POLARITY OF THE RNA IN THE TOBACCO MOSAIC VIRUS PARTICLE AND THE DIRECTION OF PROTEIN STRIPPING IN SODIUM DODECYL SULPHATE

T. M. A. WILSON, R. N. PERHAM, J. T. FINCH and P. J. G. BUTLER

Department of Biochemistry, University of Cambridge, Tennis Court Road, Cambridge, CB2 1QW and M.R.C. Laboratory of Molecular Biology, Hills Road, Cambridge, CB2 2QH, UK

Received 17 February 1976

1. Introduction

The degradation of TMV in the presence of the detergent, SDS, occurs by sequential loss of protein sub-units from one end of the virus particle [1], although prolonged exposure to the detergent has been shown to cause uncoating at both ends of the rod [2]. It has been reported that the principal direction of stripping is from the 3′- to 5′-terminus of the viral RNA [3] and much of the later work aimed at locating specific genes [4,5] and certain ribonuclease T1-resistant nucleotide sequences [6] is founded on this conclusion.

However, it has recently been demonstrated that under alkaline conditions the comparable degradation of TMV takes place by sequential loss of protein sub-units beginning at the 5′-end of the RNA [7]. Since it has been claimed that alkali and detergent induce stripping from the same end of the RNA [8], at that time thought to be the 3′-end, we have reinvestigated the direction of detergent stripping. We find that alkali and detergent do cause stripping from the same end of the virus rod but that it is the 5′-terminus of the RNA that is uncovered first. Our experiments permit us to specify the direction of the RNA chain in the helical array of protein subunits in the intact virus.

Abbreviations: TMV, tobacco mosaic virus; SDS, sodium dodecyl sulphate.

2. Materials and methods

2.1. *SDS-stripping of TMV and preparation of RNA fragments*

Partly stripped TMV rods were prepared by a slight modification of the procedure described by May and Knight [3]. Equal volumes of a suspension of TMV *vulgare* (20 mg/ml in sodium phosphate buffer, pH 7.0, ionic strength 0.05 M) and a buffer containing 1 mM EDTA (Na_4 salt), 1% (w/v) SDS, 8 mM Tris-HCl, pH 7.5, or a buffer of the same composition but omitting SDS, were brought to 30°C, mixed and left at 30°C for 35 min. The degradation was stopped by adding one third of a volume of 4 M KCl and chilling on ice. The KDS precipitate was removed by low-speed centrifugation and the supernatant placed in a siliconized Corex tube. Calcium chloride was added to a final concentration of 2 mM. Any exposed RNA 'tails' were then removed by adding micrococcal nuclease (0.05 μg/mg of starting TMV) and incubating at 20°C for 40 min [7]. The nuclease activity was terminated by adding EDTA (Na_4 salt) to a final concentration of 10–20 mM. The viral RNA was prepared immediately by a standard two-phase phenol extraction and allowed to precipitate in 75% ethanol/50 mM NaCl at −20°C for several hours. Having been centrifuged and washed, the nucleic acid was dissolved in a small volume of buffer (100 mM NaCl, 1 mM EDTA, 10 mM Tris–HCl, 0.1% SDS, pH 7.5) and loaded (0.2 ml/gradient) on to 10–40% sucrose density gradients (5 ml). The gradients were made up with the same buffer except that 0.1% SDS was omitted from the 40% sucrose

solution. The sucrose was pre-treated with diethylpyrocarbonate to inhibit stray ribonucleases [9]. The gradients were spun at 50 000 rev/min for 3 h at 15°C in a Beckman SW 50.1 rotor and fractionated by pumping out the contents of each tube from the bottom through a canula. The RNA was located by following the absorbance at 260 nm.

2.2. *Histidine-acceptor activity*

The ability of TMV RNA fragments to form aminoacyl derivatives with ^{3}H-labelled histidine (55 mCi/μmol; The Radiochemical Centre, Amersham, Bucks, UK) in the presence of yeast amino acyl tRNA synthetases was tested exactly as described previously [7].

