Observations on the Structure of Particles of White Clover Mosaic Virus

(Accepted 1 December 1977)

SUMMARY

X-ray diffraction studies of oriented specimens of white clover mosaic virus particles suggest that the particles have a helical structure of pitch 3·25 ± 0·05 nm. The X-ray diffraction patterns indicate that there is an integral, or near integral, number of subunits in four turns of the helix and that the number is 4q + 3. Optical diffraction from micrographs of the virus particles are in agreement with the X-ray results and suggest that the number of subunits in four turns is close to 27.

White clover mosaic virus (WCMV), cryptogram R/I: 2·4/(6): E/E: S/(Ap), is a flexuous filamentous virus belonging to the same group as potato virus X (PVX). The particles have a modal length of 480 nm and a diameter of 13 nm (Bercks, 1971). Electron microscope studies by Varma et al. (1968) suggested that the particles were helical with a pitch of 3·4 nm and with perhaps 11 subunits per turn.

In the present study oriented dry specimens and oriented gels of WCMV were examined by X-ray diffraction, and electron micrographs of the particles by optical diffraction.

Oriented dry specimens were prepared by allowing the wet virus gel to dry between a slotted slide and a coverslip (Tollin et al. 1968), and oriented gels were prepared in thin quartz capillaries (Gregory & Holmes, 1965). Both methods of preparation are derived from those used by Bernal & Fankuchen (1941).

An X-ray diffraction pattern of a dry specimen at 70% relative humidity is shown in Fig. 1. Although the orientation is not as good as in the case of narcissus mosaic virus (NMV; Tollin et al. 1968), the pattern is sufficiently similar to suggest that the present structure is helical—a fact confirmed by the optical diffraction studies.

The pitch of the helix, derived from a set of layer-lines whose spacings are related by a simple sequence of integers, is 3·25 ± 0·05 nm, somewhat less than that previously reported. There are other layer-lines between those corresponding to the pitch, and therefore there is a non-integral number of subunits per turn of the helix. The position of these intermediate layer-lines indicates that the structure repeats in approximately four turns. If the layer-lines corresponding to the pitch are designated 4n, then the intermediate intensities lie on layer-lines which can be designated 4n + 1 and 4n + 3 but not 4n + 2. If this approximate designation is exact, then the particle repeats exactly in four turns. Adopting this simplifying assumption for the present, the intensities on the intermediate layer-lines mean that there is either 4q + 1 or 4q + 3 subunits in the true repeat. By considering the positions of the first maxima on the 9th and 11th, and the 15th and 17th layer-lines and assuming that the dominant scattering radius is the same for each pair of layer-lines, it appears that the number 4q + 3 fits the diffraction pattern better. Because of the disorientation, the position of the truly meridional intensity, required to determine q, cannot be determined.

The evidence from the X-ray diffraction patterns that the virus repeats in exactly four turns is not conclusive. Large changes in the true repeat in a helical structure can produce only very small variations in the diffraction pattern. The structure certainly repeats approxi-
Fig. 1. X-ray diffraction pattern of a dry WCMV specimen at 70% relative humidity.

mately in four turns and the number of subunits per turn is close to \((4q + 3)/4\), where \(q\) is an integer to be determined.

In contrast with PVX and NMV, it was possible to form oriented gels of WCMV. The diffraction pattern is still very poor but clearly shows a few layer-lines which enable the pitch of the helix to be determined. In the case of NMV and PVX the pitch of the helix was found to change markedly and progressively with humidity in the solid specimen, the pitch changing from 3.3 to 3.6 nm in going from low to high humidity in the camera (Tollin et al. 1967). One might expect that in a gel the pitch would change by an even greater amount; however, with WCMV this is not the case. Although an accurate determination of the pitch of the helix in the gel is difficult because of poor orientation in the specimens, we can say that if there is a change in the pitch of the helix in the gel compared with that in a dry specimen, it is certainly no larger than the 10% observed with PVX and NMV. All attempts to form oriented liquid specimens of NMV failed because of the formation of liquid crystals (Wilson & Tollin, 1970). It is tempting to suggest that the lack of a large increase in pitch with wetting in WCMV, suggesting stronger bonds between turns of the helix and therefore a stiffer structure, allows the virus particles to align themselves in a regular way in the liquid and prevent the formation of the liquid crystal phase.

