# Reovirus: RNA Polymerase Activity in Purified Virions*

J. Borsa** and A. F. Graham

The Wistar Institute of Anatomy and Biology,
Philadelphia, Pa.

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SUMMARY. Purified reovirus particles contain a polymerase which transcribes virus-specific mRNA in vitro from the double-stranded, viral RNA genome. The enzyme activity is markedly increased after heat-shocking the virions.

Shortly after infection of L cells with reoviruses, a newly synthesized, single-stranded species of RNA (ssRNA) is detectable (1). This ssRNA hybridizes efficiently with the denatured double-stranded RNA (dsRNA) of reovirus and is viral messenger RNA (mRNA) transcribed from the ten dsRNA segments of the viral genome (2-7). Some transcription of the parental viral genome occurs even when an inhibitor of protein synthesis is added at the time of infection (7), although later events in the replicative cycle of the virus are blocked. This observation indicates that the RNA polymerase (transcriptase) responsible for this early transcription is a pre-existing enzyme. Theoretically, such an enzyme could be a host-cell enzyme or it could be an integral part of the infecting virion. We report here the finding of an RNA polymerase activity in purified reovirus particles.

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## METHODS

The plaque assay of reovirus, type 3 (1), its growth in suspension cultures (4) and purification (7) have all been previously described, as have the procedures for extracting RNA from purified virus (7) and from *in vitro* enzyme reaction mixtures (8), and the method for analysis of reovirus RNA by electrophoresis on polyacrylamide gels. The conditions for *in vitro* assay of the RNA polymerase activity of purified virions are given in the legend to Table 1. RNA made *in vitro* by the enzyme was hybridized with viral dsRNA by methods already described in detail (4, 5, 7, 8). Essentially, the total RNA of the *in vitro* reaction mixture, which included a large excess of viral dsRNA template, was extracted with phenol and denatured with dimethyl sulfoxide (DMSO). Hybridization was performed by heating the denatured products at 73.2° C for 18 hr in 0.3 M STE buffer (7).

## RESULTS

*Uptake of Labeled Ribonucleoside Triphosphates by Purified Reovirus in vitro.* Table 1 shows that purified virions have the ability to polymerize ribonucleoside triphosphates into acid-insoluble material. This incorporation is dependent on $Mg^{++}$, ATP (omission of GTP or CTP also reduces the incorporation, but to a lesser extent) and temperature. The reaction proceeded linearly for at least 5 hours, which was the longest time tested.

*Increase in RNA Polymerase Activity of Purified Reovirus Particles by Heat Shock.* It has recently been reported that heat shock of adenovirus resulted in formation of infectious cores (9). Following this report we tested the effects of heat shock on reovirus RNA polymerase activity. Samples of purified virus were heated for 20 seconds to the required temperature, quick-chilled in an ice-bath, and were then assayed for polymerase activity.

TABLE 1

Incorporation of Labeled Ribonucleoside Triphosphates into TCA-insoluble Material by Purified Virions

| Labeled precursor | Condition | cpm incorporated |
|---|---|---|
| $^{3}$H-UTP (2.0 μc, 2.0 c/mmole) | complete mixture | 465 |
| | -ATP | 88 |
| | -$MgCl_2$ | 28 |
| | -virus | 21 |
| | complete, 0° C | 43 |
| $^{14}$C-UTP (0.5 μc, 48 mc/mmole) | complete mixture | 432 |
| | complete, 0° C | 9 |
| $^{14}$C-CTP (0.5 μc, 316 mc/mmole) | complete mixture | 896 |
| | -ATP | 166 |
| | complete, 0° C | 15 |

The complete reaction mixture contained in a final volume of 0.25 ml: purified reovirus (0.9 $O.D._{260}$ units); ATP, GTP, CTP, UTP (each at 0.2 μmoles); mercaptoethanol (3 μmoles); actinomycin D (2 μg); $MgCl_2$ (3 μmoles); tris-HCL (25 μmoles, pH 8.0). Labeled XTP's were substituted one at a time for the corresponding XTP. Incubation was for 2 hr at 37° C and the reaction was terminated by adding 1 ml of ice-cold 5% TCA, containing 0.02 M sodium pyrophosphate (NaPP). The precipitate was filtered through 0.45 μ pore-size, Schleicher and Schuell membrane filters, washed with 5% TCA containing 0.02 M NaPP and then with 95% ethanol. The filters were placed in scintillation vials, dried and assayed for radioactivity in a Tricarb scintillation counter as described (8). For quantitative purposes 1 $O.D._{260}$ unit is taken as being equivalent to 45 μg of viral RNA.

