JOURNAL OF VIROLOGY, Mar. 1974, p. 762-764

Vol. 13, No. 3
Printed in U.S.A.

Fragmentation of RNA in Virus Particles of Rhinovirus Type 14

C. J. GAUNTT

Department of Microbiology, Arizona Medical Center, University of Arizona, Tuscon, Arizona 85724

Received for publication 29 October 1973

Purified particles of rhinovirus type 14 lose infectivity during incubation at 34.5 C as a result of fragmentation of RNA genomes within intact virions.

The infectious genome of several different rhinoviruses has been characterized as a single-stranded RNA molecule with a molecular weight of 2.2×10^6 to 2.8×10^6 (1, 3, 6, 7, 9, 11). Extraction of RNA from purified preparations of different rhinovirions has yielded, in addition to genomic RNA, appreciable quantities of RNA molecules which are much smaller in size than genomic RNA (3, 8, 9). Data is presented which shows that incubation of purified particles of rhinovirus type 14 (RV14) at 34.5 C, a temperature optimum for growth of this virus (4), promotes formation of subgenomic RNA fragments. The results of several experiments suggest that fragmentation of genomic RNA occurs in situ within virus particles during incubation.

[^{3}H]uridine-labeled RV14 was prepared in HeLA cell cultures and purified by banding in CsCl gradients, as previously described (3), or by centrifugation (47,500 rpm for 36 min in the Spinco SW50L rotor at 4 C) into 5 to 30% sucrose gradients in TST buffer (0.2% tryptose-phosphate broth, 0.1 M NaCl, and 0.05 M Tris, pH 7.4). Fractions containing coincident maxima in radioactivity and plaque-forming units (PFU) were dialyzed versus TST buffer containing penicillin (100 U/ml) and streptomycin (100 μg/ml) for 16 h at 4 C. Methods for cultivation of HeLa and KB cells and plaque assay of RV14 have been reported (10). Viral RNA or ribosomal RNA were extracted from particles and KB cells, respectively, with phenol and sodium dodecyl sulfate (3). The methods for plaque assay and radioactivity measurements of viral RNA were previously outlined (3).

Loss of infectivity in virus particles after incubation at 34.5 C for 24 or 48 h is shown in Table 1. Portions of each virus preparation were sealed in plastic tubes and held in 34.5 C water-jacketed incubators for the designated times or stored at −90 C (0 h control). Approximately 53 to 60% and 68 to 83% of infectivity were lost in either purified or crude virus preparations after incubation at 24 and 48 h, respectively. Viral RNA extracted from incubated particles showed even greater losses in infectivity, i.e., 59 to 96% and 98.4 to 99.3% of infectivity were lost in RNA extracted from particles incubated for 24 and 48 h, respectively. These results are in agreement with the earlier results of Dimmock (2), suggesting that inactivation involved a mechanism directed primarily against viral RNA.

The loss in infectivity in RV14 particles could be attributed to fragmentation of viral RNA to noninfectious subgenomic fragments (Fig. 1). Virus was purified by the CsCl method and incubated for 24 h at 34.5 or held at −90 C. In this experiment, incubation reduced the original virus titer (3.8×10^9 PFU/ml) by 64%. RNA was extracted and centrifuged into linear 5 to 20% sucrose gradients. Each fraction was assayed for infectivity and radioactivity. Most infectivity was associated with the major 31*S* genomic RNA (3) peak of radioactivity in either sample. Subgenomic RNA fragments of approximately 3.5×10^4 daltons in size were never found in any significant quantities in these experiments, even when purified particles were incubated for 120 h at 34.5 C. Analysis of the size of subgenomic RNA by polyacrylamide gel electrophoresis (3) showed that subgenomic RNA was heterogeneous in size, varying from 1.5×10^6 to approximately 360,000 daltons. As recovery of trichloroacetic acid-insoluble ^{3}H counts was equivalent in the two gradients (4,151 and 3,818 counts/min), whereas recovery of infectivity in the major peak was 12.4×10^5 and 5.7×10^5 PFU in gradients containing unincubated and incubated RNA samples, respectively, the loss in infectivity can be attributed to fragmentation of infectious 31*S* RNA to noninfectious subgenomic RNA fragments. In a similar experiment with a different preparation of CsCl-purified ^{3}H-labeled virus, incubation for 24 and 72 h resulted in 35 and 79% inactivation of virus. Recovery of counts per minute in 31*S* RNA/total counts per minute in these sucrose gradients was a comparable 40, 24, and 16%, respectively, for RNA extracted from unincubated virus particles or virus particles incu-

TABLE 1. *Effect of incubation at 34.5 C on infectivity in RV14 virus particles*

Virus[a]	Virus titers after incubation times (PFU/ml × 10^{-8})		
	0[b]	24	48
CsCl-purified (4)	16.0 ± 7.3[c]	6.4 ± 1.8	2.7 ± 0.7
Sucrose-purified (3)	3.9 ± 2.3	ND[d]	1.01 ± 0.67
Crude lysate (4)	4.7 ± 2.2	2.2 ± 1.5	1.5 ± 1.0

[a] Numbers in parentheses denote the number of experiments.
[b] Hours of incubation.
[c] ± Standard deviation.
[d] ND, Not determined.

