Release of Rubella Virus Ribonucleic Acid from Ribonucleoprotein by Polyanions

TAPANI HOVI

Department of Virology, University of Helsinki, Helsinki, Finland

Received for publication 20 December 1971

Rubella virus ribonucleoprotein was accessible to pancreatic ribonuclease, Pronase, and certain polyanions. Most of the ribonucleic acid (RNA) label was made acid-soluble by ribonuclease, whereas Pronase and the polyanions liberated 40S RNA from the ribonucleoprotein.

Rubella virus is an enveloped ribonucleic acid (RNA) virus and is morphologically (5, 17), but not serologically (10, 16), related to groups A and B of the arboviruses. After treatment of purified rubella virus with nonionic detergents, a 150S ribonucleoprotein (RNP) was isolated in sucrose gradients (15, 16). The RNP contained single-stranded 40S RNA and one of the three viral polypeptides (15). The RNP disintegrated in the presence of polyvinylsulfate (PVS) (7, 15). According to Pons and co-workers PVS also dissociated influenza virus RNP in a similar way (4, 12).

To discover whether dissociation of rubella virus RNP is a general property of polyanions, several related substances were tested. ³H-uridine-labeled purified (7) rubella virus (≤ 0.05 optical density units at 280 nm) was treated for 5 min in an ice bath with 1% Triton X-100. One-fourth volume of an aqueous solution of the test substance was added to the mixture, and after further incubation for 10 min the sedimentation of the tritium label was analyzed by centrifugation in a sucrose gradient (see legend for Fig. 1). Most polyanions containing either sulfate or phosphate groups were able to dissociate the RNP (Table 1). As an example, release of the 40S RNA after incubation of the RNP with sodium dodecyl sulfate (SDS)-phenol-extracted rubella virus RNA (7) is shown in Fig. 1. This type of sedimentation of polyanion-released 40S RNA is indicated by a plus (+) in Table 1. Unexpectedly, incubation of the RNP with calf thymus deoxyribonucleic acid (DNA) or soluble yeast RNA repeatedly failed to alter the sedimentation of RNA label. The reason might be the relatively rigid secondary structure of these nucleic acids caused by base pairing. The discrepancy between yeast soluble RNA and mammalian 4 to 5S RNA (Table 1) could be explained by differences in the proportion of transfer RNA. The mammalian RNA ob-

viously contained mostly ribosomal 5S RNA and breakdown products of the larger RNA species, which are not constantly base-paired. However, since heat-denatured DNA also did not dissociate the RNP (Table 1), a rigid secondary structure cannot be the only reason. The results suggest that some property of the polyanion other than the regularly repeating anionic groups on a macromolecular skeleton has a role in dissociation of rubella virus RNP.

The polycationic substances tested, diethylaminoethyl (DEAE) dextran and histone, did not dissociate the RNP but instead caused some aggregation (Table 1). None of the substances tested altered the sedimentation of intact rubella virions.

With all the polyanions tested, the protein label remained at the top of the gradient after dissociation of the RNP. This differs from the results of Pons and co-workers (4, 12), who found that PVS and influenza virus protein formed a complex morphologically similar to the native RNP.

The polyanion-induced disintegration of rubella virus RNP was further characterized by analyzing the sedimentation of double-labeled RNP (see legend for Fig. 2) after incubation with different concentrations (0.5 to 500 μ g/ml) of heparin, a natural polyanion with a relatively low molecular weight (17,000 to 20,000 daltons). With 30 μ g or more of heparin per ml, the sedimentation patterns of RNA and protein labels were like those after incubation with rubella virus RNA in Fig. 1. RNA appeared to be completely separated from protein. With lower heparin concentrations, some of the protein label remained associated with the RNA peak, which sedimented with increasing velocity as the heparin concentration decreased. This result suggests that dissociation of rubella virus RNP by heparin is not a "one hit" reaction, but that protein sub-

FIG. 1. Dissociation of rubella virus RNP by autologous RNA. ^3H -uridine- or ^3H -Leucine-labeled purified rubella virus (7, 15) ($\text{OD}_{280} \leq 0.05$) was treated with Triton X-100 for 5 min in an ice bath. Equal portions were further incubated for 10 min with (A) 2.5 μg of purified (7) rubella virus RNA per ml in an ice bath, or with (B) 20 mg of SDS per ml at room temperature, or were left as control. Samples were layered on 15 to 30% sucrose gradients in TES buffer [0.01 M tris-(hydroxymethyl)aminomethane-hydrochloride, pH 7.5; 0.001 M ethylenediaminetetraacetic acid; 0.1 M NaCl] and centrifuged for 75 min in a Spinco rotor SW50.1 at 45,000 rev/min and 4 C. Fractions (0.25 ml) were collected and acid-insoluble radioactivity was assayed as previously described (6). Symbols: (○) RNA label, (●) protein label, (△) RNA label of the control.

units are gradually detached from the remaining particle.