2.3. *Preparation of specimens for electron microscopy*

TMV partly stripped with SDS was prepared by incubation of virus as described above. The dodecyl sulphate was removed by the addition of 0.2 ml 3 M KCl per ml incubation mixture followed by centrifugation (10 min, 5000 *g* at 4°C). Virus stripped to a comparable extent with alkali was prepared by incubation at 10 mg/ml in 10 mM Na_2CO_3 buffer, pH 9.5, at 0°C for 18 hours. The reaction was stopped by the addition of 0.5 ml Tris–HCl, pH 8.0, ionic strength 0.1 M, per ml of incubation mixture.

Specimens of these partly stripped TMV preparations were prepared by allowing the particles to adsorb onto carbon coated grids from a drop of solution, followed by rinsing with distilled water and negative staining with 1% (w/v) uranyl acetate. The grids were examined and photographed in a Philips EM 300 electron microscope at a magnification of approx. 25 000.

3. Results and discussion

3.1. *Direction of coat protein-stripping from TMV in the presence of SDS*

A typical separation by sucrose density centrifugation of the fragments of TMV RNA produced by means of the SDS-stripping procedure described above is shown in fig.1. The sedimentation coefficient of intact TMV RNA is 27s, corresponding with its molecular weight of 2.2×10^6 [10]. Shorter defined pieces of TMV RNA can be obtained from the frag-

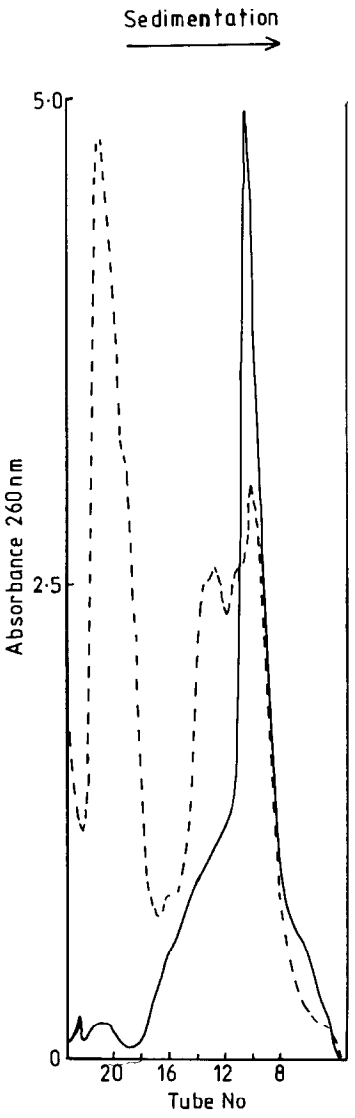

Fig.1. Sucrose density gradient centrifugation (10–40% sucrose) of the RNA extracted from TMV before (——) and after (– – – –) stripping coat protein from the virus particles with SDS. Samples of 0.25 ml were collected. Tubes 8–11 (Fractions I*a* and I*b*) and tubes 12–14 (Fractions II*a* and II*b*) were pooled separately: fractions I*a* and II*a* came from the untreated virus and fractions I*b* and II*b* came from virus stripped with SDS. Exposed RNA 'tails' were removed from partly stripped rods by treatment with micrococcal nuclease before the RNA was extracted. For other details, see the text.

ments of TMV produced by means of the alkaline degradation of the virus [7,11] and it was found that when these pieces of RNA were analysed by sucrose density centrifugation, a straight-line graph could be obtained by plotting log (molecular weight) versus mobility. Using this technique to calibrate the sucrose density centrifugation, the average mol.wt. of the RNA comprising fraction II*b* in fig.1 was estimated to be approx. 1.15×10^6.

