The Structure of Narcissus Mosaic Virus

By H. R. WILSON, P. TOLLIN and A. RAHMAN

Carnegie Laboratory of Physics, University of Dundee, Dundee, Scotland

(Accepted 3 October 1972)

SUMMARY

Analysis of the X-ray diffraction patterns from oriented specimens of narcissus mosaic virus is described. This shows that there is a marked feature in the virus particle at a radial position of about 33 Å, and it is suggested that this corresponds to the sugar–phosphate backbone of the RNA. Electron microscope studies of stained transverse sections of the virus particles are not incompatible with this interpretation. Some aspects of the possible RNA conformation in the virus particle are discussed.

INTRODUCTION

Narcissus mosaic virus (NMV) is an elongated flexible particle about 5500 Å long and about 130 Å in diameter (Tollin et al. 1967). X-ray diffraction patterns from oriented specimens of NMV can be interpreted in terms of a helical arrangement of protein subunits, with an integral, or near integral number, $5q + 4$, in five terms of the helix (Tollin, Wilson & Young, 1968). The pitch of the helix is 33 Å in dry specimens and 36 Å at 98 % relative humidity. Here we describe further studies of NMV which suggest the possible location of the nucleic acid in the virus.

METHODS

X-ray diffraction. Oriented specimens of NMV 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), using nickel-filtered Cu K$\alpha$ radiation. Humidity was controlled with saturated salt solutions. The specimen-to-film distance was about 58 mm. Intensity measurements were made with a Joyce–Loebl recording microdensitometer.

Electron microscopy. The oriented dry specimens used for X-ray diffraction are suitable for preparing ultrathin sections for electron microscopy. Pieces of the oriented specimens, about 0.5 mm long, were fixed in glutaraldehyde and postfixed with osmium tetroxide. The material was dehydrated in alcohol, stained with uranyl acetate during dehydration, embedded and sectioned. Sections were poststained with lead citrate before examination in the electron microscope.

RESULTS

X-ray diffraction

The earlier X-ray studies (Tollin et al. 1968) showed that there were $5q + 4$ protein subunits in five turns of the helix, but the difficulty of distinguishing between a truly meridional and a near-meridional reflexion made it impossible to determine the value of $q$ direction from the diffraction patterns, although they indicated that $6 \leq q \leq 9$. Because the reflexion on the 44th layer-line appeared shorter than the other apparently meridional reflexions, Tollin
Fig. 1. A cylindrical Patterson synthesis for NMV using data to a resolution of about 8 Å and a large artificial temperature factor in order to eliminate effects due to the abrupt termination of data. The dashed line which starts at the origin shows where features would be expected from a helical arrangement of scattering matter at a radius of 33 Å.

Fig. 2. The squared Fourier transform, cylindrically averaged, of a helical arrangement of spherical blobs of radius 2.5 Å, with centres at a radial position of 33 Å. Observed intensity is indicated by vertical lines.
et al. (1968) made the tentative suggestion that the value of \( q \) was 8. At that time no information was available about the mol. wt. of the protein subunit. Recently, however, M. Mayo (personal communication) has determined the subunit mol. wt. using the technique of polyacrylamide gel electrophoresis, and obtains a value of 30,000 (±10%). Accepting this value, and using an argument similar to that used by Wilson & Tollin (1969) for potato virus X (PVX), it is possible to estimate the number of protein subunits/turn of helix in NMV as 6.8 ± 0.7. Since the X-ray diffraction patterns show that there is an integral, or near integral, number in five turns of the helix, this suggests that the number of protein subunits/turn is 6.8. This corresponds to a \( q \) value of 6, which is within the range suggested by the X-ray diffraction pattern.

Further analysis of the NMV diffraction patterns can be made by calculating the cylindrical Patterson synthesis (MacGillavry & Bruins, 1948) and by using helical diffraction theory (Cochran, Crick & Vand, 1952). The cylindrical Patterson function, calculated using data to a resolution of about 8 Å is shown in Fig. 1. A large artificial temperature factor was applied in order to eliminate effects due to the abrupt termination of data. The main feature of the Patterson synthesis is a positive region at a position corresponding to a helical arrangement of scattering matter at a radius of about 33 Å. It is reasonable to assume that this marked feature at a radial position of about 33 Å is the sugar-phosphate backbone of
the RNA because this has a higher electron density than the protein. This position is close to
the suggested radial position of the RNA in PVX, and from the similarities in the structures
of NMV and PVX (Tollin et al. 1967) it might be expected that the RNA would be similarly
situated in the two viruses.

