of purified preparations of raspberry ringspot virus (Debrot, 1964; Murant, Taylor & Chambers, 1968) were found to have sedimentation coefficients $S_{20,w}$ (at infinite dilution) of 52, 92 and 130 s, respectively. These components were separated by two cycles of centrifugation in sucrose density gradients. RNA was not detected in preparations of T, which had $A_{260}/A_{180}$ ratios of about 0.7 and contained particles all of which were penetrated by phosphotungstate when examined by electron microscopy. These results, and analogy with tobacco ringspot virus (Stace-Smith et al. 1965), indicate that T is the RNA-free protein shell of the virus. Hence it can be calculated (Reichmann, 1965) that M and B particles contain 28% and 43% RNA respectively. M particles contain about $1.4 \times 10^6$ daltons and B particles contain about $2.4 \times 10^6$ daltons of RNA as determined by polyacrylamide gel electrophoresis (M. A. Mayo, unpublished results). These figures therefore give estimates of $3.6 \times 10^6$ and $3.2 \times 10^6$ daltons for the protein shells of M and B particles, respectively.

Another type of estimate (Markham, 1967) can be made using the Svedberg equation, calculating the partial specific volumes of the three components from their percentage composition of protein and RNA, and assuming their diffusion coefficients are $1.5 \times 10^{-7}$ cm$^2$/sec., the approximate value found for turnip yellow mosaic virus (Markham, 1951), which has particles of similar diameter to those of nepoviruses. The resulting estimates of particle weight are then $3.3 \times 10^6$ (T), $4.8 \times 10^6$ (M) and $6.2 \times 10^6$ (B) daltons, which, when the percentage of RNA is deducted, give $3.3$, $3.5$ and $3.5 \times 10^6$ daltons for the protein part of each kind of estimate involves some assumptions, both methods of calculation suggest that particles. Although the total weight of protein per particle of raspberry ringspot virus is about $3.4 \times 10^6$ daltons.
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The molecular weight of the protein subunits of raspberry ringspot virus was estimated by polyacrylamide gel electrophoresis. Protein, prepared by boiling purified virus for 1 min. in 1% sodium dodecyl sulphate + 1% 2-mercaptoethanol, was analysed by electrophoresis in 7.5% acrylamide gels, using 0.1M-sodium phosphate buffer, pH 7.0. The molecular weights of proteins were estimated by comparing their rates of migration with those of well-studied proteins run simultaneously in control gels. The proteins used were a mixture of

![Electrophoresis of virus and marker proteins](image)

*Fig. 1. Electrophoresis of virus and marker proteins in gels of 7.5% acrylamide containing sodium dodecyl sulphate; run for 5 hr at 4 v/cm. and stained with Coomassie brilliant blue: (a) protein from raspberry ringspot virus, T particles; (b) protein from raspberry ringspot virus, B particles; (c) protein from arabis mosaic virus; (d) bovine serum albumin (bands 1) and polymerized β-lactoglobulin (bands 2).*
\(\beta\)-lactoglobulin (Koch–Light, Ltd), polymerized using diethylpyrocarbonate (Wolf et al. 1970), and bovine serum albumin (Calbiochem Ltd). Purified virus preparations, and preparations of T or B particles, yielded one polypeptide with a molecular weight of 54,000 ± 2,000 (Fig. 1).

This estimate was not altered when gels containing 0.1 % 2-mercaptoethanol were used. Nor was it altered when the virus was disrupted in medium containing 8M-urea, 1 % sodium dodecyl sulphate, 1 % 2-mercaptoethanol and 0.07 M-tris at pH 8.3, and/or the protein alkylated by incubating the preparation with 0.3 M-iodoacetamide at 37°C for 20 min. These results suggest that the polypeptide observed is not composed of smaller polypeptides joined by disulphide linkages.

Evidence obtained by electron microscopy indicates that particles of raspberry ringspot

<table>
<thead>
<tr>
<th>Sedimentation coefficient at infinite dilution ((s))</th>
<th>Raspberry ringspot virus</th>
<th>Arabis mosaic virus</th>
<th>Tobacco ringspot virus</th>
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<tbody>
<tr>
<td>T</td>
<td>53</td>
<td>53†</td>
<td>53‡</td>
</tr>
<tr>
<td>M</td>
<td>93†</td>
<td>93†</td>
<td>91‡</td>
</tr>
<tr>
<td>B</td>
<td>126†</td>
<td>126†</td>
<td>126‡</td>
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RNA in particles\((%\) | M | 28 | 28 |
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<tbody>
<tr>
<td>B</td>
<td>41</td>
<td>41</td>
<td>41</td>
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Weight of RNA in particles\((\text{million daltons})\) | M | 1.4 |
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<tr>
<td></td>
<td>B</td>
<td>2.4</td>
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Calculated weight of protein shell\((\text{million daltons})\) | M | 3.6 |
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<tbody>
<tr>
<td></td>
<td>B</td>
<td>3.2</td>
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Calculated partial specific volume** | T | 0.74 |
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<tbody>
<tr>
<td>M</td>
<td>0.69</td>
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<tr>
<td>B</td>
<td>0.66</td>
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Diffusion coefficient\((\times 10^{-7}\text{ cm.$^2$.sec.$^{-1}$})\) | T, M and B | 1.5 |

Calculated weight of protein shell\((\text{million daltons})\) | T | 3.3 |
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<tbody>
<tr>
<td></td>
<td>M</td>
<td>3.5</td>
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<td></td>
<td>B</td>
<td>3.5</td>
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Weight of polypeptide subunit\((\text{daltons})\) | Raspberry ringspot virus | 54,000 (30) |
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<tr>
<td>Estimated no. polypeptide molecules/virus particle</td>
<td>57 to 69</td>
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Figures in parentheses are the number of estimates averaged.

* T, M and B particles are the three components found by analytical centrifugation, in order of increasing weight.

† R. Stace-Smith, personal communication.

‡ Stace-Smith (1970).

§ Calculated using Reichmann's (1965) formula.

‖ Determined by polyacrylamide gel electrophoresis (M. A. Mayo, unpublished results).

¶ Calculated from the percentage and weight of RNA in the particles.

** Assuming RNA = 0.55 and protein = 0.74.

†† Assumed value for particles of 28 nm diameter.

‡‡ Calculated using the Svedberg equation, and the percentage of RNA in the particles.
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virus are icosahedral (Harrison & Nixon, 1960); and icosahedral structures must be built of 60 asymmetric units, each of which may contain one or more chemical subunits (Caspar & Klug, 1962). The ratio of the total weight of the protein shell to that of the polypeptide molecule, calculated from the figures obtained above, lies in the range 57 to 69, and we therefore think that the correct value is 60. Thus the experimentally determined figures suggest that the virus particles contain 60 structural subunits, each consisting of a single polypeptide molecule. They are incompatible with an icosahedral structure composed of 42 morphological subunits, which would contain at least 240 polypeptide molecules (Caspar & Klug, 1962). Less detailed work with arabis mosaic and tobacco ringspot viruses (Table 1) suggests that the particles of these viruses similarly contain 60 identical polypeptide molecules. Thus the structure we propose is probably common to all nepoviruses.

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REFERENCES


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