The Effect of Urea on Tobacco Mosaic Virus: Polarity of Disassembly

(Accepted 18 February 1982)

SUMMARY

Urea acts to disassemble tobacco mosaic virus (TMV) predominantly from the 5'-end of the virus RNA. Extensively uncoated particles exhibit some degree of bi-directional uncoating as judged by a reduced ability to bind [3H]histidine. The translational activities of the RNA fragments which remained encapsidated in the urea-resistant fraction of TMV rods were investigated.

Various agents bring about the sequential disassembly of tobacco mosaic virus (TMV) in vitro. Alkali (Perham & Wilson, 1976; Ohno & Okada, 1977; Pelcher & Halasa, 1979), SDS (Ohno & Okada, 1977; May & Knight, 1965; Wilson et al., 1976) or dimethyl sulphoxide (Nicolaieff & Lebeurier 1979; Wilson, 1978; Wilson et al., 1981) remove coat protein subunits from the virus RNA in a polar fashion, predominantly from the 5'-terminus. A small and variable number of rods are stripped bi-directionally by these agents; uncoating from the 3'-end appears to begin at a later stage and proceeds for a shorter distance (Wilson et al., 1976, 1981).

6 M-urea at 0 °C is known to disassemble TMV (Buzzell, 1960, 1962). During this process, shorter rods which are more stable to further stripping than the parent TMV rods have been identified (Buzzell, 1960). By examining the RNA which remains encapsidated after urea treatment, we have determined the principal polarity of disassembly.

A TMV suspension, isolated and stored as described previously (Wilson et al., 1981), was dialysed extensively against distilled water, followed by 1 mM-EDTA (Na+ salt) pH 7, each for 18 h at 4 °C. Different preparations were thus rendered uniformly susceptible to uncoating by urea. The suspension was incubated at 0 °C for 7 min in 6 M-urea, 60 mM-sodium phosphate pH 6-9, at a virus concentration of 3-6 mg/ml. Uncoating was stopped by adding 2 vol. ice-cold 20 mM-sodium phosphate buffer pH 6-9 (Buzzell, 1960). Intact and partially stripped virus particles were collected by centrifugation at 112000 g for 16 h at 4 °C. The virus was resuspended gently at a final concentration of 30 mg/ml in 20 mM-sodium phosphate buffer pH 6-9, and dialysed extensively against this buffer at 4 °C. The exposed RNA tails were removed by treatment with micrococcal nuclease (EC 3.1.31.1; Boehringer, Mannheim) at 15 U/ml in 1 mM-CaCl2. The ‘tailing’ was stopped by addition of EGTA (Na+ salt) pH 7, to a final concentration of 5 mM. Encapsidated (and thus protected) RNA molecules were extracted and fractionated on linear 10 to 40% (w/v) sucrose density gradients as described previously (Wilson, 1978).

Control RNA sedimented as a single peak (27S) on the sucrose gradient (Fig. 1a), whereas the absorbance profile at 254 nm of encapsidated RNAs from urea-treated virus particles showed a population of intermediate-sized RNA fragments, in addition to a peak of 27S TMV RNA and a small peak of RNA fragments which arise after micrococcal nuclease digestion of exposed RNA tails. RNA fractions A to E from the urea-treated virus preparation were taken as indicated in Fig. 1(a). RNA from untreated virus was prepared as a control. RNAs were precipitated with ethanol at −20 °C and analysed by SDS–polyacrylamide gel electrophoresis (SDS–PAGE) on a 2 to 10% (w/v) linear gradient gel (Wilson et al., 1981). Fraction A (Fig. 1b) showed one major band which co-migrated with 27S RNA from untreated virus (Fig. 1b, lane T). However, fractions B to D (Fig. 1b)
Fig. 1. (a) Sucrose density-gradient centrifugation of the protected RNA fragments extracted from 6 M-urea-treated TMV particles following micrococcal nuclease digestion of exposed RNA tails (---). The sedimentation profile of RNA from untreated TMV is also shown (-----). Convenient RNA fractions (A to E) were collected as indicated. (b) SDS-PAGE (Laemmli, 1970) of RNA fractions A to E and total RNA (T) from untreated virus particles on a 2 to 10% (w/v) linear gradient gel. The positions of marker RNA molecules are shown [mol. wt. (M_r) x 10^-6]. The specific histidine acceptor activities (ct/min [3H]histidine/pmol RNA) of the RNA molecules in fractions A to E (Fig. 1a) and in 27S RNA from untreated virus were as follows: 27S TMV RNA (control), 5374 ± 255; fraction A, 5029 ± 263; fraction B, 4760 ± 108; fraction C, 4526 ± 330; fraction D, 3322 ± 20; fraction E, 6.

revealed a complex pattern of many discrete major and minor bands. The process of uncoating in 6 M-urea at 0 °C is clearly discontinuous and ranges in extent from no stripping in approx. 50% of rods, up to (approx.) 75% of the length of the particle in other cases (as estimated from the relative sedimentation behaviour of the slowest migrating RNA fragments in fraction D). RNA fragments in fraction E (Fig. 1a) were smaller than co-electrophoresed tRNA markers and so do not appear on a 2 to 10% (w/v) gel (Fig. 1b).

