SPECIFIC RNA FRAGMENTS IN DEFECTIVE PARTICLES OF BACTERIOPHAGE MS2

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1. Introduction

Amber mutants of A-protein of RNA-containing bacteriophages form defective phage particles under restrictive conditions of growth [1]. Different types of defective particles have been also observed among wild type RNA bacteriophages [2] including those multiplying under normal conditions [3].

In this communication it has been shown that some of the bacteriophage MS2 virions grown in *E. coli* AB 259 host cells both in the peptone and synthetic media are defective. Defective virions differ from the native phage by their lower buoyant density, negligible infectivity and as mainly containing fragmented RNA. However, RNA: protein and A-protein: coat protein ratios in the defective and native MS2 particles are approximately the same.

RNA contained in the defective particles is composed mainly of three specific fragments, their length being about 60, 50 and 40% of that of the native MS2 RNA, what obviously results from several ruptures in the native RNA molecule included in defective phage particles. The major rupture occurring in the 3′-terminal half of MS2 RNA leads to the formation of short 3′-terminal and long 5′-terminal fragments with 40 and 60% of the whole RNA length.

2. Methods

MS2 bacteriophage was prepared according to the method previously described [4] omitting the column chromatography stage. ^{3}H- and ^{14}C-labelled phages were grown in the synthetic medium [5]; to prepare [^{32}P]MS2 phages the same medium with twice lower phosphate concentration was used. Electrophoresis of proteins was carried out as described in [6]. Preparative electrophoresis of RNA fragments was performed in buffer E [7] in 1.6 × 16 cm gel tubes containing 2.2–3.0% gradient of polyacrylamide at 40 mA, with subsequent electroelution. For re-electrophoresis of enriched individual fractions 2% gels containing 0.5% agarose were used as well. Reduction with sodium [^{3}H] borohydride (sp. act. 1.5 Ci/mmole fig. 3a–c or 6.4 Ci/mmole, fig. 3d) was carried out according to [8]. Alkaline hydrolysates of [^{32}P]RNA were analysed by two-dimensional chromatography on Whatman AE 81 paper similarly to [9].

3. Results

Two types of MS2 particles with different buoyant density and infectivity have been normally revealed by cesium chloride density centrifugation (table1). Sedimentation analysis in a sucrose gradient has revealed no difference between defective and normal MS2 particles. The ratio [^{3}H]histidine: [^{14}C]lysine in the purified double-labelled defective particles is only 1.3 times that of native phage. Indeed, ^{3}H-labelled A-protein with relative electrophoretic mobility 0.3 of that of coat protein, has been detected in similar amounts in both particle types. Both the defective and normal phage particles are nearly equal in RNA content.

Three high-molecular weight specific RNA fragments were isolated from the defective phage particles (fig. 1) with molar ratios I : II : III = 5 : 4 : 9. Their approximate molecular weights as calculated

Table 1
Characteristics of phage MS2 particle types

Type of MS2 particles	Density in CsCl* (g/cm³)	Infectivity PFU/A_{260}	Labelling with [³H] ura and [¹⁴C] phe** Ratio of ³H to ¹⁴C	Labelling with [³H] His and [¹⁴C] Leu*** A-protein ³H, cpm	Coat protein ¹⁴C cpm
Native MS2	1.46	3.8×10^{11}	24.6	8862	50 492
Defective MS2	1.44	3.0×10^{8}	25.8	5377	42 396

*The refractive index of the phage bands was determined and buoyant density was calculated using equation $\rho^{25°} = 10.8601 \times \eta^{25°} D - 13.4974$.
**Double-labelled MS2 particles were twice banded in CsCl density gradient and dpm in both bands of MS2 were detected.
***Coat protein and A-protein content in double-labelled phage particles was detected by electrophoresis in 10% polyacrylamide gel.