Species of TMV RNA that contain the intact 3′-terminal nucleotide sequence CpCpCpA-OH found in the viral RNA can be charged with histidine in the presence of yeast histidyl-tRNA synthetase [12,13,7]. The ability of the RNA species in fractions I and II (fig.1) to be charged with histidine was therefore tested. The results are listed in table 1. It is reassuring to note first that the control 27s RNA from intact viral rods obtained in the absence (fraction I*a*) and presence (fraction I*b*) of SDS have identical specific radioactivities in the charging assay. The same specific radioactivity is observed for the RNA fragments obtained by partial SDS-stripping (fraction II*b*). Little RNA occurs in the same position of the gradient (fraction II*a*) for TMV that was not exposed to detergent and its specific radioactivity is approx. half that of the other three species. These results are easily explained by supposing that the effect of SDS is to cause protein stripping from the 5′-end of the RNA in TMV, leaving the 3′-end intact in the RNA fragments. The small amount of RNA in fraction II*a* presumably consists of fragments of viral RNA that have the same average molecular weight as the RNA in fraction II*b* but which arise by adventitious shearing of the viral rod, since no SDS has been present in the preparation of this sample. It is therefore equally likely to derive from the 5′-end or the 3′-end of the intact RNA and its specific radioactivity in the charging assay is consistent with this view. We calculate from the incorporation of radiolabelled histidine that the RNA species in fractions I*a*, I*b* and II*b* have an average acceptor activity of approx. 0.5 mol histidine/mol RNA, in comparison with the earlier values of 0.32 and 0.37 mol/mol obtained with intact TMV RNA and the fragments produced by alkaline degradation [12,7].

Table 1
Histidine-acceptor activity of TMV RNA and its fragments

Fraction of RNA from sucrose density gradient centrifugation	Molecular weight	Histidine-acceptor activity (cpm ^{3}H-labelled histidine/pmol RNA)
I*a* (tubes 8–11)	2.2×10^6	9900
II*a* (tubes 12–14)	1.15×10^6	5250
I*b* (tubes 8–11)	2.2×10^6	9800
II*b* (tubes 12–14)	1.15×10^6	10 300

The various species of RNA were incubated for 30 min at 30°C with ^{3}H-labelled histidine and the aminoacyl-tRNA synthetase system described previously [7].

3.2. *Comparison of SDS and alkaline stripping by particle morphology*

Since the charging experiments showed that the direction of stripping in SDS was from the 5′-OH end of the RNA, as previously established more rigorously for stripping in alkali [7], an attempt was made to compare partly stripped particles in the electron microscope. Under suitable conditions, the ends of negatively stained TMV particles can be distinguished: the one appears rounded or convex and the other appears as a sharp transverse line or is slightly concave. This distinction can still be observed on many of the partly stripped particles from which the RNA tails have not been removed, despite the presence of free protein which had been stripped off. Puffs corresponding to the uncoated RNA, probably made visible by some attached protein, can sometimes be seen at the end of the rod. A selection of such particles is shown in fig.2.

All of the particles in a number of fields of TMV partly stripped by SDS or alkali were inspected and the free RNA assigned to the different ends whenever this was unambiguously possible. This could not be done in all cases owing to overlap of particles where they were too thick upon the specimen grid or to imperfections in the staining. The resulting counts are given in table 2 and clearly show that the free RNA protrudes from the concave end of most particles irrespective of whether the partial stripping was performed with SDS or alkali, although a small percentage of the particles show stripping from both ends (5% and 10% for SDS and alkali respectively). The dominant direction of stripping is therefore the same for both treatments.

