JOURNAL OF VIROLOGY, Mar. 1978, p. 781-787
0022-538X/78/0025-0781$02.00/0

Vol. 25, No. 3
Printed in U.S.A.

Characterization of an RNA-Dependent RNA Polymerase Activity Associated with Measles Virus

A. S. SEIFRIED, P. ALBRECHT, AND J. B. MILSTIEN*
Bureau of Biologics, Food and Drug Administration, Bethesda, Maryland 20014

Received for publication 16 September 1977

An RNA-dependent RNA polymerase activity has been found copurifying with measles virus infectivity and complement-fixing antigen in three Vero cell-grown variants of measles virus: the attenuated Edmonston B strain, the natural non-attenuated Edmonston strain, and a subacute sclerosing panencephalitis isolate, IP-3. Incubation of purified measles virions with immunoglobulin G derived from sera of monkeys hyperimmunized against measles specifically removes activity sedimenting in the density region of measles virions. The requirements of the reaction, which is RNase sensitive, are similar to those reported for other paramyxovirus-associated activities, including detergent, divalent cation, ribonucleoside triphosphates, and a reducing agent. The size classes of RNA synthesized correspond to those found in measles-infected cells, including 50, 35, and 16 to 20S. The product RNA of the Edmonston B virus-stimulated reaction was rendered RNase resistant by annealing with RNA extracted from purified Edmonston B virions. RNA from uninfected Vero cells was ineffective in the annealing reaction.

Measles virus has been classified as a paramyxovirus on the basis of morphology and RNA size (8, 19, 23, 24). Reports in the literature of RNA-dependent RNA polymerase in the virions of other paramyxoviruses (4, 11, 18, 22) suggested that measles virus might contain a similar activity. Here we describe and characterize a measles virus-associated RNA-dependent RNA polymerase activity.

MATERIALS AND METHODS

Cells and viruses. Vero cells, a continuous line of African green monkey kidney cells, were supplied by the Cell Biology Section, Bureau of Biologics, and were used both for virus production and infectivity assays (2). They were grown and maintained in Eagle minimal essential medium supplemented with 10% fetal calf serum, 0.03% glutamine, 50 μg of gentamicin per ml, and 0.4 μg of amphotericin B per ml.

The attenuated strain of measles virus was strain Edmonston level B (Edmonston B), derived from a commercial vaccine (Eli Lilly & Co., Indianapolis, Ind.) and passaged twice in Vero cell cultures. Infectivity ranged from 3×10^7 to 10×10^7 PFU/ml for cell-associated virus and 1×10^7 to 3×10^7 PFU/ml for extracellular virus.

The natural strain of measles virus was obtained from John F. Enders after six passages in human embryonic kidney cells and was adapted to Vero cells through six consecutive passages. The infectivity of cell-associated virus was 10^6 PFU/ml.

The subacute sclerosing panencephalitis (SSPE) strain IP-3 was isolated by T. Bernstein from a brain biopsy of a 14-year-old child (7). It received 22 cell culture passages followed by one passage in rhesus monkey brain (1). Since isolation from human brain, the virus maintained the cell-restricted, nonproductive character of growth in cell cultures and spread by cell fusion, gradually involving the whole cell sheet. Infectious seeds of the IP-3 virus were prepared in suckling hamster brain; they titrated 10^6 PFU/ml of a 10% brain homogenate.

Virus infection and infectivity assay. Vero cell monolayers were infected with a multiplicity of infection of 1.0 for the attenuated strain and a multiplicity of 0.01 for the natural and the SSPE strains. All three viruses caused an almost complete cytopathic effect 2 days after infection, the latter two viruses due to a rapidly spreading process of cell fusion.

Virus infectivity was determined by plaque assay in Vero cell cultures in 35-mm petri dishes under 0.5% agarose (2).

Serological techniques. Hemagglutinating activity of virus material was assayed in microtiter plates, using 0.5% African green monkey erythrocytes. Viral nucleoprotein was assayed with rabbit serum directed specifically against purified measles nucleocapsids. Twofold dilutions of the virus material were tested against 4 U of antibody and 2 U of guinea pig complement. The indicator system was a 2% suspension of sheep erythrocytes sensitized with 6 U of rabbit amboceptor.