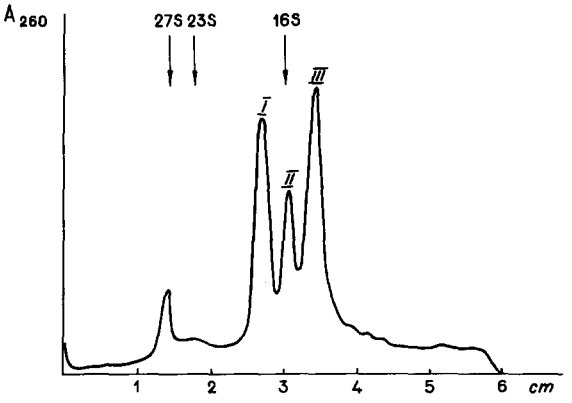

Fig. 1. Electrophoresis of RNA from defective particles of MS2 in 3% polyacrylamide gel (5% bis-acrylamide) for 2 hr at 8 mA per a gel of 6 mm diameter. Arrows indicate the positions of 27 S MS2 RNA and 23 S and 16 S *E. coli* ribosomal RNA.

from electrophoretic mobility in respect to 27 S MS2 RNA and *E. coli* 23 S and 16 S ribosomal RNAs, are 6.5×10^5 (I), 5.5×10^5 (II) and 4.4×10^5 (III). The RNA fragments retain their individuality and relative electrophoretic mobility

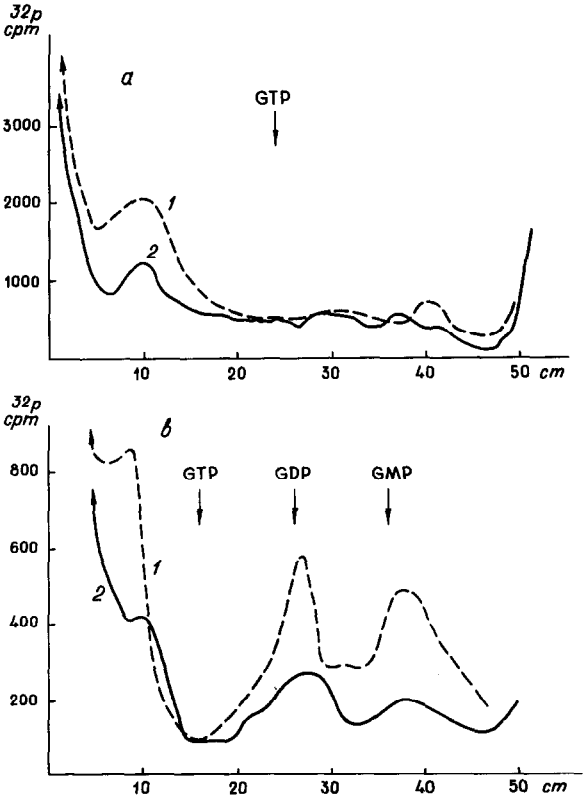

Fig. 2. Detection of pppGp in alkaline hydrolysates of [³²P]RNA from native (1) (3.7×10^7 cpm) and defective (2) (1.8×10^7 cpm) particles of MS2 phage: (a) Distribution of ³²P-radioactivity along Whatman AE 81 diagonal after two-dimensional chromatography (20 hr, 16 hr); (b) Identification of pppGp by the treatment with snake venom phosphodiesterase (SVP). 6–14 cm region of (a) chromatogram-diagonal was moistened with 0.15 ml of SVP solution (9 mg/ml; 0.021 units/mg) in 0.078 M Tris–HCl, pH 8.9, 0.0167 M $MgCl_2$, incubated in moist chamber for 2 hr at 37°C, applied to Whatman AE 81 and chromatographed for 10 hr. Arrows indicate the position of endogeneous ³H-markers.

even after denaturation with urea [10].

Chromatographic analysis of alkaline hydrolysates of [^{32}P]RNA from normal and defective phage particles has revealed radioactive material (fig. 2a) moving slower than endogeneous marker [^{3}H]GTP and being undetectable in the hydrolysates of [^{32}P]ribosomal RNA. In parallel experiments the treatment with snake venom phosphodiesterase has resulted in the transfer of ^{32}P-radioactivity of the spot to the positions of GTP (pGp) and GMP (PP_i) markers (fig. 2b). This may be regarded as an evidence of pppGp presence in the initial preparation.

The presence of free 3′-terminal hydroxylic groups in the unfractioned preparation of the RNA fragments is detectable by reduction of periodate oxidized RNA with tritiated sodium borohydride. The analysis of [^{3}H] nucleoside derivatives (purinyl- and pyrimidyl-substituted hydroxymethyl diethylene glycol) has shown adenosine to be the only 3′-terminal nucleoside of RNA in the defective phage particles (fig. 3a, b).

