Diffraction Studies of Clover Yellow Mosaic Virus

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SUMMARY

X-ray and optical diffraction evidence is given which indicates that the particle of clover yellow mosaic virus has a helical structure of pitch 3.4 nm which repeats in approx. 11 turns with 11q - 2 subunits in the true repeat and with the value of q most likely to be 9. The position of the nucleic acid at 3.6 nm from the axis of the particle is deduced from the low resolution X-ray diffraction pattern.

The classification of viruses depends on determining as many properties and characteristics as possible (Harrison et al., 1971). In our studies of the Potexvirus group we have carried out X-ray and optical diffraction studies of clover yellow mosaic virus (CYMV). The virus was grown and purified as before (Bancroft et al., 1979) and the electron microscopy, optical diffraction and specimen preparation for the X-ray studies were performed as described previously (Tollin et al., 1979).

The X-ray diffraction pattern shown in Fig. 1(a) is from a dry specimen of CYMV at 70% relative humidity. The measurements obtained from this pattern are given in Table 1. The first, second and fourth near-meridional maxima lie on layer-lines of mean spacing 0.303 nm⁻¹ and appear to be from a helical particle of 3.3 nm pitch. There are other layer-lines in the diffraction pattern which are closer together, indicating that the particle does not have an integral number of protein subunits in one turn. Measurements given in Table 1 suggest that the true repeat is a multiple of a value lying between five and six turns of the helix.

In the near-meridional region far out in the pattern the maxima occur closer to the centre of the pattern than they would if they lay on layer-lines corresponding to 3.3 nm. This indicates that the number of subunits/turn is slightly less than, rather than slightly more than, an integer. In the latter case the maxima would be at larger reciprocal distances than the layer-lines corresponding to the pitch.

Poorly oriented specimens of a solution of the virus showed maxima in the X-ray diffraction pattern only on the first and second layer-lines and at spacings comparable with those of the dry specimens.

Optical diffraction patterns were obtained from some 20 individual particles, one of which is shown in Fig. 1(b). All showed maxima on a layer-line at 0.294 nm⁻¹, almost always on both sides of the meridian, and on a layer-line at a spacing of usually between a fifth and a sixth of the 0.294 nm⁻¹ layer-line. Sometimes the maxima on this latter layer-line lay on only one side of the meridian, indicating that even when stain has penetrated into both sides of the primary helix it may only penetrate into one side of the secondary helix. The mean value of the ratio of the distance of the two layer-lines from the equator was 5.66 ± 0.13, but with extreme values of 4.26 and 6.98. A similar average ratio was obtained for reconstituted CYMV and for CYMV protein assembled around polyadenylic acid.

Measurements of the diam. of about 80 negatively stained particles gave an average value of 14.0 ± 1.4 nm. Using transverse sections through dry specimens of CYMV (similar to those obtained for papaya mosaic virus; Tollin et al., 1979) the centre-to-centre distance between the particles was measured as 11.6 nm, indicating some inter-penetration of the particles similar to that found in specimens of other viruses of this group (Tollin et al., 1968).
Fig. 1. (a) X-ray diffraction pattern for CYMV at 70% relative humidity. (b) Optical diffraction pattern for a single particle of CYMV.

Table 1. The axial repeat period in clover yellow mosaic virus at 75% relative humidity as determined from the X-ray diffraction pattern

<table>
<thead>
<tr>
<th>Distance on film (nm)</th>
<th>Spacing (nm)</th>
<th>Layer-line (nm)</th>
<th>Repeat period (nm)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.5</td>
<td>3.24</td>
<td>11</td>
<td>35.7</td>
</tr>
<tr>
<td>10.9</td>
<td>1.64</td>
<td>22</td>
<td>36.1</td>
</tr>
<tr>
<td>12.0</td>
<td>1.50</td>
<td>24</td>
<td>35.8</td>
</tr>
<tr>
<td>15.4</td>
<td>1.17</td>
<td>31</td>
<td>36.1</td>
</tr>
<tr>
<td>16.4</td>
<td>1.09</td>
<td>33</td>
<td>36.1</td>
</tr>
<tr>
<td>21.4</td>
<td>0.84</td>
<td>42</td>
<td>35.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(44)</td>
<td>37.1</td>
</tr>
<tr>
<td>26.8</td>
<td>0.68</td>
<td>53</td>
<td>35.9</td>
</tr>
<tr>
<td>37.5</td>
<td>0.49</td>
<td>75</td>
<td>37.0</td>
</tr>
<tr>
<td>43.5</td>
<td>0.43</td>
<td>86</td>
<td>37.0</td>
</tr>
<tr>
<td>50.4</td>
<td>0.38</td>
<td>97</td>
<td>36.6</td>
</tr>
</tbody>
</table>

* The positions of the layer-lines on the X-ray diffraction pattern interpreted in terms of a true repeat of 36.3 nm.