To estimate the frequency of uncoating from the 3'-end of the virus rods, the ability of protected RNA fractions A to E (Fig. 1a) to bind L-[2,5-3H]histidine was assayed as before (Perham & Wilson, 1976; Carriquiry & Litvak, 1974; Oberg & Philipson, 1972). The average mol. wt. of the RNA molecules in fractions A to D were estimated by comparison with the relative sedimentation positions of RNA fragments prepared by partial disassembly in alkali (Perham & Wilson, 1978). The results are shown in the legend to Fig. 1. RNA fractions A and B (Fig. 1a) showed almost the same average histidine-acceptor activity as 27S RNA from untreated virus (mean value, 5200 ct/min/pmol). Furthermore, 90% of the RNA molecules in fraction C and 65% of the RNA molecules in fraction D retained their tRNA-like function. In vitro translation of RNA fractions A to D (Fig. 1a) in a prokaryotic cell-free protein-synthesizing system (Glover & Wilson, 1981, 1982) showed incorporation of L-[4,5-3H]leucine into a polypeptide of M_r 17500 which co-migrated with authentic TMV coat protein on SDS-PAGE. Efficiencies with which each RNA fraction (A to D) encoded the M_r 17500 product [measured by excising and solubilizing the relevant gel bands in H_2O_2 (30%, v/v) and counting in Triton X-100/toluene scintillant; data not shown] paralleled almost exactly their abilities to bind [3H]histidine covalently at the 3'-terminus. Where uncoating had occurred from the 3'-end, to affect both histidine acceptor and prokaryotic...
Fig. 2. (a) Electrophoresis of products of RNase T1 digestion of RNA fractions A to E (Fig. 1a) and untreated TMV RNA (T). The positions of the large T1-resistant oligonucleotides $\Omega$, $\psi_1$, and $\psi_2$ are marked. Small T1 digestion products migrate at the front (F). $\Omega$, $\psi_1$, and $\psi_2$ are located in the 5'-half of the virus RNA. (b) SDS–PAGE (Laemmli, 1970) of the $[^3]$Hleucine-labelled products encoded by RNA fractions A to D in the wheat germ cell-free system (Roberts & Paterson, 1973) and visualized by fluorography (Bonner & Laskey, 1974; Laskey & Mills, 1975). The positions of $^{14}$C-labelled marker proteins are shown [mol. wt. ($M_r$) $\times 10^{-3}$]. Twenty-$\mu$l incubations were prepared in duplicate as described previously (Wilson et al., 1978) and the total TCA-precipitable incorporations (ct/min $[^3]$Hleucine/pmol RNA) were as follows (corrected for $-\psi$RNA background): untreated 27S TMV RNA, 187 500 $\pm$ 10 000 (products not shown); fraction A, 199 500 $\pm$ 11 000; fraction B, 178 000 $\pm$ 14 000; fraction C, 47 500 $\pm$ 13 000; fraction D, 22 300 $\pm$ 4 800; fraction E, 0 (no products to show).

To confirm that uncoating had occurred from the 5'-terminus during urea treatment, 20·5 pmol of each of the RNA fractions was digested with ribonuclease T1 and the products analysed by electrophoresis on a 12% (w/v) polyacrylamide gel buffered with 90 mm-tris–borate pH 8·3, containing 2·5 mm-EDTA and 7 M-urea as described previously (Wilson et al., 1981). A large and unique product of extensive T1 digestion called omega, ($\Omega$) is 72 nucleotides in length and is located almost at the 5'-end of intact TMV RNA (Richards et al., 1978). The yield of omega ($\Omega$) per pmol RNA declined with decreasing fragment size, as judged by fluorescent staining with 2 mg/ml ethidium bromide (Fig. 2a).

Further support for these results was obtained by examining the level of incorporation of $\text{L-}[4,5-^3$H]leucine into trichloroacetic acid (TCA)-precipitable polypeptides in the wheat germ cell-free system (Roberts & Paterson, 1973) using RNA fractions A to E (Fig. 1a) as exogenous templates. The system selectively translates overlapping genes at the 5'-end of intact TMV RNA which encode a polypeptide of $M_r$ 110 000, and occasionally a read-through protein of $M_r$ 165 000. RNA fractions A to C (Fig. 1a) supported synthesis of

---

Short communications

139
the Mr 110000 product, but in rapidly diminishing amounts (Fig. 2 b). The total incorporation of [3H]leucine/pmol added RNA from fraction C is only 24% that of fraction A, or control 27S TMV RNA (see legend to Fig. 2 b), indicating that a large number of rods have been uncoated from the 5'-end of the RNA (Richards et al., 1978). The translational activity of fraction D is lower still (11% that of fraction A) with the largest product of mol. wt. (approx.) 90000. It is likely, therefore, that 11% of the RNA molecules in fraction D are derived from rods which have been uncoated uni-directionally from the 3'-end for a distance of at least 3.9 kilobases to account for the synthesis of truncated polypeptides of Mr 90000 and less. However, up to 36% of RNA molecules in fraction D have lost the aminoacylatable 3'-terminus (legend to Fig. 1 b); therefore, a proportion (approx. 25%) of RNA molecules in fraction D must be derived from bi-directionally uncoated virus rods. There is no evidence for enhanced synthesis of an internally coded polypeptide of Mr 30000 which has been observed during translation of alkali-generated 3'-terminal TMV RNA fragments (Wilson et al., 1978).

From these results, we conclude that uncoating of TMV in 6 M-urea at 0 °C can commence from either end of the rod, but that the majority of even the most extensively stripped particles arise by uncoating only from the 5'-terminus of the RNA molecule, in common with the effects of other chemical agents on TMV.

We are grateful to Mrs Victoria Derbyshire for carrying out an initial study on the optimal conditions for disassembly. University of Liverpool Botanic Gardens, Ness were generous suppliers of Nicotiana tabacum var. Samsun for virus propagation. This work was supported in part by SRC grant number GR/B 23656.

Department of Biochemistry
University of Liverpool
P.O. Box 147, Liverpool L69 3BX, U.K.

L. E. BLOWERS
T. M. A. WILSON*

REFERENCES

Short communications


(Received 3 November 1981)