Purification of virus and viral RNA. Extracellular virus was recovered by pelleting the clarified medium (1,000 × *g* for 10 min) in a Beckman type 19 rotor at 19,000 rpm (35,900 × *g*) for 2 h. Cell-associated virus was harvested by gently scraping cells into a minimum volume of phosphate-buffered saline, washing two times in 5 ml of phosphate-buffered saline, and sonically treating cells suspended in 2 ml of phosphate-buffered saline two times for 30 s each, in a

Branson Sonifier fitted with a microprobe at full output, to release the virus from the cells. Cell debris was recovered by low-speed centrifugation (1,000 × *g* for 10 min). The virus was then partially purified on a discontinuous sucrose gradient: 15 ml of cell extract was layered over 15 ml of 15% sucrose (0.01 M Tris–0.03 M NaCl), which had been layered over 5 ml of a 65% sucrose cushion (0.01 M Tris–0.03 M NaCl in D_2O). After spinning for 50 min at 27,000 rpm (96,300 × *g*) in an SW 27 rotor, virus was recovered from the interface of the two sucrose layers. Both extracellular and cell-associated virus were further purified by centrifuging to equilibrium on a linear 15 to 65% sucrose gradient in a Beckman SW 27.1 rotor at 25,000 rpm (81,000 × *g*) for 16 h. Thirty equal fractions were collected from the bottom of the tube. Density was determined from the refractive index, with a standard curve constructed by using density gradient marker beads (Reproductive Systems Inc.). The visible band, at a density of 1.25 to 1.26 g/cm^3, was pooled. On some occasions, the virus was further purified through a second sucrose gradient in like manner, or by adsorption to and elution from vervet erythrocytes by the arginine elution technique of Lebon et al. (15). Protein concentration was determined by the method of Lowry et al. (16).

For preparation of nucleocapsids, virions purified by linear equilibrium sucrose gradients were dialyzed against 0.1 M $NaHCO_3$, pH 10, adjusted to 2% Tween 20, and were then sedimented on a 15 to 30% sucrose gradient, at 4°C, in 0.005 M Tris–0.001 M EDTA–0.5% sodium deoxycholate, for 3 h at 27,000 rpm (95,400 × *g*) in an SW 27.1 rotor. Rat liver ribosomes, 80S, prepared by the method of Blobel and Potter (6), were used as a marker.

In the case of IP-3, the purification of nucleocapsids was performed as outlined above for virions by using cellular extracts. Nucleocapsids were then centrifuged on deoxycholate-sucrose gradients.

Endogenous RNA-dependent RNA polymerase assay and preparation of ^{3}H-labeled reaction products. Measles virions or purified nucleocapsids were added in 10-μl amounts (10 to 40 μg of virus protein) to 40 μl of the following reaction mixture: 0.05 M Tris-hydrochloride (pH 7.8)–0.1 M NaCl–0.003 M dithiothreitol–0.004 M $MgCl_2$–7 × 10^{-4} M each ATP, CTP, and UTP–2 μg of yeast RNA per ml–2 × 10^{-5} M [^{3}H]GTP (1,700 cpm/pmol). The reaction was allowed to proceed for 2 h at 28°C. For analysis of enzymatic activity, the reaction was stopped by addition of 10% trichloroacetic acid containing 0.017 M sodium pyrophosphate, and trichloroacetic acid-precipitable counts were collected on filters. For the experiment depicted in Table 2, trichloroacetic acid precipitates were collected by centrifugation at 300 × *g* for 10 min, suspended in 0.1 M Tris, pH 8.5, precipitated with 20% trichloroacetic acid, and collected on filters. For preparation of complementary RNA (cRNA), the products were adjusted to 1% sodium dodecyl sulfate (SDS), extracted with an equal volume of buffer (0.1 M NaCl–0.001 M EDTA–0.05 M Tris, pH 7.0)-equilibrated phenol with intermittent shaking at room temperature and centrifuged at 6,000 × *g* for 10 min to separate the layers. The aqueous layer was ether extracted and dialyzed. [^{3}H]cRNA was sized on 15 to 30% sucrose gradients (0.01 M Tris[pH 7.8]–0.1 M NaCl–0.01 M EDTA–0.5% SDS) in an SW 27.1 rotor, at 24,000 rpm for 16 h at 20°C, with 18 and 28S ribosomal RNA markers. One-half of each fraction was treated with RNase (10 μg/ml, 20 min) before trichloroacetic acid precipitation. For sizing dimethyl sulfoxide (Me_2SO)-treated RNA, the RNA sample was made to 80% Me_2SO (Sigma, grade 1), incubated at room temperature for 10 min, and diluted 1:4 with 0.01 M Tris (pH 7.4)–0.1 M NaCl–0.001 M EDTA (TNE) before centrifuging on a sucrose velocity gradient.

