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RNA Polymerase Activity Associated with Bacteriophage $\phi 6$[1]

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The *Pseudomonas phaseolicola* bacteriophage $\phi 6$ incorporated labeled UTP into an acid-insoluble precipitate. Incorporation was dependent on the presence of manganese acetate, ATP, GTP, CTP, and a short heat treatment of the phage; the reaction was stimulated by NH_4Cl. The substitution of ^{14}C-ATP, -CTP or -GTP for UTP, together with the appropriate unlabeled ribonucleoside triphosphates, disclosed that CMP was incorporated to the greatest extent followed by GMP, UMP, and AMP. Radioactive RNAs formed by the reaction were resistant to RNases A and T_1 in high salt but susceptible to these nucleases in low salt. The labeled RNA co-sedimented and co-electrophoresed with $\phi 6$ double-stranded (ds) RNA. However, the distribution of the radioactivity into the three ds-RNA components varied depending on the ^{14}C-ribonucleoside triphosphate used in the reaction. The incorporation of UMP was primarily into the two smaller ds-RNA segments, GMP primarily into the large ds-RNA segment, and CMP and AMP were about equally distributed into all three ds-RNA segments.

Double-stranded RNA (dsRNA) containing viruses such as wound tumor, reovirus, cytoplasmic polyhedrosis, and a virus-like particle from *Penicillium stoloniferum* have been reported to contain RNA polymerase activity (2, 4, 6, 11-14, 21-23, 26). Recently it was reported that a large-particle fraction obtained from reovirus-infected L cells contained two RNA polymerases; one enzyme was designated transcriptase and synthesized a single-stranded RNA (ssRNA), while the second enzyme was designated replicase and mediated the synthesis of a dsRNA (16-18, 28).

Previously, we reported the isolation of a lipid-containing bacteriophage, $\phi 6$, whose host is *Pseudomonas phaseolicola* (24). The phage genome consists of three components of dsRNA (Semancik, Vidaver, and Van Etten, Abstr. Annu. Meet., Amer. Soc. Microbiol. p. 220, 1972; Semancik, Vidaver, and Van Etten, J. Mol. Biol., in press). The present report describes the characteristics of an RNA polymerase associated with phage $\phi 6$. The radioactive product formed with the enzyme co-sedimented and co-electrophoresed with native $\phi 6$ dsRNA. Portions of these results have been published in abstract (Van Etten, Vidaver, Koski, and Semancik, Abstr. Annu. Meet., Amer. Soc. Microbiol. p. 235, 1973).

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MATERIALS AND METHODS

Materials. Tritiated UTP and the ^{14}C-ribonucleoside triphosphates were obtained from New England Nuclear Corp.; unlabeled ribonucleoside triphosphates and RNase A were obtained from Sigma Chemical Co. Rifampin, α-amanitin, and actinomycin D were purchased from Calbiochem, Henley and Co., and Merck and Co., respectively. Tobacco rattle virus (TRV) RNA (20) and brome mosaic virus (BMV) RNA (7) were prepared in our laboratory.

Phage $\phi 6$ was isolated from lysates 160 min after infection at a phage-to-bacterium ratio of 5:1. The phage was purified after DNase treatment and concentration with polyethylene glycol by equilibrium centrifugation in CsCl as described previously (24). The phage $\phi 6$ band from the CsCl gradient was diluted fourfold with 0.01 M potassium phosphate (pH 7.1), pelleted by centrifugation, resuspended in the phosphate buffer, and stored in portions at −70 C. Antiserum to phage $\phi 6$ was prepared as described previously (25).