The enriched RNA fractions of several preparative electrophoretic runs have been pooled and used for the second round of electrophoresis which gives about 60% yield of individual RNA fragments containing not more than 10% admixtures of the adjacent fragments.

The 3′-terminal analysis of the individual RNA fragments reveals the presence of adenosine only in fragment III (fig. 3c, d). After periodate oxidation, the latter incorporated 2.4 times more [^{14}C]isonicotinic acid hydrazide (^{14}C-INH) than the native MS2 RNA what well agrees with its molecular weight. No 3′-terminal nucleosides have been detected in fragments I and II, though fragment I do incorporate varying quantities of ^{14}C-INH, what was probably due to an artifactual binding of the latter.

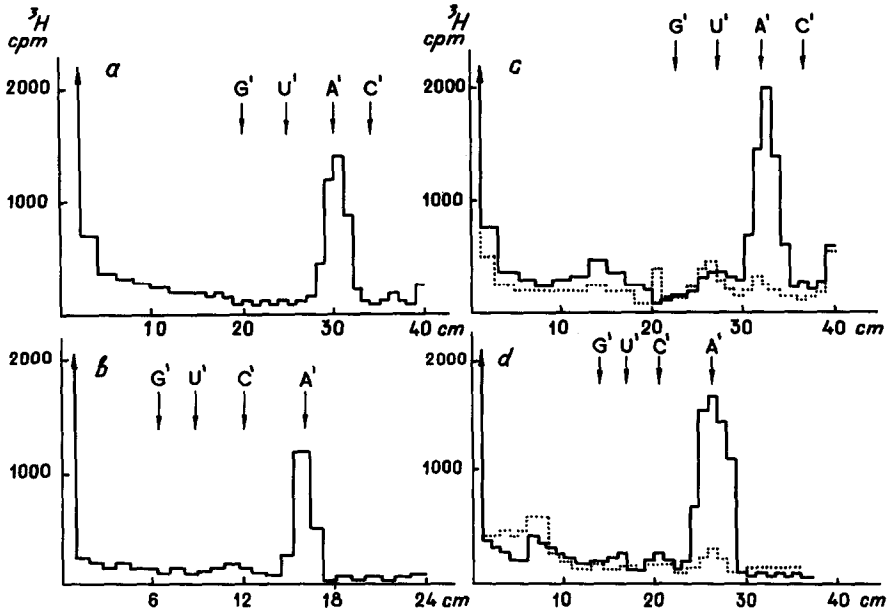

Fig. 3. Chromatography of alkaline hydrolysates of RNA and individual fragments after modification of their 3′-terminus with sodium [^{3}H]borohydride. The following hydrolysates were analysed along with standard nucleoside derivatives (A′, G′, C′, U′): (a) 7 A_{260} units of native MS2 RNA on Whatman DE 81, developed with re-distilled water; (b) 1.4 A_{260} units of total RNA from defective particles on a thin layer of MN 300 cellulose in the system: methyl ethyl ketone–*t* - butanol–ammonia–water (30:40:10:15); (c) 7 A_{260} units of fragment III (———) and 5 A_{260} units of fragment II (. . . . .) on Whatman DE 81; (d) 2 A_{260} units of fragments III (——) and I (. . . . .) on Whatman 1 in the system isobutyric acid–*n*-butanol–ammonia–water (15:30:1:10).

4. Discussion

Our defective MS2 particles isolated from crude phage preparations differ from the defective ones of A-protein amber mutants [1] and from so-called light MS2 particles isolated by Rohrmann and Krueger [3]. RNA fragmentation seems to be related to the action of intracellular RNAses on the structurally imperfect virions. A somewhat similar phenomenon has been observed with A-protein mutants [1]. MS2 RNA sites subjected to RNAse attack in imperfect virions have been shown to be differing from those in isolated RNAs [11–13]. The rupture of an MS2 RNA molecule in its 3′-terminal half results in the formation of short (40% of the whole length) 3′-terminal and long (60% of the whole length), 5′-terminal fragments of MS2 RNA. The intermediate (50%) fragment may be possibly formed from the long one by means of its additional fragmentation. However, the latter possiblility needs experimental verification.

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