EVIDENCE FOR POLAR RECONSTITUTION OF TMV

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1. Introduction

During reconstitution experiments conducted with Tobacco Mosaic Virus (TMV), Stussi, Lebeurier and Hirth [1] described incompletely coated TMV particles (IC) of 700 Å length. It was demonstrated that the free RNA of these particles was able, under certain conditions, to be coated and to give infectious TMV particles. These observations suggested a polar process of reconstitution. But conflicting results were reported on this subject by several research workers. Fraenkel-Conrat [2] was unable to demonstrate by chemical means the polarity of self-assembly in the case of TMV. On the other hand, Butler and Klug [3] and Guilley, Stussi and Hirth [4] by controlled digestion of TMV-RNA with exonucleases, indicated that reconstitution of TMV began by the 5'OH end. However, difficulties exist in the use of exonuclease and some ambiguities appeared in the interpretation of the results. The present paper reports experiments which suggest strongly that TMV protein recognises the 5'OH end of the TMV-RNA and that reconstitution is a polar process.

2. Materials and methods

2.1. *Reconstitution procedure*

RNA and protein of TMV were prepared according to methods already described in detail [1]. Reconstitution experiments were conducted as previously described [1], except that the Prot/RNA ratio was 10. Incompletely coated TMV particles (IC) and whole reconstituted virus particles (WP) were separated by using hydroxyapatite columns [Guilley et al., to be published, 5, 6] or sucrose density gradient centrifugation [1]. The RNA tails of IC were eliminated by incubation with pancreatic ribonuclease (RNase 3.7 μg/mg RNA, 1 hr at 37°); the fragments of TMV-particles obtained in this way by removal of the RNase sensitive tails (partially coated particles or PC) were sedimented by ultracentrifugation. The RNA of PC was then extracted (PC-RNA).

2.2. *Preparation of ^{14}C Dimedone*

Dimedone ^{14}C was prepared according the method described by Glitz [7], using 0.5 mCi diethylmalonate 2-^{14}C (10 mCi/mM) and mesityloxide. The obtained ^{14}C Dimedone was tested by infrared and UV spectra and had a specific activity of 1.7×10^6 cpm/μM.

2.3. *Labelling of the 3'OH end of the RNA*

The 3'OH end of RNA was labelled in WP and in PC-RNA by means of ^{14}C Dimedone fixation after $NaIO_4$ oxydation, exactly as described by Glitz and Sigman [7] for transfer, ribosomal or f_2 viral RNA. The molecular weight of ^{14}C Dimedone treated RNA was controlled by means of polyacrylamide gel electrophoresis according to Loening [8].

Radioactivity was counted after drying on planchets using a gas flow counter Beckman Lowbeta 2.

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Table 1
^{14}C Dimedone fixation on WP- and PC-TMV-RNA.

	$NaIO_4$ treatment	μM of fixed ^{14}C Dimedone / μM of treated RNA
Standard TMV-RNA	+	0.70
	-	0.04
TMV-RNA extracted from RNase treated TMV	+	0.59
WP-RNA	+	0.88
PC-RNA (separation on hydroxyapatite)	+	0.035
	-	0.027
PC-RNA (separation on sucrose gradient)	+	0.044

The counted radioactivity is expressed for 1 μM of treated RNA. Molecular weight of standard TMV-RNA: 2×10^6 daltons. Molecular weight of PC-RNA: 4.6×10^5 daltons. Each value given in the table represents the average of 3 experiments with 1 to 7 mg TMV-RNA. ^{14}C Dimedone (specific activity 1.7×10^6 cpm/μM) was fixed after $NaIO_4$ oxydation as described by Glitz [7].

3. Results

3.1. *Fixation of ^{14}C Dimedone on WP- and PC-RNA*

In a first step it was necessary to estimate if the fixation of ^{14}C Dimedone gave rise to degradation of TMV-RNA: polyacrylamide gel electrophoresis of standard TMV-RNA and of ^{14}C-Dimedone-TMV-RNA showed that no degradation occurred during the treatment and that its molecular weight was 2×10^6 daltons. PC-RNA was not degraded as demonstrated by analytical ultracentrifugation; on the basis of the value of $S_{20,w}$ and considering the mean length of these particles (700 Å) their molecular weight corresponded to an average value of 4.6×10^5 daltons.

The results, reported in table 1, show that without preliminary $NaIO_4$ oxydation of RNA, no fixation of ^{14}C Dimedone occurred.

Standard TMV-RNA and WP-RNA fixed ^{14}C Dimedone in the same way. The RNase treatment of TMV before the extraction of RNA did not alter significantly the fixation of ^{14}C Dimedone.

^{14}C Dimedone was not fixed on PC-RNA. This result shows that the RNA present in the 700 Å length particles (PC) obtained after RNase treatment of incompletely coated TMV (IC) did not contain the 3'OH end of the RNA chain but only the 5'OH end.

3.2. *Reconstitution of ^{14}C Dimedone marked RNA*

Protein and ^{14}C Dimedone RNA were reconstituted and IC-particles were isolated as described before. After RNase treatment PC particles were isolated by ultracentrifugation. Radioactivity of the pellet (PC) and of the supernatant (digested RNA tails) was estimated: 98.5% of the radioactivity was found in the supernatant and 1.5% in the pellet. This result shows that the 3'OH end is in the RNA tail.

4. Discussion

The present results seem to demonstrate unambiguously that the polar process of reconstitution suggested by Stussi, Lebeurier and Hirth [1] is a real one. The fact that ^{14}C Dimedone is fixed specifically by the 3'OH free end of RNAs after treatment by $NaIO_4$ is of great interest: in fact we never observed in this system the type of unspecific fixation described with ^3H-sodium borohydride especially with high molecular weight RNA. The fact that no degradation of the TMV-RNA occurs during fixation of Dimedone is also worth noticing.

Very recently Guilley, Stussi and Hirth [4] and Butler and Klug [3] suggested from digestion of the 5'OH end of TMV-RNA by exonucleases that reconstitution of TMV begins by this end. However, the fact that some endonuclease activity exists, even in very pure enzyme preparations of exonucleases, introduces some difficulties in the interpretation of the results. The present paper proves that the suggestions of Guilley, Stussi and Hirth [4] and Butler and Klug [3] are right and that a particular configuration of the TMV-RNA exists at the 5'OH end which is recognized specifically by TMV-protein. The polarity of reconstitution in the case of TMV allows one to expect that in the near future the nucleotide sequence of TMV-RNA near the 5'OH end will be determined by stepwise reconstitution experiments.

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