Inhibition of polymerase activity by specific antiglobulin. Immunoglobulin G (IgG) was purified from heat-inactivated (56°C, 30 min) monkey sera by passing it over a DEAE-cellulose (DE-52) column, eluting it with 0.01 M potassium phosphate buffer, pH 8.0, and precipitating it with 50% $(NH_4)_2SO_4$. The control sera were obtained from a pool of two monkeys with no history of exposure to measles; the measles-positive sera came from three monkeys having had natural measles infection and twice hyperimmunized with measles antigen. The virus-neutralizing titer of the measles-positive sera was 1:27,000, and the complement-fixing titer with purified measles nucleocapsids was 1:256 compared with a titer of less than 1:8 for the measles-negative sera. The measles-positive IgG preparation had a virus-neutralizing antibody titer of 1:33,000 and a protein concentration of 17.6 mg/ml; control IgG was 12.1 mg of protein per ml.

For experiments testing the effect of anti-measles IgG on the endogenous polymerase activity, control and measles-positive IgG's were incubated with equal volumes of isopycnic gradient-purified measles virions at room temperature for 1 h and then at 4°C overnight. The suspensions were filtered through 0.45-μm cellulose filters to remove antigen-antibody complexes, and the filtrates were centrifuged to equilibrium on a 15 to 65% sucrose gradient and assayed across the gradient for endogenous RNA-dependent RNA polymerase activity.

Annealing of ^{3}H-labeled reaction products. A constant amount of ^{3}H-labeled reaction products (600 cpm) and varying amounts of unlabeled measles virion RNA or Vero cell RNA were denatured in 0.05% SDS and 2.5 mM EDTA in a volume of 0.05 ml at 100°C for 3 min and then quick cooled by plunging into ice. The samples were adjusted with 0.1 M Tris and 0.2 M NaCl to a final concentration of 0.05 M Tris and 0.16 M NaCl in a volume of 1 ml and incubated for 16 h at 60°C to allow annealing, followed by RNase treatment (Worthington RAF, 50 μg/ml, 37°C, 30 min). Trichloroacetic acid-precipitable counts were collected on filters. For annealing experiments, unlabeled RNA was extracted from Edmonston B virions purified on a sucrose gradient. The virus preparation was dialyzed against TNE, adjusted to 1% SDS, and extracted with buffer-equilibrated phenol at room temperature.

The aqueous phase was ether extracted to remove phenol and dialyzed against TNE. The virion RNA recovered in this manner was shown to contain 50S RNA on sucrose velocity gradient analysis. Vero cell RNA was prepared from uninfected Vero cells by hot-phenol extraction (12) and was treated with DNase and Pronase before use. It had an absorbance ratio (260 nm/280 nm) of 2.0.

Hydroxylapatite separation of single-stranded and double-stranded products. Hydroxylapatite (Bio-Gel HTP, Bio-Rad Laboratories lot 13201) was slurried in water and packed under pressure to 1 to 2 cm in a 1-cm-diameter jacketed column. The column was maintained at 60°C. Samples were added and allowed to equilibrate. All sample not adhering to the column was collected in the first fraction. Single-stranded products were collected in three 1-ml elutions with 0.14 M phosphate buffer (PB) containing 0.4% SDS, allowing the column to equilibrate to 60°C with each addition. The column was washed with 10 ml of 0.14 M PB, and the double-stranded products were then eluted in two 1-ml volumes with 0.4 M PB. Fractions were trichloroacetic acid precipitated onto filters, and the counts were determined. Recovery from hydroxylapatite was nearly quantitative. This separation was tested, using single- and double-stranded poliovirus RNA prepared by the method of Bishop and Koch (5). The 0.14 M PB eluates were totally susceptible to S_1 nuclease digestion; the 0.4 M PB eluates were resistant to S_1 nuclease.

S_1 nuclease digestion. Samples to be nuclease digested were incubated in a mixture of 2.5 mM $ZnSO_4$, 10 μg of denatured calf thymus DNA per ml, and 0.03 M Na acetate, pH 4.5, with the single-strand-specific nuclease from *Aspergillus oryzae*, S_1, for 1 h at 50°C. Trichloroacetic acid-precipitable counts were collected on filters. S_1 was obtained from Miles Laboratories and further purified on Sephadex G-75 (3).