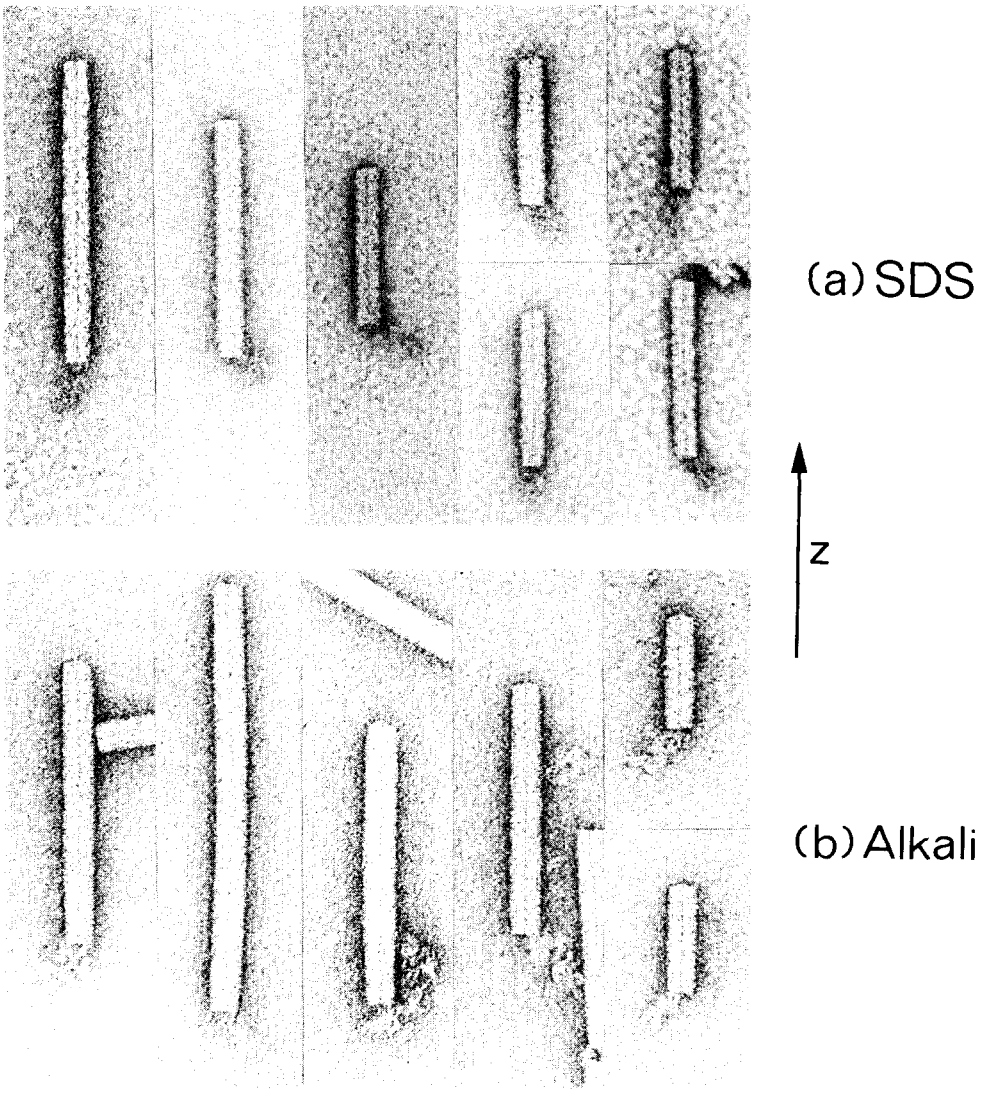

Fig.2. Electron microscope images of particles of TMV partly stripped or protein with (a) SDS and (b) alkali. The images are aligned so that the rounded or convex ends are at the top and the concave ends at the bottom. In each case, puffs of uncoated RNA can be seen at the concave end. The *z*-axis indicated corresponds in direction with that used by Holmes et al. [19] and Champness et al. [20]. (Magnification approx. 170 000 X).

3.3. *Direction of the RNA compared with the protein*

The nucleoprotein helix of TMV is right-handed [14] and, because of the asymmetry of the protein and the sugar–phosphate linkages of the RNA, each defines a direction of the helix. The results reported above show that the 3′-end of the RNA, terminating in CpCpCpA-OH [15,16], must occur at the convex

Table 2
Numbers of particles for which the end with the exposed RNA is morphologically distinguishable

Method of stripping	End showing RNA		
	Convex	Concave	Both
SDS	0	45	2
Alkali	1	62	6

end of the particle, while the 5′-end, with the $m^7G^{5'}ppp^{5'}Gp$ 'cap' [17,18] occurs at the concave end. In view of the current investigations of the structures of the virus and of the protein disks, with electron density maps available at about 7Å [19] and at 5Å [20] respectively, it is interesting to correlate the morphology of the particle ends with the electron density maps.

