THE POLARITY OF STRIPPING OF COAT PROTEIN SUBUNITS FROM THE RNA IN TOBACCO MOSAIC VIRUS BY DIMETHYLSULPHOXIDE

T. M. A. WILSON

Department of Biochemistry, University Hospital and Medical School, Clifton Boulevard, Nottingham, NG7 2UH, England

Received 24 November 1977

1. Introduction

A variety of agents such as urea [1], SDS [2–5] and alkali [6–9] bring about a progressive, polar disassembly of TMV. Frequently, a series of more or less stable, intermediate-length particles can be observed during the stripping of the virus [10]. Contrary to [4,11] both alkali [9] and SDS [5] strip TMV from the 5′- to the 3′ end of the viral RNA. DMSO strips TMV from the 3′- to the 5′-end of the RNA [12]; the coat protein gene was thus assigned to within 2000 nucleotides of the 3′-end of TMV RNA [13]. Since the initial assumption required to make this assignment, namely that viral assembly is a polar process [14] beginning at the 5′-end of the RNA [15–18], is now known to be wrong [19,20], it seemed important to reinvestigate the polarity of stripping in DMSO and to consider its implications for the location of the coat protein gene.

The results presented in this paper suggest that at least the principal direction of uncoating in DMSO is from the 5′- to the 3′-terminus of the RNA.

2. Materials and methods

TMV *vulgare* strain, was a generous gift from P. J. G. Butler, MRC Lab. Molec. Biol. Cambridge. The virus was stored at a concentration of 30 mg/ml at 4°C in sodium phosphate buffer, pH 7.0, ionic strength 0.05–0.10 mol/l.

Abbreviations: TMV, tobacco mosaic virus, DMSO-dimethylsulphoxide; SDS, sodium dodecyl sulphate; EDTA, ethylenediaminetetra-acetic acid; EGTA, ethyleneglycol-bis-(2-aminoethyl ether) *N,N′*-tetraacetic acid

2.1. *Treatment of TMV with DMSO*

TMV suspension, 10 ml, 2.5 mg/ml, in 0.01 M phosphate buffer, pH 7.0, was added to 115 ml water–DMSO solution to give final conc. 72% DMSO (by vol.). Incubation was carried out at 24°C for 30 min. The procedure followed [12] except that the exposed RNA tails were removed with micrococcal nuclease (EC 3.1.4.7; Boehringer) in the presence of 1 mM calcium chloride [5,9]. No bentonite was required, the nuclease activity was terminated by adding EGTA (neutralized with KOH) to final conc. 5 mM. The protected RNA fragments were then extracted by the standard 2-phase phenol procedure in the presence of SDS (0.5–1.0%, w/v) and EDTA (5–10 mM) as recommended [21]. The extracted RNA was precipitated with 75% ethanol/50 mM NaCl at −20°C for several hours. It was harvested in an MSE 18 refrigerated centrifuge at 10 000 rev/min for 20 min at −5°C. The pellets of RNA were washed with fresh ethanol/NaCl and taken up in a small volume of buffer (100 mM NaCl, 1 mM EDTA, 10 mM Tris–HCl, pH 7.5, 0.1% SDS). The total RNA extract was then fractionated by sucrose density-gradient centrifugation on linear 10–40%, w/v, diethylpyrocarbonate-treated [22] sucrose density gradients (6.5 ml). The gradients were made up with the same buffer except that 0.1% SDS was omitted from the 40% sucrose solution. The gradients were spun at 50 000 rev/min for 3.5 h at 15°C in an MSE Superspeed 65 ultracentrifuge. The contents of each tube were fractionated by pumping out from the

bottom through a canula and the RNA peaks were located by following the A_{260}. The RNA fragments were precipitated once again with ethanol/NaCl and the pellets resuspended in water to give final RNA conc. 1 μM (see section 3).

A control preparation of TMV RNA was prepared simultaneously, following an identical procedure but omitting DMSO.

2.2. *Histidine-acceptor abilities*

The ability of TMV RNA fragments to form aminoacyl derivatives with [^{3}H]histidine (55 mCi/μmol; The Radiochemical Centre, Amersham, Bucks) in the presence of yeast aminoacyl-tRNA synthetases was tested exactly as in [9].

3. Results and discussion

A typical separation by sucrose density-gradient centrifugation of the TMV RNA fragments produced by the DMSO-stripping procedure described above is shown in fig.1. The sedimentation coefficient of intact TMV RNA is 27 S, corresponding with mol. wt 2.2×10^6 [23]. The disassembly of TMV at alkaline pH produces a series of defined nucleoprotein fragments [10]. The pieces of RNA obtained from these intermediates can be analysed by sucrose density-gradient centrifugation, in which case a straight-line graph is obtained by plotting log (mol. wt) versus mobility. Using this technique to calibrate the sucrose density centrifugation, the average molecular weights of the RNA fragments comprising fractions (1) and (2) in fig.1 were estimated. The results are shown in table 1.