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Table 2 indicates that the short heat treatment at 70° C enhanced the polymerase activity by a factor of 13 over that at 40° C. The requirements for the polymerase reaction are presented in Table 3. Incorporation was completely dependent on $Mg^{++}$, ATP, GTP and CTP, and its rate was proportional to virus concentration. Mercaptoethanol inhibited the reaction. Thus, Tables 1, 2 and 3 clearly show that the purified reovirus preparations contained an RNA polymerase, and that the activity was markedly increased by heat

shocking the virus. Further studies on the heat activation phenomenon will be reported elsewhere. The remainder of this report establishes that the observed polymerase activity is an integral part of the reovirus virion and that the product is a virus-specific ssRNA.

TABLE 2

Effect of Heat Shock on RNA Polymerase Activity of Purified Reovirus

| Temp. of shock (20 sec) | TCA insoluble cpm |
|---|---|
| 40° C | 664 |
| 50° C | 684 |
| 60° C | 2533 |
| 70° C | 8916 |
| 80° C | 188 |
| 90° C | 144 |

The reaction mixture was similar to that of Table 1; 0.5 $O.D._{260}$ units of heat-shocked virus and 2.0 μc (2.0 c/mmole) of $^{3}H$-UTP were used. Incubation time at 37° C was for 30 minutes.

TABLE 3

Requirements of the Incorporation Reaction Catalyzed by Heat-Shocked Reovirus

| Conditions | cpm incorporated |
|---|---|
| Complete reaction mixture | 35645 |
| -ATP | 432 |
| -GTP | 579 |
| -CTP | 364 |
| $-Mg^{++}$ | 253 |
| -virus | 137 |
| + mercaptoethanol (3 μmoles) | 18672 |
| one-half virus concentration | 18123 |

Samples of purified virus (0.4 $O.D._{260}$ units) were heated for 30 seconds at 70° C, chilled and assayed for polymerase activity. The complete reaction mixture was as in Table 1 except that mercaptoethanol was omitted from it. Incubation was for 30 minutes at 37° C with 2.0 μc of $^{3}H$-UTP.

Association of Polymerase Activity with the Virion. Reovirus preparations used routinely were purified by isopycnic centrifugation on CsCl gradients (7), and the polymerase could have banded fortuitously with the virions in the gradient. To check this possibility, virus purified through the CsCl gradient stage was analyzed by sedimentation through a sucrose gradient and by column chromatography on DEAE-cellulose. The results (Fig. 1) show that plaque-forming units (PFU), polymerase and O.D.$_{260}$ remained closely associated in both analyses and probably were properties of the virion. The results with reovirus antiserum prepared in rabbits gave further evidence for this point. Prior to heat shock the antiserum had little

