The Structure and Characterization of a Closterovirus, Beet Yellows Virus, and a Luteovirus, Beet Mild Yellowing Virus, by Scanning Transmission Electron Microscopy, Optical Diffraction of Electron Images and Acrylamide Gel Electrophoresis

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SUMMARY

Attempts were made to determine more precisely the structure and properties of a closterovirus, beet yellows virus (BYV), and a luteovirus, beet mild yellowing virus (BMYV), by scanning transmission electron microscopy, optical diffraction of electron images and acrylamide gel electrophoresis. It was shown that BYV has a mol. wt. of $76.5 \times 10^6$, including an RNA of $4.15 \times 10^6$. The pitch of the helix is $3.7 \text{ nm}$ and there would be $8.5$ protein subunits per turn. BMYV particles have a diameter of $26 \text{ nm}$ and a total mol. wt. of $6.5(\pm 0.45) \times 10^6$. The mol. wt. of the protein subunit is about $24000$ and that of the RNA $2 \times 10^6$.

Beet yellowing viruses present in Europe can be divided into two groups according to the morphology of the particles: viruses with an elongate form such as beet yellows virus (BYV) (see Fig. 1), a closterovirus, and those of nearly spherical shape such as beet mild yellowing virus (BMYV), a luteovirus (see Fig. 2).

BYV particles are about 1250 nm long, characteristically flexuous filaments with clearly defined helical cross-banding (Brandes & Wetter, 1959). The structure of the filaments has been determined previously by several authors (Brandes & Zimmer, 1955; Horne et al., 1959; Russell & Bell, 1963; Varma et al., 1968; Bar-Joseph & Hull, 1974; Carpenter et al., 1977). However, these data were mostly obtained from measurements of micrographs and varied considerably. Probably because of the difficulty in obtaining sufficient quantities of purified virus, the physical properties of BMYV are unknown. Two other luteoviruses, the type virus barley yellow dwarf virus (BYDV) and potato leafroll virus (PLRV), are characterized in more detail. The particles of both viruses contain single-stranded RNA of mol. wt. about $2 \times 10^6$ (Brakke & Rochow, 1974; Rowhani & Stace-Smith, 1979). PLRV RNA is linked to a small protein (Mayo et al., 1982).

A new approach to characterizing both BYV and BMYV by means of scanning transmission electron microscopy (STEM), optical diffraction and polyacrylamide gel analysis is described in this paper and the results obtained are discussed.

Isolates of BYV and BMYV were kindly supplied by Y. Bouchery (Station de Zoologie, INRA, Colmar, France) and were maintained in sugar beet (Beta vulgaris L.) and Claytonia perfoliata Donn. Purification methods were as described previously (Chevallier & Putz, 1982). The preparations of viruses were negatively stained with $2\%$ uranyl acetate or $2\%$ uranyl formate, after adsorption to carbon-coated collodion film substrates. The micrographs were recorded using a Philips 301 electron microscope at 80 kV, with a nominal magnification of $\times 44000$.

Optical diffraction of these micrographs was performed as described by Aebi et al. (1973). A scanning transmission electron microscope (Vacuum Generators STEM, HB-5) interfaced to a
Fig. 1. Beet yellows virus (BYV). (a) Conventional bright field image of negatively stained virus; (b) scanning dark field image of unstained virus. The bending of the filaments suggests that BYV rods are flexible. (c) Optical diffraction patterns from occasional straight and well-preserved stretches showing the main reflections at (3.7 nm)$^{-1}$ and frequently a layer-line at (7.4 nm)$^{-1}$. To determine the filament mass per unit length from STEM dark field images, homogeneous stretches were selected [see boxes in (b)]. Box coordinates and filament directions (horizontal or vertical centre lines of the boxes) are transmitted to the computer which tracks the filament (d) and calculates mass and length of the selected stretch. Mass per unit length values of 34 single filament stretches represented in histogram (e) yield an average of 6.12 (± 0.55) × 10$^4$ per nm. The bar marker in (c) represents 10 nm and the ordinate values in (e) are percentages.

cigital data acquisition system (minicomputer UNIVAC) as described by Engel et al. (1981) was used to determine the filament mass per unit length of BYV and the total mass of BMYV, following the procedure of Engel (1979). The validity of the method was checked using tobacco mosaic virus (TMV) as a reference for mass measurements. Precision of this method compared favourably with the best method hitherto used: the analytical ultracentrifuge.

Proteins were prepared for gel electrophoresis and analysed according to the method of Weber & Osborn (1969) and silver-stained as described by Merril et al. (1981).
RNA was obtained by phenol–SDS extraction of purified virus and the molecular weights of glyoxal-denatured RNAs were determined by 0·8% agarose gel electrophoresis as described by McMaster & Carmichael (1977).