RESULTS

The Edmonston B strain of measles virus grown in Vero cells showed RNA-dependent RNA polymerase activity in the 1.25 to 1.26 g/cm^3 density region of an isopycnic sucrose gradient corresponding to the position of peak virus infectivity and a visible band. Gradients of uninfected Vero cells showed none of these phenomena. Figure 1A illustrates the pattern obtained when samples of such a gradient performed on Edmonston B measles virus extract from infected Vero cells were assayed in the presence of detergent (Triton N-101, 0.08%). The arrow indicates the fraction containing the visible band.

Infectivity titers of the total cell-associated virus were three to five times higher than those of the total extracellular virus. Polymerase activity was considerably higher in gradients of virus material obtained from cell extracts (Fig. 1A) than from cell supernatants (data not shown), i.e., 13,000 cpm/10 μl as compared with 4,600 cpm/10 μl. This observation suggests that the activity correlated with virus titer and indeed was associated with the presence of measles virus.

In Fig. 1A, polymerase activity had a peak in the 1.28 g/cm^3 density region of the gradient which was not associated with maximum measles infectivity. This region of the gradient contains significant amounts of measles complement-fixing antigen and may contain measles nucleocapsids. To test this, the virion pool was isolated from the gradient shown in Fig. 1A, treated with Tween 20 (2%), and recentrifuged on a similar isopycnic sucrose gradient. Figure

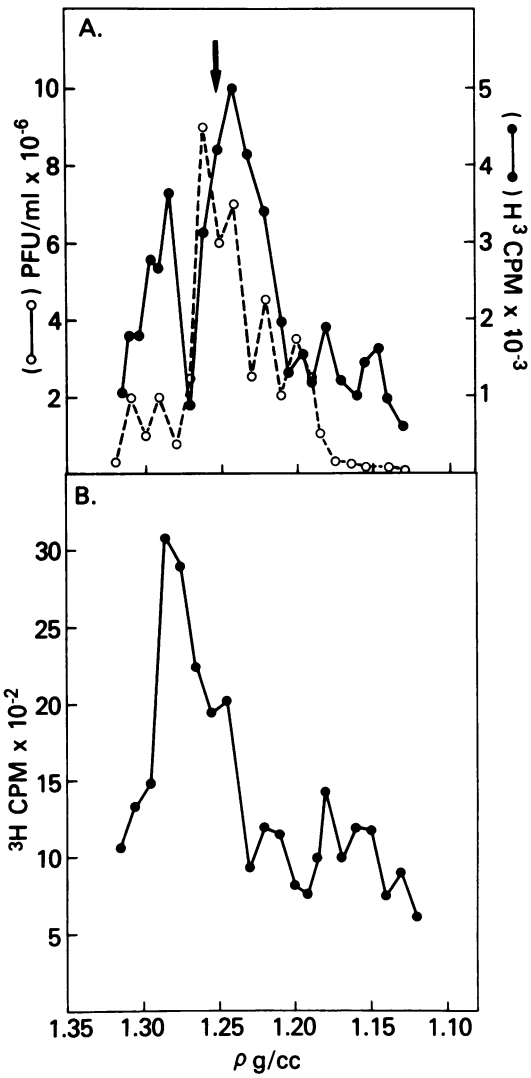

FIG. 1. *Purification of RNA-dependent RNA polymerase activity associated with measles virus. (A) Isopycnic sucrose (15 to 65%) gradient of Edmonston B measles virus showing measles infectivity titer* (○) *peaks at buoyant density of 1.25 to 1.26 g/cm^3, as does endogenous RNA-dependent RNA polymerase activity* (●) *assayed as described in the text. The arrow indicates the position of a visible band of measles virions in the gradient. (B) The 1.25 to 1.26 g/cm^3 density region in Fig. 1A was pooled, treated with Tween 20 to yield nucleocapsids as described in the text, and centrifuged on an isopycnic sucrose gradient as in Fig. 1A.* (●) *RNA-dependent RNA polymerase activity.*

1B shows that the RNA polymerase activity shifted to the density region of nucleocapsids. Nucleocapsids prepared in this manner had associated polymerase activity, which sedimented at 200S in a sucrose velocity gradient (data not shown) as reported for measles nucleocapsids (13). The activity was associated with a positive complement fixation test for measles nucleocapsids.

To further show that the activity was associated with measles virions, rather than a cellular fraction of measles-infected cells, virus purified on a discontinuous sucrose gradient followed by a linear isopycnic gradient was further purified by adsorption to vervet monkey erythrocytes and eluted with arginine. Eluted virus again contained polymerase activity that copurifies with the measles infectivity.