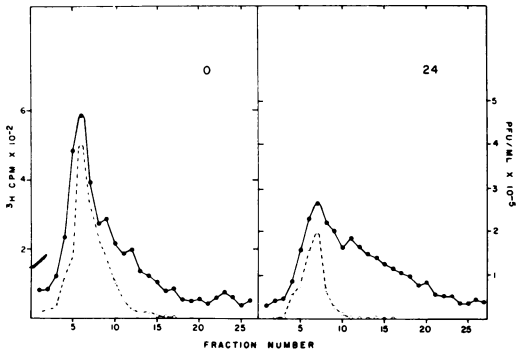

FIG. 1. *Sedimentation of RNA extracted from CsCl-purified virus after incubation of particles at 34.5 C in sucrose gradients. RNA was extracted from virus particles held at −90 C (panel labeled 0) or incubated 24 h at 34.5 C (panel labeled 24) and centrifuged into 5 to 20% sucrose gradients. Each fraction of the gradient was assayed for infectivity (O) and counts in trichloroacetic acid-insoluble RNA (●).*

bated for 24 or 72 h. Eight hours of incubation of CsCl-purified particles was sufficient to result in detectable fragmentation of extracted genomic RNA.

Centrifugation of rhinoviruses into CsCl gradients has resulted in loss of infectivity (9) and also resulted in an artificial increase in the sedimentation coefficient of RV14 virus particles (5). To rule out that CsCl was involved in fragmentation of RV14 RNA, [^{3}H]uridine-labeled virus was purified by centrifugation into sucrose gradients. Virus particles purified by this technique were held at −90 C or incubated for 48 h at 34.5 C. The loss in infectivity in the particles after incubation was 74%. Recovery of counts per minute in 31*S* RNA/total counts per minute in sucrose gradients was 43 and 23% for RNA extracted from unincubated and incubated virus particles, respectively.

The fragility of isolated viral RNA during incubation at 34.5 C was examined in the following experiment. RNA was extracted from CsCl-purified virus particles and held at −90 C or incubated at 34.5 C for 48 h prior to centrifugation into sucrose gradients. The results (Fig. 2) show that genomic RNA per se does not undergo the extensive fragmentation observed for RNA extracted after incubation of virus particles. The specific infectivities of RNA in the unincubated and incubated samples were 23.5 and 21.9 PFU per count per min, respectively.

Several properties of purified, incubated virus particles were measured to determine if incubation at 34.5 C resulted in physical alteration of the particles. All virus preparations in the following four experiments showed reductions in titers of 65 to 88% after incubation at 34.5 C. Buoyant densities of unincubated and incubated (48 h) particles in CsCl were 1.410 ± 0.002 and 1.411 ± 0.002 g/ml for two different virus preparations. Incubated (48 h) [^{3}H]uridine-labeled purified virus particles were found to cosediment with unincubated ^{32}P-labeled virus particles after centrifugation into linear 5 to 30% sucrose gradients (identical results were obtained when the labeled precursors were re-

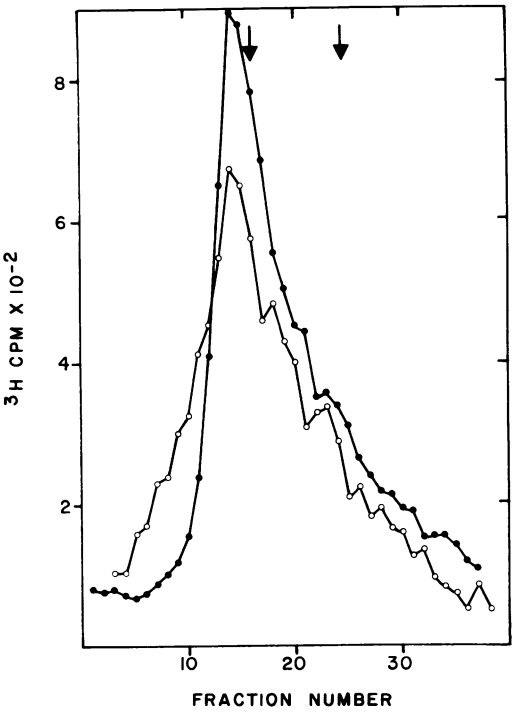

FIG. 2. *Sedimentation of RNA extracted from CsCl-purified unincubated virus and subsequently incubated at 34.5 C. Composite profiles of control RNA held at −90 C (●) or RNA incubated at 34.5 C for 48 h (O). The left and right arrows indicate positions of sedimentation of 28 and 18S KB cell ribosomal RNA, respectively.*