An alternative interpretation of the diffraction data can be made using the helical diffrac-
tion theory. The observed diffraction reflexions in the centre of the NMV pattern (at 57 %
r.h.) fall under the maxima of the transform of a helical arrangement of scattering matter
situated at a radius of about 33 Å (Fig. 2). This again suggests that there is a marked feature
in the virus at this radial position. The sugar-phosphates of an RNA molecule at low resolu-
tion approximates to a continuous helix of scattering matter and this enhances layer-lines
at the centre of the pattern corresponding to the pitch of the helix. The fifth and tenth layer-
lines are particularly strong compared with adjacent layer-lines as would be expected from
this enhancement (Fig. 3).

Electron microscopy

Electron microscopy of stained transverse sections of tobacco rattle virus (TRV) showed
two dark rings, one of which could possibly correspond to the RNA (Tollin & Wilson, 1971).
In the hope that similar studies of NMV would give support to the X-ray results, stained
sections were examined in the electron microscope. Fig. 4 shows a stained section cut per-
pendicular to the virus axis and shows the hexagonal close-packed arrangement of virus
particles. Some cracking of the specimen due to severe drying has occurred and this shows
up as clear regions between the virus domains. To demonstrate more clearly the hexagonal
packing of the particles, Fig. 5 shows a portion of Fig. 4 which has been rotated on itself
six times at angular intervals of 60°. From these photographs it is possible to measure the
interparticle distance as 106 Å (± 10 Å) which is in good agreement with the value of 106 Å
(± 4 Å) for the interparticle distance estimated from the X-ray diffraction pattern from dry
specimens (Tollin et al. 1967).
The main feature of the stained particles are an inner region of diameter 20 to 30 Å which is relatively free from stain, a circular region, of mean diameter ~ 50 Å which is heavily stained, and an outside region with again little staining. This pattern of stain can be interpreted as being due to positive staining of some features of the virus particles. The particles have an outside diameter of ~ 130 Å and therefore interlock in the dry state, hence the outer lightly stained region must represent coat protein. It is interesting that the interparticle distance of 106 Å is about as close as interlocking helices of diameter 130 Å can come without having two helices trying to share the same gap in a third. The lightly stained region in the centre may represent a hole down the particle but might equally well correspond to protein.

One possible interpretation of the darkly stained ring is that this represents the mean diameter of the nucleic acid. However, this would not agree with the conclusions from the X-ray diffraction studies. An alternative interpretation is that both the RNA and part of the protein may be staining and that the observed ring is due to a combination of these. This interpretation is suggested by the fact that similar sections of TRV show two dark rings, only one of which corresponds to the probable RNA position. TRV is a much larger particle than NMV, with an outer diameter of about 250 Å and the dark rings have diameters of ~ 160 Å and ~ 82 Å. The outer ring corresponds to the possible RNA position. It may be that the NMV sections have stained in a similar way, but because of the smaller size of the virus particles the two rings are not resolved in this case. If this were so then the outer part of the stained ring in NMV could correspond to the RNA and this would be compatible with the X-ray diffraction results. However, it cannot be claimed that the electron microscope results support the X-ray results, but merely that they are not incompatible with them.
DISCUSSION

We can consider whether the radial position of 33 Å for the RNA sugar–phosphate is a reasonable value. If we assume that the phosphate–phosphate separation along the RNA has the same value of 5·15 Å in NMV as in TMV, we can estimate the number of nucleotides/turn of the helix. This gives a value of 40·8 which corresponds to a 6% RNA content, and, if there are 6·8 protein subunits/turn of the helix, to six nucleotides associated with each subunit. A 6% RNA content is similar to that found in most of the PVX group of viruses, and corresponds to a mol. wt. of 2·2 × 10^6. This value compares reasonably well with the value of 2·5 × 10^6 estimated by M. Mayo (personal communication) using the technique of acrylamide gel electrophoresis.

