A Re-evaluation of the Structure of Narcissus Mosaic Virus and Polymers Made from its Protein

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SUMMARY

The structure of narcissus mosaic virus (NMV) particles and its protein polymers have been re-examined. The virus particle contains 44 coat protein subunits in five turns of its helix and has a mol. wt. of about $36 \times 10^6$. Its protein forms stacked discs or rings at pH 5 which, with time, are replaced by two-start helices with 26 subunits in three turns or nine in one.

Particles of viruses belonging to the potato virus X family such as papaya mosaic virus (PMV; Tollin et al. 1979) and potato virus X (PVX; Tollin et al. 1980) have between eight and nine subunits per turn of their helices, the fraction depending on the true repeat. Narcissus mosaic virus (NMV) is an apparent exception (Tollin et al. 1975) and we have re-examined its structure. We confirm the report that NMV protein assembles as two-start helical particles (Robinson et al. 1975) and re-describe their structure. We also report on an additional structure.

NMV, a kind gift from Dr W. P. Mowat, was purified as for clover yellow mosaic virus (Bancroft et al. 1979) and disassembled at pH 8 in 2 M-LiCl after freezing (Robinson et al. 1975). The RNA was removed by centrifugation at 10000 rev/min for 15 min in a Sorvall centrifuge followed by centrifugation at 36000 rev/min for 4 h in a Beckman Model L centrifuge to remove residual virus. The resulting supernatant solution had a 280/250 nm ratio of 1.82. The protein, at 2 to 4 mg/ml, was dialysed overnight at 4 °C against pH 4, 5 and 6 buffers composed of $0.01\, M$- or $0.1\, M$-sodium citrate containing $0.01\, M$, $0.2\, M$- and $0.3\, M$-$\left(\text{NH}_4\right)_2\text{SO}_4$ or against pH 8, $0.1\, M$-tris containing $0.3\, M$-$\left(\text{NH}_4\right)_2\text{SO}_4$ (Robinson et al. 1975) measured at 4 °C. The dialysed solutions were then kept at room temperature before electron microscopy in 1% uranyl formate in a Philips EM 201 or an AEI EM6B. Best re-assembly results, determined by electron microscopy, were obtained with $0.01\, M$- or $0.1\, M$-citrate at pH 5 in $0.1\, M$-$\left(\text{NH}_4\right)_2\text{SO}_4$. Optical diffraction was as described by Tollin et al. (1979) and calibrations were made using catalase (Luftig, 1967).

NMV particles have a single-start helix repeating in five turns (Tollin et al. 1968). Tollin et al. (1975) deduced that there were $6\frac{4}{5}$ subunits per turn by using the ratio of the distances from the meridian of the first intensity maxima on the fifth and first layer lines and applying the helical selection rule (Cochrane et al. 1952). We have measured the pitch of NMV to be $3.4\, \text{nm}$ using catalase as the internal standard rather than more indirect estimates and the average unpacked diam. to be $14.1\, \text{nm}$ with a standard deviation of $0.8\, \text{nm}$ (60 particles), probably partly reflecting different degrees of flattening. Using these figures, taking the diffraction pattern in Fig. 1(b) of Tollin et al. (1975) and employing a calculation less subject to error than the ratio of distances from the meridian, we find that $2\pi r R = 10.34$, where $r$ is the radius of the particle and $R$ the position of the maximum on the first layer line. This value lies within the range of values obtained by us from measurements of diffraction patterns obtained from 19 single particles which gave an average value of $10.9$ with an error about the mean of $0.6$ (95% confidence interval). The values of $2\pi r R$ as the arguments for the first maxima of Bessel contributions $J_8$ and $J_9$ for a cylindrical particle are 9.65 and 10.71,
Fig. 1. Electron micrographs of NMV protein polymers after (a) 1 and (b) 3 days at 20 °C in 0.1 M-citrate, pH 5, containing 0.1 M-(NH₄)₂SO₄. The inset is a diffraction pattern showing stacked rings. Electron micrographs of end-on discs or rings of PMV protein are shown in (c).

respectively (Moody, 1967), so our results correspond most closely to J₈. If it is assumed that the stain penetrated to a depth of 0.5 to 1.0 nm, values between J₈ and J₉ are obtained. We confirm that there is an approximate true repeat of the structure after five turns, (Tollin et al. 1975) since we obtained a value of 5.12 ± 0.26 for the 19 particles. Since the first and fifth layer lines usually occupied the same quadrant in single-sided images, we infer that the helix is composed of 8\(\frac{3}{4}\) subunits per turn. A radial projection of such a helix and its corresponding transform are given in Fig. 2(c).

Knowing the above and taking the mol. wt. of the RNA and protein subunits as 2.2 × 10⁶ and 24,000 (M. Short, unpublished data) respectively, and the particle length as 550 nm (Mowat, 1971) we calculate that the virus is composed of about 1400 subunits, has a mol. wt. of about 36 × 10⁶ and contains five nucleotides per protein subunit. These values are consistent with those found for PMV (Tollin et al. 1979) and PVX (Tollin et al. 1980).

We also re-examined the polymers made by the protein in the absence of nucleic acid and found that protein samples after 1 day at 20 °C contained circular rings as well as short tubes (Fig. 1a). The short tubes are composed of stacks of rings or discs (see inset, Fig. 1a) and have a repeat of 3.6 nm as measured from 12 particles. We attempted to confirm the value of between 8 and 9 subunits per turn deduced for the virus by examining end-on discs. Fig. 1(c) shows such particles and between 8 and 9 subunits can be directly counted. Since the diameter of the discs and short rings is within the range found for the virus, we conclude that 9 is the more likely value.
Short communications

Fig. 2. Radial projections (left) and transforms (right) of (a) double helix with 9 subunits repeating in one turn; (b) double helix with 26 subunits repeating in three turns; (c) single helix with 44 subunits repeating in five turns, as for the virus.

After incubation at room temperature for an additional 2 to 3 days, stacked ring particles were replaced by long flexuous tubes (Fig. 1b). These are two-start helices repeating either every turn or third turn as evidenced from diffraction patterns which were similar to those shown in Fig. 2 of Robinson et al. (1975). The helices have a pitch of 7.6 nm as measured from 11 particles and a diameter like that of the virus. Knowing this, we have generated radial projections and transforms for the helical polymers, using the measured dimensions, fraction between 8 and 9 subunits per turn and assuming a spherical subunit shape and no lattening (Fig. 2).

The positions of the maxima in the present Fig. 2 are consistent with those found by us and in Fig. 2 of Robinson et al. (1975). We have interpreted Fig. 2(c) of Robinson et al. (1975) to indicate that there are $8\frac{3}{4}$ rather than $9\frac{1}{2}$ subunits per turn since in the latter case, the maxima on the first layer line would be expected to be further away from the meridian than those on the second layer line and this was not observed.

Protein added to NMV RNA in 0.01 M-tris, pH 7, formed single-start helices like those of the virus. This means that the formation of the discs or double helices does not result from protein altered in the isolation process.

We conclude that the structure of NMV is not inconsistent with that of other viruses in the PVX family and that the double-helical structures repeating in every one and three turns have, respectively, 2 and 1$\frac{3}{2}$ more protein subunits per turn than originally described (Robinson et al. 1975).
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