Diffraction Studies of Tulip Virus X Particles

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SUMMARY

X-ray diffraction from oriented tulip virus X (TVX) particles and optical diffraction from electron micrographs show that the virus particles have a helical structure with a pitch of about 3.25 nm and a true repeat in five turns of the helix. Both X-ray and optical diffraction indicate that the number of subunits in the repeat period is 5q + 4, where q is an integer. The X-ray diffraction patterns suggest that 9 ≥ q ≥ 6, and the optical diffraction patterns suggest that q is probably 7 or 8. The X-ray diffraction patterns indicate that there is a marked feature in the virus particle at a radial position of about 3.3 nm, which is probably the RNA. The equatorial diffraction further suggests that the virus has an axial hole of about 1.5 nm radius.

INTRODUCTION

Tulip virus X (TVX) is a recently discovered virus with flexuous filamentous particles about 500 nm long and 13 nm in diameter (Mowat, 1979). As judged by its particle morphology, biological properties, particle composition and serological behaviour, it is a definitive potexvirus (W. P. Mowat, unpublished results). In common with many members of this group (Koenig, 1978), TVX attains high concentrations in some of its experimental host plants. Preparations of virus particles, which are strongly birefringent, are readily obtained from inoculated leaves of Chenopodium quinoa either by clarification of sap with n-butanol or by precipitation with polyethylene glycol 6000 in the presence of 0.2 M-NaCl and further purified by cycles of differential centrifugation.

METHODS

X-ray diffraction. Oriented specimens of TVX were prepared by the coverslip method (Bernal & Fankuchen, 1941; Tollin et al., 1968). X-ray diffraction patterns were obtained with pin-hole cameras of the type described by Langridge et al. (1960) and with a low-angle camera with slit collimation, using nickel-filtered CuKα radiation. Humidity was controlled with saturated salt solutions. Intensity measurements were made with a Joyce-Loebl recording densitometer and the discrete equatorial reflections were corrected for randomness, disorientation and multiplicity (Langridge et al., 1960).

Electron microscopy. TVX samples were air-dried on to carbon-filmed support grids and negatively stained. Uranyl formate/sodium hydroxide [I. M. Roberts, personal communication (in Barnett & Murant, 1970)] was preferred to other negative stains tried, as it gave good contrast and little deformation of the particles. Preparations were examined in a Philips 301G electron microscope at 80 kV accelerating voltage, with instrumental magnification × 43 000 and a fixed objective lens current. The magnification was calibrated using catalase crystals.

Optical diffraction. The optical diffractometer was similar to that developed by Taylor & Lipson (1964). Regions of the micrographs where the virus particles formed ‘rafts’, and where
the virus particles were straight, were masked off and used as diffraction objects (Klug & Berger, 1964).

RESULTS

X-ray diffraction

An X-ray diffraction pattern of an oriented dry specimen at 75% relative humidity is shown in Fig. 1. The pattern is very similar to that from narcissus mosaic virus (Tollin et al., 1968) and can be interpreted in the same way. It is characteristic of that from a helical structure which repeats in five turns of the helix, with 5q + 4 subunits in the repeat period, where q is an integer lying in the range 9 > q > 6. The pitch of the helix is 3.24 ± 0.03 nm at 75% relative humidity and 3.07 ± 0.05 nm in the very dry state.

Discrete Bragg reflections are only observed in the inner region of the equator of the diffraction pattern and these can be indexed on a hexagonal lattice. In the very dry specimens, with phosphorus pentoxide in the X-ray camera, the separation of the virus particles is 9.9 ± 0.1 nm and at 75% relative humidity the separation is 11.0 ± 0.1 nm. The diameter of TVX particles, as measured in electron micrographs of stained particles is 13 ± 0.5 nm. Although this may be affected by flattening or stain edge effects it suggests that there is a high degree of interlocking of the helical particles in the dry specimens, as observed with other viruses (Wilson et al., 1973).

The intensity distribution on the fifth layer-line (the one corresponding to the pitch of the helix) can be described by the first-order Bessel function J₁(2πRr₀), where r₀ = 3.25 ± 0.15 nm, suggesting that there is a marked feature in the virus particle at this radial position (Table 1). This feature is probably the sugar–phosphate backbone of the RNA because this has a higher electron density than the protein. This radial position is close to the proposed position of the RNA in potato virus X (Wilson & Tollin, 1969) and narcissus mosaic virus (Wilson et al., 1973).

The contribution of the RNA to the equatorial diffraction depends on J₀ (2πRr₀), where J₀ is the zero-order Bessel function. However, a strong equatorial reflection is observed at the position of the first zero of J₀(2πR3.3), so some other factor must also be affecting the equatorial diffraction. This could be an axial hole in the virus particle. Since, in the dry specimens, the virus particles interlock, at low resolution the specimen can be pictured as a uniform distribution of protein in which there is a hexagonal arrangement of holes coaxial with helical RNA molecules embedded within the protein (Fig. 2). The equatorial diffraction at low resolution can then be represented by

\[ J₀(2πRr₀) - \frac{J₁(2πRr₁)}{2πRr₁} \]

where r₁ is the radius of the axial hole and x is a scaling factor. If the strong first reflection were to be explained in terms of a J₀ Bessel function alone, the value of r₀ would have to be changed by at least 0.5 nm, which is well outside the range indicated by the fifth layer-line measurements. Moreover, the agreement further out along the equator would be poorer.