RNA polymerase assay. The standard reaction mixture consisted of 70 mM *N,N*-bis (2-hydroxyethyl) glycine (Bicine), pH 8.5; 13 mM manganese acetate; 83 mM NH_4Cl; 0.67 mM each of ATP, CTP, and GTP; 2 μCi of ^{3}H-UTP (22.2 Ci/mmol); and 0.3 A_{260} units of the phage preparation in a total volume of 0.3 ml. This amount of phage contained 47 μg of protein. The reaction mixtures were incubated at 37 C. At various time intervals, 0.05-ml samples were removed, applied to filter paper disks, and quickly

dried, and trichloroacetic acid-insoluble radioactivity was determined as described by Bollum (5). In some experiments, 0.1 μCi of ^{14}C-ATP, ^{14}C-UTP, ^{14}C-GTP, or ^{14}C-CTP, together with the appropriate unlabeled ribonucleoside triphosphates, were substituted for the ^{3}H-UTP. The results are expressed as pico-moles of ribonucleoside monophosphate incorporated per milligram of virus protein per 10 min of incubation time. The values given were corrected for a zero time reaction.

Association of the enzyme activity with phage. The purified phage was recentrifuged to equilibrium (105,000 $\times$ *g* for 16 h) in CsCl gradients (30% [wt/vol]) with a fixed angle rotor (Spinco Ti50, Beckman Instruments Co.) or centrifuged at 70,000 $\times$ *g* for 75 min in 10 to 35% (wt/vol) linear sucrose gradient columns in a Spinco SW27 rotor. Fractions were collected and assayed for infectivity (24), for enzyme activity, and for A_{260}. Prior to the assay for enzyme activity, the samples were dialyzed overnight against 0.01 M potassium phosphate, pH 7.1.

Analyses of the polymerase product. Radioactive RNA, synthesized in the standard reaction, was isolated by the single phase phenol procedure of Diener and Schneider (10). The RNA was analyzed on linear log sucrose density gradient columns (8) with the sucrose dissolved in 0.3 M NaCl and 0.03 M sodium citrate, pH 7.0 (2 $\times$ SSC), or by polyacrylamide gel electrophoresis. In some instances the radioactive RNA was incubated with RNase A (10 μg/ml) at 37 C for 30 min in 0.01 $\times$ or 2 $\times$ SSC prior to analysis. After centrifugation of the sucrose density gradients on a Spinco SW41 rotor for 9 h at 190,000 $\times$ *g* at 14 C, the gradient columns were scanned photometrically with an ISCO model 222 Density Gradient Fractionator (Instrumentation Specialties Co.), and 0.33-ml fractions were collected, diluted with 2 ml of water, and counted in 15 ml of counting solution. The counting solution was composed of 536 ml of toluene; 464 ml of Triton X-100, 4.44 g of 2,5-diphenyloxazole, and 81 mg of 1,4-bis-2-(4-methyl-5-phenyloxazolyl)-benzene.

Gel electrophoresis was performed in 2.4% polyacrylamide with 0.5% agarose in 0.04 M Tris; 0.033 M sodium acetate; 0.001 M sodium EDTA, and 0.2% sodium lauryl sulfate, (pH 7.2) (3). After subjecting the 6-mm diameter gels to pre-electrophoresis for 3 h at 5 mA per gel, 0.05-ml samples in 10% sucrose were applied and subjected to electrophoresis at room temperature for 10 h at 5 mA per gel. The gels were scanned at A_{260} with a Gilford model 2410 linear transport system (Gilford Instruments), which was coupled to a Beckman DU spectrophotometer. After scanning, the gels were frozen with solid CO_2 and sliced into 1-mm sections. The radioactive material was eluted from the gel slices (27), and the samples were counted in 10 ml of counting solution. The counting solution was composed of 1 liter of toluene, 4 g of 2,5-diphenyloxazole, and 50 mg of 1,4-bis-2-(4 methyl-5-phenyloxazolyl)-benzene.

Other determinations. Possible interconversion of ribonucleoside triphosphates by phage $\phi 6$ was tested by hydrolyzing the ^{14}C-RNA synthesized from each of the ^{14}C-ribonucleoside triphosphates in 1 M KOH for 18 h at 37 C. The hydrolysates were subjected to high-voltage electrophoresis on Whatman 3 MM paper for 10 h at 1000 V in 0.2 M sodium acetate buffer (pH 3.8) (9). The paper was dried, and the ribonucleoside monophosphates were observed with a UV lamp. The paper was then cut into small sections, and the radioactivity was determined.