Such maps of the nucleoprotein and of the protein show four rods of protein in each subunit running between radii of 40Å and 70Å, two of the rods being slewed and two radial. The upper and lower boundaries of the subunits are clearly defined by the symmetry of the protein crystals, while the direction of slew of the rods shows that Holmes et al. [19] and Champness et al. [20] have chosen to view the subunits the same way up and have therefore adopted the same convention for the direction of the vertical axis *(z)* of the structures. In both cases, the rods are roughly parallel to the long axis of the subunit (fig.4 of reference 19 and fig.2 of reference 20). Thus in the virus, where the rods dip to lower z values at higher radii, the overall slope of the subunits is downwards, like the branches of a fir tree.

If, as is probable, the apex of this helix composed of sloping subunits corresponds with the morphologically convex end of the virus particle, then the RNA runs from the bottom to the top of the helix, as pictures in [19], in a 5′- to 3′-direction. Similarly, during the nucleation reaction between the TMV RNA and a protein disk [21], on the maps shown in reference [20] the 5′- to 3′-direction of the RNA is anti-clockwise. These directions will give an essential guide for any model building to try to understand the details of the RNA-protein interaction.

Acknowledgements

We are grateful to St. John's College, Cambridge for the award of the Hutchinson Research Studentship to T.M.A.W. and we thank Dr R. J. Jackson for the use of some facilities provided by a grant from the Medical Research Council. We are indebted to Drs A. C. Bloomer and J. N. Champness for pointing out to us the slope on the subunits of the virus.

References

[1] Hart, R. G. (1955) Proc. Natl. Acad. Sci. US 41, 261–264.
[2] Symington, J. (1969) Virology 38, 309–316.
[3] May, D. S. and Knight, C. A. (1965) Virology 25, 502–507.
[4] Kado, C. I. and Knight, C. A. (1966) Proc. Natl. Acad. Sci. US 55, 1276–1283.
[5] Kado, C. I. and Knight, C. A. (1968) J. Mol. Biol. 36, 15–23.
[6] Mandeles, S. (1968) J. Biol. Chem. 243, 3671–3684.
[7] Perham, R. N. and Wilson, T. M. A. (1976) FEBS Lett. 62, 11–15.
[8] Onda, H., Taniguchi, T. and Matsui, C. (1970) Virology 42, 551–553.
[9] Fahnestock, V. E. and Nomura, M. (1974) Methods in Enzymology 30, 555.
[10] Caspar, D. L. D. (1963) Adv. Protein Chem. 18, 37–121.
[11] Perham, R. N. (1969) J. Mol. Biol. 45, 439–441.
[12] Carriquiry, E. and Litvak, S. (1974) FEBS Lett. 38, 287–291.
[13] Öberg, B. and Philipson, L. (1972) Biochem. Biophys. Res. Commun. 48, 927–932.
[14] Finch, J. T. (1972) J. Mol. Biol. 66, 291–294.
[15] Mandeles, S. (1967) J. Biol. Chem. 242, 3103–3107.
[16] Glitz, D. G., Bradley, A. and Fraenkel-Conrat, H. (1968) Biochim. Biophys. Acta 161, 1–12.
[17] Zimmern, D. (1975) Nucleic Acid Res. 2, 1189–1201.
[18] Keith, J. and Fraenkel-Conrat, H. (1975) FEBS Lett. 57, 31–33.
[19] Holmes, K. C., Stubbs, G. J., Mandelkow, E. and Gallwitz, U. (1975) Nature (London) 254, 192–196.
[20] Champness, J. N., Bloomer, A. C., Bricogne, G., Butler, P. J. G. and Klug, A. (1976) Nature (London) 529, 20–24.
[21] Butler, P. J. G. and Klug, A. (1971) Nature New Biol. 229, 47–50.