The first three equatorial reflections, indexed as (1,0) (1,1) and (2,0) appear much stronger than the others. The (2,1) (3,0) and (2,2) reflections are also observed, but the (3,1) reflection is absent or very weak, indicating that there is a zero of the transform in that region. This is close to a zero of J₀(2πR3.3), the first term in the equatorial diffraction expression, and must also be close to a zero of the second term in order to give a resultant zero. This would correspond to r₁ about 1.5 nm, which is a very reasonable value for the radius of an axial hole and is similar to that observed in potato virus X particles (Varma et al., 1968). The value of
Fig. 1. (a) X-ray diffraction pattern of a TVX specimen at 75% relative humidity. (b) Low-angle equatorial X-ray diffraction pattern of a dry specimen of TVX showing the (1,0) (1,1) and (2,0) reflections.

Table 1. Observed and calculated positions of diffraction maxima on the fifth layer-line of the X-ray diffraction pattern

<table>
<thead>
<tr>
<th>$R$ (nm$^{-1}$)</th>
<th>Observed</th>
<th>Calculated*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.095</td>
<td>0.090</td>
<td></td>
</tr>
<tr>
<td>0.273</td>
<td>0.262</td>
<td></td>
</tr>
<tr>
<td>0.395</td>
<td>0.418</td>
<td></td>
</tr>
<tr>
<td>0.578</td>
<td>0.573</td>
<td></td>
</tr>
</tbody>
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* The calculated values correspond to the maxima of $J_1(2\pi R r)$ where $r = 3.25$ nm.

$x$, which relates the scattering of the RNA and the hole, is 0.8 in Fig. 3. This is a reasonable value when compared with that estimated on the basis of the radial electron density distribution for tobacco mosaic virus (Franklin & Holmes, 1958). It can be seen from Fig. 3
Fig. 2. Diagrammatic representation of a low-resolution projection along the virus axis within a dry TVX specimen. The clear regions represent the axial holes, the dark rings represent the RNA and the background represents the protein.

Fig. 3. Calculated and observed equatorial X-ray diffraction. ●, Observed amplitude of diffraction. Only the magnitude is determined experimentally—the sign has been made the same as that of the calculated transform.

\[ J_0(2\pi R_{3.3}) - 0.8 \frac{J_1(2\pi R_{1.5})}{2\pi R_{1.5}} \]

that the effect of the hole is to displace the first zero position of the transform and this accounts for the observed (1,0) reflection. Thus, the low resolution diffraction on the zero and first layer-lines can be interpreted. There is evidence of an axial hole in electron micrographs of some of the virus particles (see Fig. 4 a), but an accurate measurement of its diameter is
difficult. However, estimates made from enlargements are not inconsistent with the X-ray results.

**Optical diffraction**

Optical diffraction patterns were obtained from electron micrographs of groups of parallel virus particles and from individual particles (Fig. 4). Those from the groups of particles were clearest, and they all showed near-meridional maxima on a layer-line at 0.30 ± 0.01 nm⁻¹, almost always on both sides of the meridian, although the intensity varied across the meridian. These diffraction maxima are due to stain between turns of the primary helix. Diffraction maxima are also observed on a layer-line at 0.061 ± 0.005 nm⁻¹, but in most cases only on one side of the meridian. The ratio of the two layer-line spacings is very close to 5, indicating that the structure repeats in approx. five turns of the primary helix. The intensity on the 0.30 nm⁻¹ layer-line is stronger in the same quadrants as the observed intensity on the 0.061 nm⁻¹ layer-line, confirming the X-ray results that there are 5q + 4 subunits in the true repeat period (Tollin *et al.*, 1975).

The distance of the diffraction maximum on the 0.06 nm⁻¹ layer-line from the meridian (R) indicates the order of the Bessel function contributing to this layer-line and this depends on the number of subunits per turn of the primary helix. Measurements of R combined with the assumption that the stain is close to the outside of the particle at r = 6.2 nm, gives an average value of 2πRr for this diffraction maximum of 10.0 ± 0.5. The first maximum of $J_4(x)$ occurs at $x = 9.6$ and that of $J_5(x)$ at $x = 10.7$, which suggests that the order of the Bessel function on the 0.06 nm⁻¹ layer-line is probably 8 or 9. The corresponding values of q would then be 7 or 8 (Tollin *et al.*, 1975), which implies 7-8 or 8-8 subunits per turn of the helix.
A fair amount of information is now accumulating about the structural parameters describing particles of members of the Potexvirus group. These studies show that the number of protein subunits per turn of the helix is very similar in the different viruses of the group, and in most of them is close to, but fractionally less than, 9 (Richardson et al., 1981). Thus, for example, in potato virus X itself, the evidence suggests that there are 8.875 subunits per turn (Tollin et al., 1980). This has led Richardson et al. (1981) to put forward the hypothesis that the number of subunits per turn in all members of the Potexvirus group is close to, but slightly less than 9, and that different viruses mainly differ in the fractional departure from 9. The possible value of 8.8 for TVX would thus fit in with this hypothesis whereas 7.8 would not. Further studies should make it possible to decide between these two values.

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REFERENCES


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