We found a mol. wt. of 25 000 for the BYV protein subunit; this value is close to the published data (23 500, Bar-Joseph & Hull, 1974; 22 500, Carpenter et al., 1977; 24 000, Short et al., 1977). The mol. wt. of BYV RNA was estimated to be 4·15 × 10^6 by gel electrophoresis using the DNA of phage λ cut with HindIII (7·8, 3·07, 2·18, 1·42, 0·73 and 0·66 all × 10^6), and the RNAs of TMV (2·19 × 10^6) and alfalfa mosaic virus (1·3, 1·0, 0·7 and 0·3 all × 10^6) as markers. This is very similar to the value of 4·4 × 10^6 obtained for undenatured RNA by Bar-Joseph & Hull.
Short communication

(1974) who used a mol. wt. marker of $4 \times 10^6$, and also to the values derived from the sedimentation coefficients of denatured RNA ($4.2 \times 10^6$, $4.6 \times 10^6$) (Bar-Joseph & Hull, 1974; Carpenter et al., 1977). The total mass per unit length obtained by STEM is $61200 \pm 5500$ per nm, $N = 34$. Thus, the total mol. wt. of a 1250 nm-long particle is estimated to be $76.5 \times 10^6$ (Fig. 1 c). Bar-Joseph & Hull (1974) calculated a value of $84 \times 10^6$ for the BYV mol. wt.

The optical diffraction patterns from electron micrographs of BYV (Fig. 1 c) particles showed a strong layer-line at a meridional spacing of $(3.7 \text{ nm})^{-1}$ and frequently a weaker layer-line at $(7.4 \text{ nm})^{-1}$. This result is in agreement with the value of $3.75 \text{ nm}$ for BYV helical pitch reported by Bar-Joseph & Hull (1974) from particle measurements in electron microscope preparations. Measured on ten different diffraction patterns, the ratio between the first and the second layer-line position is $2 \pm 0.04$, indicating that the helix is in register every other turn. Using the mass per unit length value ($61200 \text{ per nm}$; Fig. 1) we estimate that there are $226440$ daltons per turn. Taking into account the BYV subunit mol. wt. ($25000$), the RNA mol. wt. ($4.15 \times 10^6$), the particle length ($1250 \text{ nm}$) and the helix pitch ($3.7 \text{ nm}$), we obtained a total of $8.57$ subunits per turn. The presence of a distinct layer-line at half the distance of the first meridional reflection therefore suggests $8.5$ subunits per turn to be the most acceptable figure.

Thus, the structure appears to repeat every $7.4 \text{ nm}$ after two turns of the primary helix, and to contain $17$ subunits per helical repeat. Bar-Joseph & Hull (1974) reported that they observed approximately $10$ subunits in end-on views of BYV per turn, which can be considered as a near value.

To our knowledge, no structural data concerning BMYV have been published as yet. In general, luteoviruses which are phloem-limited and not mechanically transmissible have been much less studied in their chemical and physical properties than other plant virus groups. Rochow & Duffus (1981) reviewed the data concerning BYDV, an icosahedral virus approximately $23 \text{ nm}$ in diameter with one major type of coat protein of mol. wt. $24000$. These authors reported also that an isolate of beet western yellows virus, serologically related to BMYV, yielded a single RNA species of mol. wt. $1.9 \times 10^6$. By electron microscopy, using T4 phage tails as a standard, we found that the mean diameter of BMYV particles is $26 \text{ nm}$ (Fig. 2a). Using the same markers as for the BYV, we determined the mol. wt. of the denatured RNA to be $2.0 \times 10^6$. Analysis of the protein composition of purified BMYV by gel electrophoresis showed that the virus contained a major structural protein with a mol. wt. of $24000$ (Fig. 2b, c). STEM mass determination yielded a total mol. wt. of the particle $6.5(\pm 0.45) \times 10^6$, $N = 29$ (Fig. 2d).

If we consider that BMYV is a particle with a surface structure similar to that of turnip yellow mosaic virus, with a triangulation number $T = 3$, the protein shell of BMYV would be made up of $180$ protein subunits. With a mol. wt. of $24000$ per subunit, the empty protein shell would have a total mol. wt. of $4.32 \times 10^6$, in agreement with STEM mass determination of empty particles, which gave a mol. wt. of $4.0(\pm 0.4) \times 10^6$, $N = 36$.

Thus, electron microscopical methods allow us to obtain results that accord well with those derived by biochemical methods. These methods are particularly useful for the study of structural properties of particles such as those of luteoviruses which are known to be in very low concentration in plants.

REFERENCES


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