Observations of RNA-dependent RNA polymerase activity were made on sucrose equilibrium and velocity gradients with the natural Edmonston strain of measles virus and with the SSPE isolate, IP-3, as well. The natural strain yielded a considerably lower infectivity titer than the attenuated strain. The IP-3 strain did not produce appreciable amounts of virions, and the purification steps were designed for recovery of nucleocapsids. Table 1 shows the specific activity of the RNA-dependent RNA polymerase activity associated with each of these viruses in relation to protein concentration and virus infectivity. The values given represent ratios of polymerase activity and infectious virus titer determined on the same sample of purified cell-associated virus.

The sensitivity of RNA-dependent RNA polymerase activity to inhibition by IgG against measles virions was examined. Although incubation of purified Edmonston B virions with IgG purified from a pool of measles-positive monkey sera reduced infectious virus titer to $<5 \times 10^2$ PFU/ml compared with a value of 2.1×10^7 PFU/ml obtained on incubation with measles-negative sera, no effect was seen on the polymerase activity. However, when the experiment was designed to assess effect on polymerase activity of removal of measles virions from solution, the results seen in Table 2 were obtained. Purified Edmonston B measles virions were incubated with measles-negative or measles-positive IgG, filtered to remove precipitates, and applied to 15 to 65% linear sucrose gradients. Table 2 shows that incubation with measles-positive IgG abolished infectivity and polymerase activity in those fractions of the gradient where measles virions band, whereas measles-negative IgG did not.

In general, the measles-associated polymerase activity was similar to that reported for other paramyxoviruses. Table 3 shows the requirements of the reaction, which was partially dependent on the presence of dithiothreitol and monovalent cation and totally dependent on divalent cation and the nucleoside triphosphate precursors. In early experiments the reaction appeared to be dependent on the presence of Triton N-101, but in later work this dependence was not seen, possibly because subsequent stages of purification rendered the virion envelope permeable to the components of the reaction mixture. RNase did not inhibit the reaction unless the samples were pretreated with detergent (treatment with 0.08% Triton N-101 for 20 min followed by 50 μg of RNase per ml for 20 min

TABLE 1. *Specific activity of virion-associated polymerase in three variants of measles virus*

Virus	Activity[a]		
	Per ml of purified polymerase source	Per mg of virus protein	Per PFU
Edmonston B	294	70	1.47×10^{-7}
Edmonston, natural	353	104	3.2×10^{-5}
SSPE(IP-3)	371	309	

[a] Picomoles of [^{3}H]GTP incorporated in 2 h at 28°C.

TABLE 2. *Anti-measles IgG effect on polymerase activity*[a]

Incubation	Infectious virus titer (PFU/ml)	Polymerase activity (cpm/10 μl)
Measles-negative IgG	1.7×10^5	10,300
Measles-positive IgG	$<5 \times 10^1$	1,560

[a] Edmonston B measles virus was incubated with IgG and processed as described in the text. The values presented were obtained by averaging over the measles density region (ρ = 1.25 to 1.26 g/cm^3) of the gradient.

TABLE 3. *Requirements of measles-associated RNA-dependent RNA polymerase*[a]

Assay conditions	Incorporation	
	cpm	%
Complete system, 0 time	223	0
Complete system, 2 h	1,681	100
−NaCl	1,222	68
−DTT	921	48
$-Mg^{2+}$	344	8.3
$-Mg^{2+}$, $+Mn^{2+}$	1,569	92
−ATP, −UTP, −CTP	365	9.7
−Triton N-101	872	44

[a] RNA-dependent RNA polymerase activity was assayed as described in the text, using Edmonston B measles virus purified by isopycnic centrifugation.

prior to incubation with reaction components) presumably due to sequestering of viral RNA and products by the virion coat. Under these conditions, the polymerase reaction is completely sensitive to inhibition by RNase. The polymerase reaction was stimulated 2.5-fold by addition of yeast RNA, as noted for Sendai virus-associated transcriptase (20). The addition of yeast RNA did not change either the size or the measles virus homology of the product, and was used in all experiments presented here at a concentration of 2 μg/ml.

To examine the products of polymerase activity, the reaction mixture (total volume, 2 ml) was extracted with phenol, and the RNA species were sized on a 15 to 30% sucrose gradient using 18 and 28S markers. Half of each collected fraction was RNase treated. Figure 2 shows the general size classes obtained in the IP-3-directed reaction, including mainly 50, 35, and 16 to 20S species. Measles-infected cell cytoplasm contains 50, 35, and 16 to 20S RNAs (8, 9, 19, 25). All three measles isolates synthesized these general classes in this reaction (see also Fig. 3A). There is very little RNase resistance of the products in these size classes (Fig. 2).