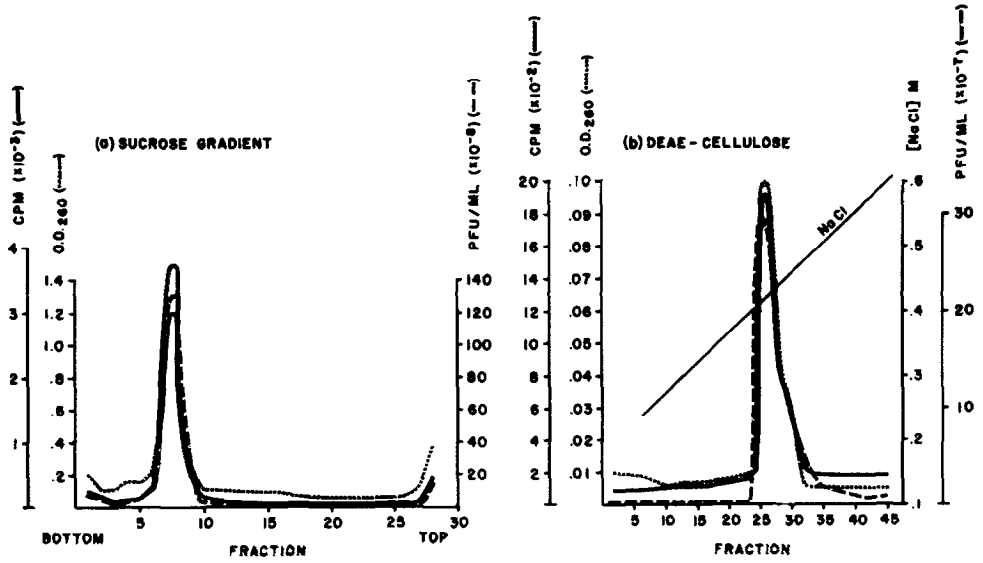


Fig. 1.

a) Analysis of PFU and polymerase activity of purified reovirus on a 10-50% sucrose gradient. Sedimentation was for 75 minutes at 20,000 rpm in SW-25.1 rotor. Aliquots of the gradient fractions were assayed for polymerase activity after heat activation and for PFU (without heat activation).

b) Analysis of PFU and polymerase activity of purified reovirus by ion-exchange chromatography on a DEAE-cellulose column. The fractions were eluted with a linear gradient of NaCl(1), the main peak of virus being at 0.43 M. Aliquots of the gradient fractions were assayed for polymerase activity after heat activation and for PFU (without heat activation).

effect on the polymerase activity of purified virus. After heat shocking the virions, the antiserum reduced the polymerase activity by 90%. This result suggests that the polymerase may be an internal constituent of the virus particle.

Analysis of the Polymerase Product. A polymerase product was synthesized with heat shocked virus and $^{3}$H-UTP under the conditions described in Table 3 and extracted with phenol. The product was found to be completely susceptible to RNase (1 μg/ml at 37° C for 30 minutes) digestion. A portion was analyzed by gel electrophore-

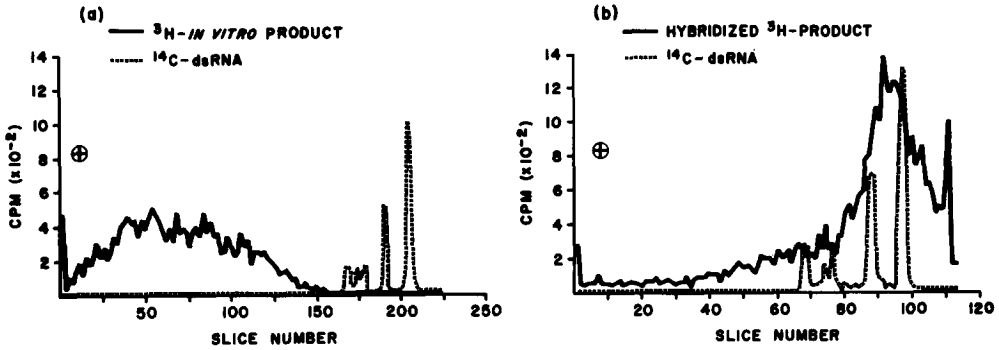


Fig. 2.

Analysis of in vitro polymerase product on acrylamide gel electrophoresis: (a) before hybridization; (b) after hybridization, as described in Methods.

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sis, along with $^{32}$P-labeled viral RNA (Fig. 2a). The remainder was denatured with DMSO, hybridized, and then analyzed in the same way (Fig. 2b). The hybridized polymerase product was insensitive to RNase, showing it was completely hybridized (7). Prior to hybridization the polymerase product migrated ahead of the dsRNA marker. After hybridization the major portion ran with the dsRNA marker. These results show that the polymerase product is virus-specific ssRNA (4, 5, 7).

## DISCUSSION

Purified reovirus particles contain an RNA polymerase which can transcribe ssRNA from the viral genome *in vitro*. In infected cells the mRNA's that are synthesized correspond in size to the ten segments of viral dsRNA (5, 6, 7), but *in vitro* much of the product consists of short fragments, indicating that our conditions do not permit efficient transcription of entire segments of viral dsRNA. A limited number of the dsRNA segments of parental viral RNA are transcribed in infected cells when protein synthesis is prevented (7). The polymerase we have described here is an obvious candidate to perform this early transcription.

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