Only in the case of TMV is there direct evidence about the location of the RNA (Franklin, 1956; Franklin & Holmes, 1958), from which information, combined with the value of the pitch of the helix and the RNA content, the mean value of the phosphate–phosphate distance along the RNA can be calculated. In the case of TRV, PVX and NMV the evidence concerning the possible radial position of the RNA is indirect, and hence conclusions based on this are tentative. Nevertheless, it is of interest that the results seem to be consistent with a similar mean phosphate–phosphate distance in all the viruses, although the number of nucleotides associated with a protein subunit is not the same in the different viruses. The simplest interpretation of such a result is that the phosphate–phosphate distance is constant along each RNA molecule, and that the nucleotide conformation is similar in the different virus RNAs. We can consider whether this is stereochemically possible.

From symmetry considerations it is to be expected that there is an integral number of nucleotides associated with each protein subunit, and, apart from differences in the bases, these groups of nucleotides will be equivalent. Within a group, however, there can be tertiary structure; that is, all the nucleotides need not be equivalent. This is the most general kind of model for the virus RNA, and in such a model the tilt of the bases relative to the virus axis need not be the same for each nucleotide, nor the phosphate–phosphate distance between neighbouring nucleotides. However, if all the nucleotides were equivalent, then the RNA would have secondary structure only, as in this case the tilt of the bases relative to the virus axis would be the same for all nucleotides and the phosphate–phosphate distance along the chain would be constant. This is the simplest possible structure for the virus RNA.

Single crystal studies of nucleosides and nucleotides have shown that the preferred rotations about bonds are limited to relatively small ranges, and the values for double-helical DNA and RNA models also lie within these ranges (Arnott & Hukins, 1969; Sundaralingam, 1969). It is reasonable to assume that the same preferred conformations will also occur in the virus RNAs, so that the single-crystal results can be used as constraints in constructing possible molecular models of the RNA. For the general kind of virus RNA model mentioned earlier, with tertiary structure within the group of nucleotides associated with a protein subunit, the single-crystal data are not restrictive enough to decide between the many possible models. However, in the case of the simpler RNA model, the equivalence of nucleotides imposes added constraints, and we can consider whether such models are stereochemically acceptable. In such RNA models the bases would be expected to lie about 3·4 Å apart, and because the rise/nucleotide parallel to the virus axis is much less than this, the bases would have to be tilted relative to the virus axis. The phosphate–phosphate distance of 5·15 Å is less than that in double-helical DNA and RNA models – in B-DNA the phosphate–phosphate distance is 6·5 Å, in A-DNA it is 5·5 Å, and in double-helical RNA it is 5·6 Å. This presents a problem if the same orientation of the C(5')–O(5') bond relative to the sugar
ring is retained in the virus RNA model as in the DNA and double-helical RNA models. This orientation, referred to as gauche–gauche, is also the orientation observed in all 5′-nucleotides which have so far been studied. However, in nucleosides, other orientations of the C(5′)–O(5′) bond relative to the sugar ring are observed, and if these are allowed in the virus RNA model the phosphate–phosphate distance can be made acceptable. Our preliminary model building shows that models with a similar nucleotide conformation can be constructed to satisfy the helical parameters of TRV, TMV, PVX and NMV, although there are certain features of the models which are not completely satisfactory. A more systematic study of such models may enable a more critical assessment of their acceptability to be made, but it should be emphasized that even if models of this kind are completely satisfactory they would not necessarily be unique. Other, more complex models, may also be equally satisfactory.

As we stated earlier, the evidence concerning the possible location of the RNA in TRV, PVX and NMV is indirect. The discussion of possible RNA conformations will be on firmer ground when more direct information is available about the RNA location and also when more is known about the preferred conformations of nucleotides.

We thank Mr W. P. Mowat for supplying the virus and Mr I. M. Roberts for preparing the sectioned specimens and for much of the electron microscopy. H. R. Wilson and A. Rahman wish to acknowledge the award of a Medical Research Council grant.

REFERENCES


(Received 21 August 1972)