To investigate whether the high-molecular-weight species were complex molecules, perhaps short sequences of newly synthesized RNA hydrogen bonded to virion RNA, the high-molecular-weight portion of the Edmonston B-directed reaction products (indicated by the bar in Fig. 3A) was pooled, treated with Me_2SO, and resedimented on sucrose (Fig. 3B). Even

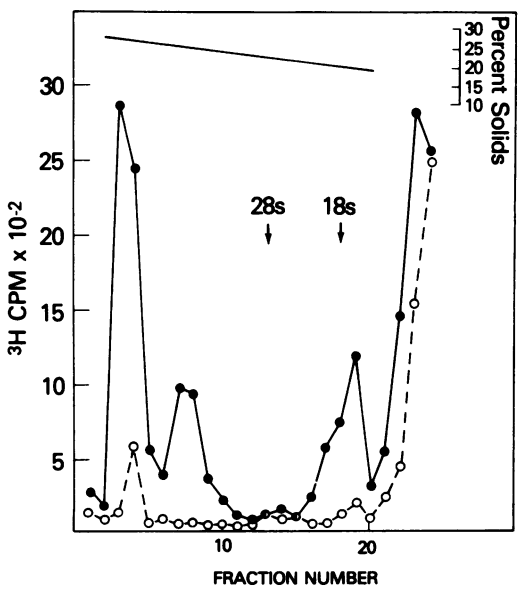

FIG. 2. *[^{3}H]RNA synthesized from IP-3 virus, as described in the text, was phenol extracted and applied to a 15 to 30% sucrose gradient containing SDS, and fractions untreated (●) or treated (○) with RNase were trichloroacetic acid precipitated.*

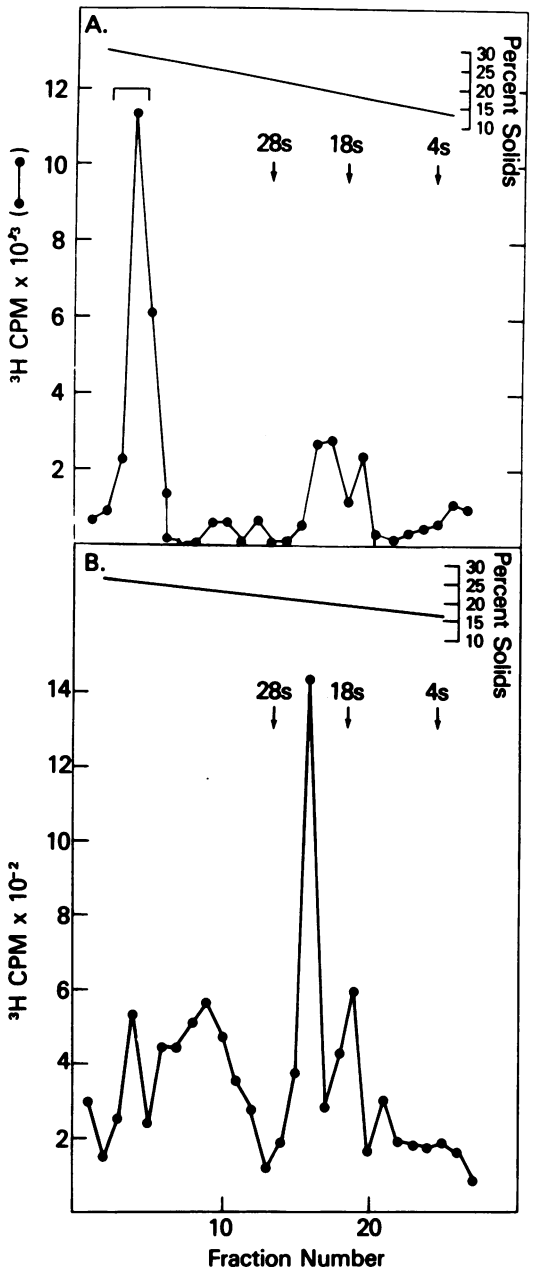

FIG. 3. *(A) [^{3}H]cRNA synthesized from Edmonston B virus, as described in the text, was phenol extracted and applied to a 15 to 30% sucrose gradient containing SDS, and fractions were trichloroacetic acid precipitated. (B) The high-molecular-weight fraction indicated by the bar in (A) was treated with Me_2SO before velocity gradient sedimentation.*

after Me_2SO treatment, a significant portion (50%) sedimented at greater than 28S, suggesting that some of the product may represent transcriptive intermediates, but that a major fraction of the newly incorporated radioactivity is stably linked to high-molecular-weight RNA.