versed). CsCl- or sucrose-gradient-purified RV14 particles were incubated at 34.5 C for 48 h or held at −90 C, and then one-half of each sample was incubated with pancreatic RNase (50 μg/ml) for 30 min at 37 C. Plaque assay of these samples showed that the RNase-treated preparations contained from 97 to 121% of the infectivity present in the corresponding untreated samples. These results show that particles incubated at 34.5 C do not undergo gross physical alterations in density, size, or capsid integrity. The possibility that RNA fragmentation was a result of RNase in the milieu which attacks sites on the genome during incubation at 34.5 C was examined as follows. Portions of unincubated and incubated (48 h) virus particles were mixed with approximately 1.0 A_{260} unit of KB cell ribosomal RNA, bromophenol blue, and sucrose to approximately 50% (wt/vol). After 30 min at 25 C, the samples were subjected to electrophoresis into columns of polyacrylamide gel (3) for 3 h. Virus particles in either sample just entered the gels. No detectable breakdown occurred in the added ribosomal RNA, suggesting that measurable amounts of RNase were not present in the milieu surrounding the purified particles.

The results show that loss in infectivity in purified RV14 virus particles after incubation at 34.5 C is accompanied by a loss in the amount of 31*S* genomic RNA that is extractable from the incubated particles. Data from several experiments have shown a direct correlation between the percentage of inactivation of infectivity in particles and percentage of loss in amount of extractable 31*S* genomic RNA, suggesting that the majority of purified virus particles initially contained 31*S* RNA. Several kinds of measurements failed to detect any biophysical differences between incubated and unincubated particles, suggesting that fragmentation of the viral RNA occurred in situ within the virus particles during incubation. Additional work will be required to determine if fragmentation of the viral RNA RV14 particles is due to a RNase present within the particles.

I thank Marshall Dinowitz for many helpful comments and suggestions during the course of this work and for a critical reading of the manuscript. I also thank Royce Z. Lockart, Jr., and Bruce D. Korant for constructive criticisms of the manuscript in its early stages. This research was supported by funds from a General Research Support Grant to the Arizona Medical Center, University of Arizona, from the Division of Research Resources.

LITERATURE CITED

1. Brown, F., J. F. E. Newman, and E. J. Stott. 1970. Molecular weight of rhinovirus ribonucleic acid. J. Gen. Virol. **8**:145-148.
2. Dimmock, N. J. 1967. Differences between the thermal inactivation of picornaviruses at "high" and "low" temperatures. Virology **31**:338-353.
3. Gauntt, C. J. 1973. Synthesis of ribonucleic acids in KB cells infected with rhinovirus type 14. J. Gen. Virol. **21**:253-267.
4. Gauntt, C. J., J. Sauck, and E. Carlson. 1973. Growth of rhinovirus type 14. Arch. Gesamte Virusforsch. **41**:382-385.
5. Korant, B. D., K. Lonberg-Holm, J. Noble, and J. T. Stasny. 1972. Naturally occuring and artificially produced components of three rhinoviruses. Virology **48**:71-86.
6. McGregor, S., and H. D. Mayor. 1971. Biophysical and biochemical studies on rhinovirus and poliovirus. I. Morphology of viral ribonucleoproteins. J. Virol. **2**:149-154.
7. Nair, C. N., and K. K. Lonberg-Holm. 1971. Infectivity and sedimentation of rhinovirus ribonucleic acid. J. Virol. **7**:278-280.
8. Rueckert, R. R. 1971. Picornaviral architecture, p. 256-306. *In* K. Maramorosch and E. Kurstak (ed.), Comparative virology. Academic Press Inc., New York.
9. Sethi, S. K., and C. E. Schwerdt. 1972. Studies on the biosynthesis and characterization of rhinovirus ribonucleic acid. Virology **48**:221-229.
10. Stoker, D., J. Kiernat, and C. Gauntt. 1973. Interferon induction by rhinoviruses and effect of interferon on rhinovirus yields in human cell lines. Proc. Soc. Exp. Biol. Med. **143**:23-37.
11. Stott, E. J., and R. A. Killington. 1972. Rhinoviruses. Ann. Rev. Microbiol. **26**:503-524.