The measurements of the position of the maxima on the equator of the X-ray diffraction patterns are consistent with the positions of a hexagonal lattice of spacing 12.0 nm, which agrees with the transverse sections. The intensities of these crystalline reflections are consistent with the successive maxima of $J_0(2\pi R)$ with a value of $r = 3.6$ nm, modified by a $J_1(x)/x$ function due to an axial hole in the virus. The maxima on the first layer-line corresponding to the pitch (layer-line 11 of Table 1) and those on the second (22 of Table 1) can be interpreted in terms of $J_1(2\pi 3.6R)$ and $J_2(2\pi 3.6R)$ respectively. Beyond the second this interpretation is no longer valid, when layer-lines other than those corresponding to the pitch occur. This suggests that, at low resolution (<2.0 nm), the X-ray diffraction pattern is dominated by scattering at a radius of 3.6 nm. This distance is a reasonable one for the phosphate backbone of the nucleic acid and agrees with the observation in the case of narcissus mosaic virus (Wilson et al., 1973). Thus, because the virus particles inter-penetrate, the structure at low resolution can be pictured as a uniform distribution of electron density,
corresponding to the protein, in which there is a lattice of holes co-axial with the helical RNA molecules embedded within it.

There is no necessity for the helical structure of a virus particle to repeat in an integral number of turns, and at high enough resolution it almost certainly does not. However, it is often convenient to describe the structure in these terms.

The distance from the meridian (R) of the diffraction maximum on the optical diffraction pattern on the closer-in layer-line indicates the order of the Bessel function contributing there and hence the approximate number of subunits/turn. Measurements of R, combined with the assumption that the stain was close to the outside of the particle at r = 6.5 nm, gave an average value of \(2\pi R\) for this reflection of \(10.8 \pm 0.02\). The first maximum of \(J_9(x)\) occurs at \(x = 10.7\). The values of 8 or 10 are not completely eliminated, since the individual values of R show considerable variation. However, they do lie within the limits for distorted helices with \(n = 9\) discussed by Moody (1967).

The optical diffraction pattern thus strongly supports a value of about 9 subunits/turn. The X-ray evidence indicates that the number must be just less than 9. The measurements of the ratio of the vertical position of the closer to the farther reflection \((x_n/x_n)\) in the optical patterns gives information on the number of subunits/turn. Following the notation of Wilson et al. (1978), if \(u\) is the number of subunits and \(t\) the number of turns in the true repeat

\[
\frac{u}{t} = n \frac{x_n}{x_1}
\]

where \(n\) is the order of the Bessel function of the closer-in layer-line. The average of \(x_1/x_n\) for CYMV from the optical diffraction patterns is 5.66 and of \(u/t\) is 8.82. The fact that \(x_1/x_n\) is not integral but close to 5.5 means that the particle must repeat in approx. 11 turns. The possible Bessel functions appearing on any layer-line are given by the selection rule: \(l = tn + um\), where \(m\) is an integer (Cochran et al., 1952).

If \(t = 11\), \(n = 9\), and \(l = 2\), then \(um = -97\); the only feasible solution then is \(m = -1\), \(u = 97\). This would give the particle 97 subunits in 11 turns or 8.82 subunits/turn, in good agreement with the optical diffraction results. Table 1 shows the allocation of layer-line numbers on the assumption of 97 subunits in the true repeat and hence the first truly meridional reflection corresponding to a spacing of 0.38 nm and the calculated true repeat distances which give a pitch of 3.3 ± 0.1 nm. The large differences in the position of the lower spot in the optical pattern correspond to very slight variations in the number of subunits/turn. The variation would explain the slightly different conclusion obtained from a limited number of observations on polymerized CYMV protein in which the number of subunits/turn was taken as 8.80 (Bancroft et al., 1979). Thus, the extreme values of 4.26 and 6.98 for \(x_1/x_n\) give values of \(u/t\) of 8.77 and 8.86, only slightly different from the mean value of 8.82.

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REFERENCES


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