There were some RNase-resistant products, especially in the small size classes (less than10S). Because of this, single-stranded RNA was separated on a hydroxylapatite column (see above); it comprised about 75% of the total reaction product. Approximately 75% of the material separated as double-stranded product was shown to be S_1 resistant even after denaturation unless it was first nicked with RNase, which rendered it almost totally S_1 sensitive, suggesting that it may contain more complicated structures than simple double strands.

To demonstrate that the products of the measles-associated polymerase activity were transcripts of the measles genome, the single-stranded ^3H-labeled reaction product isolated on hydroxylapatite was tested for annealing to either virion RNA or to uninfected Vero cell RNA. Table 4 shows that the polymerase product annealed only to measles RNA and not to Vero cell RNA.

DISCUSSION

These results demonstrate the presence of an RNA-dependent RNA polymerase activity in measles virions. This activity incorporated [^3H]GTP into acid-insoluble material under conditions described for other paramyxoviruses. Moreover, the products contained base sequences complementary to RNA extracted from virions of measles. Therefore, the activity appears to be an RNA-dependent RNA transcriptase analogous to the enzyme found in the virions of other paramyxoviruses (4, 11, 22).

The problems of working with measles virus are great compared with other paramyxoviruses due to the cell-associated nature of the virus and the fragility of the virion (17). We have tried to demonstrate that the polymerase activity is indeed associated with measles virus: (i) by showing that the activity copurifies with measles virions or nucleocapsids whether separated on the basis of density or of size; (ii) by specifically purifying measles virions on the basis of their erythrocyte-adsorbing property; (iii) by hybridizing the reaction product to measles RNA; and (iv) by showing that incubation with anti-measles IgG specifically abolishes activity associated with measles virions. Further, we have been able to demonstrate this activity associated with three different measles strains but not with uninfected Vero cells. In addition, we have noted polymerase activity in BSC-1 cells persistently infected with the IP-3 virus but not in uninfected BSC-1 cells (J. B. Milstien and P. Albrecht, unpublished observations).

TABLE 4. *Annealing of measles virus polymerase products to measles RNA and to Vero cell RNA*[a]

RNA added, μg/ml		% RNase resistant
None		8.0
Vero,	0–100	9.0
Measles,	6.5	12
	20	32
	50	63
	101	102
	200	99

[a] Edmonston B single-stranded [^3H]cRNA isolated from hydroxylapatite, 600 cpm per reaction mixture, was annealed with varying amounts of RNA as described in the text.

Besides being present in the intact (infectious) virion fraction, measles polymerase activity was also associated with measles nucleocapsids, as was also noted for other paramyxoviruses (11, 21), since it was found associated with the IP-3 isolate and was found sedimenting at a velocity and a density consistent with those reported for measles nucleocapsids (13).

The specific activity of the polymerase is about the same as that found with other paramyxovirus transcriptases, especially for the Edmonston B strain. Huang et al. (11) give a specific activity of 215 U/mg of protein for Newcastle disease virus transcriptase, a value they report as 1 to 3% that of vesicular stomatitis virus.

The size classes of products seen are as expected, based on the sizes of measles-specific RNA seen in infected cells (8, 10, 19, 25). Although some of the larger-sized reaction product was converted to a more slowly sedimenting form by Me_2SO (Fig. 3B), similar to observations on heat-treated 50S RNA from measles-infected cells (10), a significant portion of it represented high-molecular-weight RNA. Taking into account the ability of Me_2SO to fragment RNA, this amount may be even greater. Most of the RNA synthesized was single stranded.

Some of the product was RNase resistant and fractioned as double strands on hydroxylapatite. Analysis of the Edmonston B product gave structures that appeared to be hairpin structures, because they were separated as double strands on hydroxylapatite and remain S_1 resistant after denaturation unless nicked with RNase. Hairpin structures have been seen in transcription of negative-strand virus stocks containing defective interfering particles (14). Because measles virus is known to generate such particles (13), the appearance of these structures

may be related to the existence of defective interfering particles in the virus stock.

ACKNOWLEDGMENT

We are indebted to David J. Waters for making available the rabbit antiserum to measles nucleoproteins.