The equilibrium between a polyanion-protein complex and its free components is known to be affected by ionic conditions. At high ionic strengths the relative amount of free components is increased and the biological action of the polyanion is inhibited (1, 3, 13, 14). However, in 0.35 M NaCl, which did not detectably affect the RNP without heparin, the dissociative effect of heparin was slightly enhanced. Thus, stable heparin plus core protein complexes are obviously not necessary for disintegration of the RNP. The divalent cations Ca^{2+} , Mg^{2+} , and Mn^{2+} , in 100-fold molar excess to the anionic groups of heparin, did not inhibit the action of heparin in this system. The stabilizing bonds in the RNP affected by heparin are thus presumably not formed by these cations.

To investigate further the protein-RNA relationships in rubella virus RNP, samples of double-labeled isolated RNP were treated with pancreatic

TABLE 1. Effect of various polyionic substances on the stability of rubella virus ribonucleoprotein

Substance	Final concn ($\mu\text{g}/\text{ml}$)	Peak at about 150S = RNP	Peak at about 40S = RNA
Water		+ ^a	—
Polyvinylsulfate	1	±	±
	10–500	—	+
Dextran sulfate ^b	20	±	±
	100	—	+
Heparin	5	—	—
	30–500	—	+
Poliovirus RNA	2	—	+
Rubella virus RNA	2.5	—	+
Cytoplasmic RNA from BHK21/13S cells ^c	50	—	+
Ribosomal RNA from HeLa cells ^c	30	±	±
	150	—	+
4 to 5S RNA from BHK21/13S cells ^c	50	—	+
4 to 5S RNA from HeLa cells ^c	50	—	+
4 to 5S RNA from yeast ^d	20–500	+	—
Calf thymus DNA ^d	50–500	+	—
Heat-denatured DNA ^e	50–500	+	—
Hyaluronic acid	50–500	+	—
Bovine serum albumin	250	+	—
DEAE-dextran ^b	50	+	—
	500	± ^f	—
Histone ^d	100	— ^f	—

^a Symbols: +, reproducible peak with top at about 150S or 40S (See Fig. 1); ±, variable sedimentation pattern or broad peak; —, no peak at this position.

^b From Pharmacia Fine Chemicals, Uppsala, Sweden.

^c Cytoplasmic fraction prepared with Triton X-100 (11) and RNA extracted with SDS-phenol; 28S, 18S, and 4 to 5S RNA species precipitated separately with ethanol from sucrose gradient (7).

^d From Sigma Chemicals Co., St. Louis, Mo.

^e Aqueous DNA solution was boiled for 5 min and chilled rapidly.

^f Aggregation of RNP.

ribonuclease, Pronase, or deoxyribonuclease as a control. Most of the RNA label in rubella virus RNP was accessible to ribonuclease under these conditions (Table 2). Pronase made almost all of the small amount of input protein label acid-soluble, but both labels were resistant to deoxyribonuclease treatment. The RNA label, which remained acid-insoluble after a 20-min digestion, sedimented near the top of the gradient together with the protein label (Fig. 2). After Pronase treatment of RNP, the RNA label was recovered as a single peak at about 40S, like RNA extracted

from the RNP with SDS (Fig. 2). The fact that most of the RNA label was digested by a relatively low concentration of ribonuclease does not prove that this large fraction of RNA was primarily accessible to ribonuclease. It is probable that when some of the RNA has been digested the

TABLE 2. Enzymatic digestion of rubella virus RNP: results of a typical experiment^a

Enzyme	Acid-insoluble radioactivity recovered (counts/min)	
	³ H-uridine	³⁵ S-methionine
None	1,470	165
Ribonuclease (2.5 µg/ml)	196	160
Pronase (25 µg/ml)	1,455	8
Deoxyribonuclease (25 µg/ml)	1,495	172

^a Samples of isolated rubella virus RNP labeled with ³H-uridine and ³⁵S-methionine (Radiochemical Centre, Amersham, England) were incubated for 20 min at room temperature with enzymes. The reaction was stopped by chilling the mixture and adding trichloroacetic acid to give 5% (w/v).