Optical diffraction patterns from electron micrographs of WCMV show clearly that the structure is helical. Fig. 2(a) shows an electron micrograph of a particle stained with uranyl formate adjusted to pH 4.8 with sodium hydroxide (Barnett & Murant, 1970) and Fig. 2(c) shows an optical diffraction from it. There is a layer-line corresponding to a periodicity of \(3.2 \pm 0.1\) nm whose intensity is off-meridional and must be caused by stain in the groove
between the turns of the primary helix. On a few of the patterns there is another maximum on a layer-line corresponding to a periodicity of $12 \pm 0.5$ nm. This is likely to be caused by stain in one of the secondary grooves on the virus. A similar layer-line was reported by Varma et al. (1968). This layer-line suggests that the particle has an approximate repeat in four turns of the primary helix but that it may not be exact. When the staining is predominantly on one side of the particle, the maxima on both layer-lines occur in the same quadrant, which is consistent with the number of subunits being closer to $4q+3$ than to $4q+1$ for the following reason.

The orders $n$ of the Bessel functions occurring on the $l$th layer-line are given by those values of $n$ satisfying the relation (Cochran et al. 1952) $l = t m + um$, where $t$ is the number of turns in the true repeat period, $u$ is the number of subunits in the true repeat, and $m$ is an integer. In the present case we are concerned with layer-lines 1 and 4 and Bessel functions 1 and 7 (see below). For $l = 4$ and $m = 0$, $n = +1$. Since the maxima on the $l = 4$ and $l = 1$ layer-lines occur in the same quadrant, $n = +7$ on $l = 1$. Substituting in the above equation gives $1 = +4 \times 7 - u$ for $m = -1$, giving $u = 27$ (i.e. $4q+3$). If the maxima had been in opposite quadrants $n$ would have been $-7$, giving $u = 29$ (i.e. $4q+1$). Physically, the implication of the Bessel functions on the 1st and 4th layer-lines having the same sign is that the primary and secondary grooves giving rise to the two maxima have the same hand.

The positions of the maxima on the 3.2 nm layer-line correspond to the maxima of a $J_1$ Bessel function, with stain at a radius of $5.3 \pm 0.5$ nm, which means that there is some penetration of the stain into the particle. Assuming that the stain in the secondary groove is at the same radius and that there is no distortion of the particle then the Bessel function
contributing to the 12 nm layer-line is of order 7. In general, the distance from the equator to the two observed intensities can yield an estimate of the number of subunits per turn of the primary helix, i.e. $u/t$. If $x_1$ is the distance to the $J_1$ Bessel function layer-line and $x_n$ that for the $J_n$ layer-line, then $u/t = n - x_n/x_1$ when the reflexions are in the same quadrant. With $n = 7$, and taking into account the error in measuring the position of the layer-lines, the value of $u/t$ for WCMV lies between 6·72 and 6·75. Thus, while the structure is close to repeating in four turns, the true repeat may be much larger, or in fact even infinite. It should be noted that these calculations assume that there is no distortion of the particle. If there is distortion, then we can only conclude that the value of $n$ is close to 7, and the determination of its exact value requires further studies.

The number of subunits per turn is less than the estimated value of 11 given by Varma et al. (1968). Even if we assume that the stain giving rise to the diffraction maximum on the 12 nm layer-line is at the maximum possible radius (6·5 nm), the order of the Bessel function cannot be greater than 9 and the corresponding number of subunits per turn would then be between 8·72 and 8·75.

We thank Mr W. P. Mowat for the virus preparations.

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REFERENCES


(Received 28 September 1977)