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 [5,9,24]. The ability of the various RNA species in fractions (1) and (2) (fig.1) and control, 27 S, RNA to be charged with histidine was therefore tested. The results are shown in table 1. It is reassuring to note first that the control, 27 S, RNA from intact rods obtained in the absence and presence of DMSO have almost identical specific radioactivities in the charging assay. The RNA which occurs in the same position of the gradient as fractions (1) and (2), in the absence of a stripping agent arises by adventitious shearing of the viral rods, presumably during handling [5]. Thus these RNA fragments have a specific histidine-acceptor activity which is almost exactly half that of control, 27 S, RNA [5]. From the results in table 1, it appears that the enhanced numbers of RNA fragments in fractions (1) and (2), following DMSO-treatment, possess intact, functional 3′-terminal sequences in approx. 80% and 60% of cases, respectively. It would therefore seem reasonable to conclude that the effect of 72% (by vol.) DMSO upon intact TMV rods is to cause disassembly predominant-

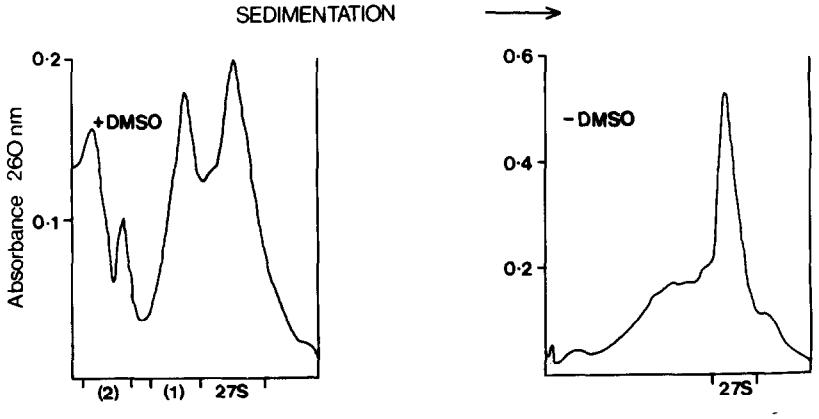

Fig.1. Sucrose density gradient centrifugation (10–40% sucrose) of the RNA extracted from TMV (*vulgare* strain) in the presence (+) or absence (–) of DMSO (72%, by vol.). Samples, 0.25 ml, were collected. The exposed RNA tails were removed from partly stripped rods by treatment with micrococcal nuclease before the RNA was extracted. For other details, see the text.

Table 1

RNA source	Mol. wt	Conc. (μM)	Histidine-acceptor activity (cpm [^{3}H]histidine/pmol RNA)
Control (+tRNA) No activating enzymes	2.5×10^6	20	63
Total *E. coli* tRNA	2.5×10^4	20	3950[a]
27 S TMV RNA (–DMSO)	2.2×10^6	1	4000
27 S TMV RNA (+DMSO)	2.2×10^6	1	4200
Fraction (1) (+DMSO)	0.9×10^6	1	3310
Fraction (2) (+DMSO)	$0.1-0.2 \times 10^6$	1–2	2480[b]

[a] Assuming $tRNA^{his}$ = 0.05 mol/mol total tRNA
[b] Average specific acceptor activity

The various species of RNA were incubated at the stated concentrations for 30 min at 30°C with [^{3}H]histidine and the aminoacyl-tRNA synthetase system [9,24]. Control incubations without any added RNA gave approx. 500 cpm [^{3}H]histidine

ly, if not exclusively at first, from the 5′-end of the viral RNA. After stripping has proceeded from the 5′-end for some distance [12], it may then commence from the 3′-terminus, resulting in the two-tailed rods [12] which occur under extreme stripping conditions (72% DMSO or above). The RNA from such particles would be unable to bind histidine, hence the reduced ability for aminoacylation among the shorter RNA fragments.

From the incorporation of radiolabelled histidine, it can be calculated that the 27 S RNA species (and *E. coli* $tRNA^{his}$, assuming it to represent 0.05 mol/mol total tRNA) have average acceptor act. 0.25 mol histidine/mol RNA. For comparison, 0.5 mol/mol was obtained with intact TMV RNA and fragments produced by disassembly in SDS [5], and 0.32–0.37 mol/mol obtained with intact TMV RNA and fragments produced in alkali [9,25]. The present decrease in histidine-binding efficiency may reflect the age of the aminoacyl-tRNA synthetase preparation.