FIG. 2. Sedimentation analysis of rubella virus RNP after enzymatic digestion. Equal samples of isolated RNP labeled with ³H-uridine and ³⁵S-methionine were treated for 20 min at room temperature with (A) 2.5 µg of ribonuclease per ml, (B) 25 µg of Pronase per ml, (C) 20 mg of SDS per ml, or left as control. Sedimentation analysis as in Fig. 1. Symbols: (○) RNA label, (●) protein label, (△) RNA label of the control.

conformation of the remaining RNP may loosen, and make more RNA accessible to the enzyme.

The present results indicate that the surface of rubella virus RNP has some exposed RNA and core protein. In this respect the RNP of rubella virus seems to resemble that of Semliki Forest virus (2, 8, 9). Furthermore, dextran sulfate can at least partially liberate the RNA from the nucleocapsid of Semliki Forest virus (L. Kääriäinen, *personal communication*).

It is interesting that the helical nucleoproteins of vesicular stomatitis virus and of the simian paramyxovirus SV5 are apparently unaffected by PVS (Obejski and Simpson, *unpublished data*; Goldstein, Pons, and Compans, *unpublished data*, cited in reference 4). On the other hand, the 60 to 70S RNA of Rous sarcoma virus can be liberated from Triton X-100-treated virus by PVS (Hovi and Vaheri, *unpublished data*). More rigorous studies are needed, however, to determine the usefulness of this kind of experiment for studies of structural relationships of viruses.

ACKNOWLEDGMENTS

I am indebted to Lena Aura and Pirkko Korpela for their excellent technical assistance.

This investigation was supported by grants from the Yrjö Jahnsson Foundation and the Finnish Medical Research Council.

LITERATURE CITED

- Bernfeld, P., J. S. Nisselbaum, B. J. Berkeley, and R. W. Hanson. 1960. The influence of chemical and physicochemical nature of macromolecular polyanions on their interaction with human serum β -lipoproteins. *J. Biol. Chem.* 235:2852-2859.
- Burge, B. W., and E. R. Pfefferkorn. 1968. Functional defects of temperature-sensitive mutants of Sindbis virus. *J. Mol. Biol.* 35:193-205.
- Godal, H. C. 1966. Precipitation of human fibrinogen with heparin. *Scand. J. Clin. Lab. Invest.* 12:56-65.
- Goldstein, E. A., and M. W. Pons. 1970. The effect of polyvinylsulfate on the ribonucleoprotein of influenza virus. *Virology* 41:382-384.
- Holmes, I. H., M. C. Wark, and M. F. Warburton. 1969. Is rubella an arbovirus? II. Ultrastructural morphology and development. *Virology* 37:15-25.
- Hovi, T., and A. Vaheri. 1970. Rubella virus-specific ribonucleic acids in infected BHK21 cells. *J. Gen. Virol.* 6:77-83.
- Hovi, T., and A. Vaheri. 1970. Infectivity and some physicochemical characteristics of rubella virus ribonucleic acid. *Virology* 42:1-8.
- Kääriäinen, L., and S. Keränen. 1970. Ribonuclease and pronase sensitivity of Semliki Forest virus core particles. *Scand. J. Clin. Lab. Invest.* 25 Suppl. 113:103.
- Kääriäinen, L., and H. Söderlund. 1971. Properties of Semliki Forest virus nucleocapsid. I. Sensitivity to pancreatic ribonuclease. *Virology* 43:291-299.
- Mettler, N. E., R. L. Petrelli, and J. Casals. 1968. Absence of cross-reactions between rubella virus and arboviruses. *Virology* 36:503-504.
- Penman, S. 1969. Preparation of purified nuclei and nucleoli from mammalian cells, p. 35-48. In K. Habel and N. P. Salzman (ed.), *Fundamental techniques in virology* Academic Press Inc., New York.

12. Pons, M. W., J. T. Schulze, and G. K. Hirst. 1969. Isolation and characterization of the ribonucleoprotein of influenza virus. *Virology* 39:250-259.
13. Tunis, M., and W. Regelson. 1963. A comparative study of the inhibiting effects of anionic polyelectrolytes on deoxy-ribonucleases. *Arch. Biochem.* 101:448-453.
14. Vaheri, A. 1964. Heparin and related polyionic substances as virus inhibitors. *Acta Pathol. Microbiol. Scand. Suppl.* 171:1-98.
15. Vaheri, A., and T. Hovi. 1972. Structural proteins and subunits of rubella virus. *J. Virol.* 9:10-16.
16. Vaheri, A., and T. Vesikari. 1971. Small size rubella virus antigens and soluble immune complexes: analysis by the platelet aggregation technique. *Arch. Gesamte Virusforsch.* 35:10-24.
17. von Bonsdorff, C.-H., and A. Vaheri. 1969. Growth of rubella virus in BHK21 cells: electron microscopy of morphogenesis. *J. Gen. Virol.* 5:47-51.