LITERATURE CITED

1. **Albrecht, P., T. Burnstein, M. J. Klutch, J. T. Hicks, and F. A. Ennis.** 1977. Subacute sclerosing panencephalitis: experimental infection in primates. Science **195**:64–66.
2. **Albrecht, P., and H. P. Schumaker.** 1972. Markers for measles virus. I. Physical properties. Arch. Gesamte Virusforsch **36**:23–35.
3. **Ando, T.** 1966. A nuclease specific for heat denatured DNA isolated from a product of *Aspergillus oryzae*. Biochim. Biophys. Acta **114**:158–168.
4. **Bernard, J. P., and R. L. Northrup.** 1974. RNA polymerase in mumps virions. J. Virol. **13**:183–186.
5. **Bishop, J. M., and G. Koch.** 1967. Purification and characterization of poliovirus-induced infectious double-stranded ribonucleic acid. J. Biol. Chem. **242**:1736–1743.
6. **Blobel, G., and V. R. Potter.** 1967. Studies on free and membrane-bound ribosomes in rat liver. I. Distribution as related to total cellular RNA. J. Mol. Biol. **26**:279–292.
7. **Burnstein, T., L. B. Jacobsen, W. Zeman, and T. T. Chen.** 1974. Persistent infection of BSC-1 cells by defective measles virus derived from subacute sclerosing panencephalitis. Infect. Immun. **10**:1378–1382.
8. **Carter, C., A. Schluederberg, and F. L. Black.** 1973. Viral RNA synthesis in measles virus-infected cells. Virology **53**:379–383.
9. **Hall, W. W., and V. ter Meulen.** 1976. RNA homology between subacute sclerosing panencephalitis and measles virus. Nature (London) **264**:474–477.
10. **Hall, W. W., and V. ter Meulen.** 1977. Polyadenylic acid [poly(A)] sequences associated with measles virus intracellular ribonucleic acid (RNA) species. J. Gen. Virol. **35**:497–510.
11. **Huang, A., D. Baltimore, and M. A. Bratt.** 1971. Ribonucleic acid polymerase in virions of Newcastle disease virus: comparison with vesicular stomatitis virus polymerase. J. Virol. **7**:389–394.
12. **Khoury, G., J. C. Byrne, K. K. Takemoto, and M. A. Martin.** 1973. Patterns of simian virus 40 deoxyribonucleic acid transcription. II. In transformed cells. J. Virol. **11**:54–60.
13. **Kiley, M. P., R. H. Gray, and F. E. Payne.** 1974. Replication of measles virus: distinct species of short nucleocapsids in cytoplasmic extracts of infected cells. J. Virol. **13**:721–728.
14. **Lazzarini, R. A., G. H. Weber, L. D. Johnson, and G. M. Stamminger.** 1975. Covalently-linked message and anti-message (genomic) RNA from a defective vesicular stomatitis virus particle. J. Mol. Biol. **97**:289–307.
15. **Lebon, P., A. Protat, and P. Molinie.** 1975. L-Arginine elution of measles virus adsorbed on monkey erythrocytes. Infect. Immun. **11**:1407–1408.
16. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
17. **Mountcastle, W. E., and P. W. Choppin.** 1977. A comparison of the polypeptides of four measles virus strains. Virology **78**:463–474.
18. **Robinson, W. S.** 1971. Ribonucleic acid polymerase activity in Sendai virions and nucleocapsids. J. Virol. **8**:81–86.
19. **Schluederberg, A.** 1973. Viral RNA synthesis in measles virus infected cells. Biochem. Biophys. Res. Commun. **42**:1012–1015.
20. **Stone, H. O., and D. W. Kingsbury.** 1973. Stimulation of Sendai virion transcriptase by polyanions. J. Virol. **11**:243–249.
21. **Stone, H. O., D. W. Kingsbury, and R. W. Darlington.** 1972. Sendai virus-induced transcriptase from infected cells: polypeptides in the transcriptive complex. J. Virol. **10**:1037–1043.
22. **Stone, H. O., A. Portner, and D. W. Kingsbury.** 1971. Ribonucleic acid transcriptase in Sendai virions and infected cells. J. Virol. **8**:174–180.
23. **Waterson, A. P.** 1962. Two kinds of myxovirii. Nature (London) **193**:1163–1164.
24. **Winston, S. H., R. Rustigian, and M. A. Bratt.** 1973. Persistent infection of cells in culture by measles virus. III. Comparison of virus-specific RNA synthesized in primary and persistent infection in HeLa cells. J. Virol. **11**:926–932.
25. **Yeh, J.** 1973. Characterization of virus-specific RNAs from subacute sclerosing panencephalitis virus-infected CV-1 cells. J. Virol. **12**:962–968.