It would appear therefore, that DMSO, in common with other stripping agents, brings about a polar disassembly of TMV which begins at the 5′-terminus of the intact viral RNA.

Fingerprint analysis [26] of the fragments of RNA obtained by partial stripping in alkali [9], located the coat protein gene in the 3′-terminal 1000 nucleotides of TMV RNA. The location of the coat protein gene in the 3′-terminal 2000 nucleotides [13] is consistent with this despite a wrong assumption as to the direction of virus self-assembly from the 5′-end [15–18] and consequently, about the direction of DMSO stripping [12]. The coat protein gene was located [13] by screening for specific encapsidation with coat protein of RNA fragments prepared by mild T1 ribonuclease treatment of the RNA tails exposed either by partial stripping in DMSO or during limited reconstitution of the virus. We now know that self-assembly proceeds initially from an internal point on the RNA, to the left [19] of the coat protein gene, predominantly in the 5′-direction [20,27,28]. The inconsistencies implicit in these studies from various laboratories can therefore be resolved by recognizing that partially reconstituted virus particles may still possess an exposed coat protein cistron which, after mild T1 ribonuclease digestion, would give rise to the RNA fragments specifically encapsidated by coat protein. There is no doubt therefore, that the coat protein gene is located in the 3′-terminal 1000 nucleotides of the viral RNA.

Acknowledgements

I am grateful to Dr R. N. Perham for his interest and encouragement and to Mrs Y. Thompson for skilled technical assistance.

References

[1] Buzzell, A. (1960) J. Am. Chem. Soc. 82, 1636–1641.
[2] Hart, R. G. (1955) Proc. Natl. Acad. Sci. USA 41, 261–264.
[3] Symington, J. (1969) Virology 38, 309–316.
[4] May, D. S. and Knight, C. A. (1965) Virology 25, 502–507.
[5] Wilson, T. M. A., Perham, R. N., Finch, J. T. and Butler, P. J. G. (1976) FEBS Lett. 64, 285–289.
[6] Schramm, G., Schumacher, G. and Zillig, W. (1955) Z. Naturf. 106, 481–492.
[7] Harrington, W. F. and Schachman, H. K. (1956) Arch. Biochem. Biophys. 65, 278–295.
[8] Perham, R. N. (1969) J. Mol. Biol. 45, 439–441.
[9] Perham, R. N. and Wilson, T. M. A. (1976) FEBS Lett. 62, 11–15.
[10] Perham. R. N. and Wilson, T. M. A. (1978) Virology in press.
[11] Onda, H., Taniguchi, T. and Matsui, C. (1970) Virology 42, 551–553.
[12] Nicolaieff, A., Lebeurier, G., Morel, M. C. and Hirth, L. (1975) J. Gen. Virol. 26, 295–306.
[13] Richards, K. E., Morel, M. C., Nicolaiff, A., Lebeurier, G. and Hirth, L. (1975) Biochemie 5, 749–755.
[14] Stussi, C., Lebeurier, G. and Hirth, L. (1969) Virology 38, 16–25.
[15] Butler, P. J. G. and Klug, A. (1971) Nature New Biol. 229, 47–50.
[16] Guilley, H., Stussi, C. and Hirth, L. (1971) CR Acad. Sci. Ser. D 272, 1181–1184.
[17] Thouvenel, J. C., Guilley, H., Stussi, C. and Hirth, L. (1971) FEBS Lett. 16, 204–206.
[18] Ohno, T., Nozu, Y. and Okada, Y. (1971) Virology 44, 510–516.
[19] Zimmern, D. and Wilson, T. M. A. (1976) FEBS Lett. 71, 294–298.
[20] Butler, P. J. G., Finch, J. T. and Zimmern, D. (1977) Nature 265, 217–219.
[21] Efron, D. and Marcus, A. (1973) Virology 53, 343–348.
[22] Ehrenburg, L., Fedorcsak, I. and Solymosy, F. (1976) Progr. Nucl. Acid. Res. Mol. Biol. 16, 189–262.
[23] Caspar, D. L. D. (1963) Adv. Protein Chem. 18, 37–121.
[23] Öberg, B. and Philipson, L. (1972) Biochem. Biophys. Res. Commun. 48, 927–932.
[25] Carriquiry, E. and Litvak, S. (1974) FEBS Lett. 38, 287–291.
[26] Hunter, T. R., Hunt, T., Knowland, J. and Zimmern, D. (1976) Nature 260, 759–764.
[27] Lebeurier, G., Nicolaieff, A. and Richards, K. E. (1977) Proc. Natl. Acad. Sci. USA 74, 149–153.
[28] Otsuki, Y., Takebe, I., Ohno, T., Fukuda, M. and Okada, Y. (1977) Proc. Natl. Acad. Sci. USA 74, 1913–1917.