The presence of RNase activity in the phage was determined by incubating the phage with fungal ^{14}C-ribosomal RNA at 37 C, and the acid-precipitable radioactivity was measured over a period of 60 min (5). The amount of protein present in the phage preparations was determined by the method of Lowry et al. (15) with bovine albumin as a standard.

RESULTS

Properties of phage $\phi 6$ RNA polymerase. Preliminary experiments indicated that the RNA polymerase activity associated with the phage $\phi 6$ preparations was low and inconsistent. Because the lipid envelope of the virion might reduce substrate permeability, the phage was subjected to short treatments with organic solvents and detergents (chloroform, ethyl ether, freon, Triton X-100, sodium lauryl sulfate, sodium deoxycholate, and Igepon T-73) in order to disrupt the lipid envelope and possibly stimulate enzyme activity. However, none of these treatments consistently enhanced enzyme activity although in some individual experiments the treatments were stimulatory. Since RNA polymerase activity was markedly stimulated in reovirus by short heat treatments (6), similar treatments were applied to phage $\phi 6$. A short heat treatment followed by quick chilling in an ice bath, markedly enhanced activity (Table 1). The highest enzyme activity was obtained with a treatment at 60 C for 10 s. However, the optimum temperature and length of the heat treatment varied slightly with different phage $\phi 6$ preparations; thus the optimum heat treatment was always established for each

TABLE 1. *Effect of short heat treatments on RNA polymerase activity in phage $\phi 6$*

Temperature	Length of treatment (s)	^{3}H-UMP incorporated[a]
22	—	6.2
50	10	15.5
50	20	59.4
50	30	83.9
60	5	29.3
60	10	176.4
60	20	60.8
70	5	144.8
70	10	30.6
80	5	4.4
80	10	4.0

[a] Picomoles incorporated per milligram of phage protein per 10 min.

preparation. All subsequent experiments were conducted with heat-treated phage.

The general characteristics of the phage $\phi 6$ RNA polymerase reaction are reported in Table 2. The reaction was dependent on the presence of ribonucleoside triphosphates, manganese acetate, and was stimulated by NH_4Cl. The reaction was insensitive to chloramphenicol and the antibiotics rifampicin, actinomycin D, and α-amanitin, which inhibit DNA-directed RNA synthesis. The addition of 2-mercaptoethanol or dithiothreitol had no effect on enzyme activity. A number of buffers at several pH values were tested; the highest activity was obtained with Bicine (pH 8.5). The effect of manganese and magnesium ion concentrations on the reaction are shown in Fig. 1A. In all experiments, manganese acetate was more effective than magnesium acetate. A concentration of 75 to 100 mM NH_4Cl stimulated enzyme activity about twofold (Fig. 1B); NH_4Cl could not be replaced with NaCl or KCl.

The reaction was dependent on phage $\phi 6$ concentration (Fig. 2). The time course of the reaction is demonstrated in Fig. 3A; the incorporation of ^{3}H-UMP was linear for about 10 min after which incorporation reached a plateau. The limiting factor was the concentration of phage since the addition of more phage at 20 min resulted in additional ^{3}H-UMP incorporation (Fig. 3B). The addition of phage $\phi 6$ dsRNA or BMV RNA (ssRNA) had no effect on ^{3}H-UMP incorporation. The addition of RNase to the standard assay mixture at the start of the reaction resulted in about a 75% decrease in the ^{3}H-UMP incorporated; however, the addition of RNase at the conclusion of the assay had no effect on the amount of radioactivity previously incorporated (Table 3). Separate experiments established that phage $\phi 6$ contained negligible RNase activity. The incorporation of ^{3}H-UMP was severely inhibited by pyrophosphate, whereas orthophosphate had no effect on the reaction (Table 3). Incorporation of ^{3}H-UMP into RNA did not occur if the ribonucleoside triphosphates were replaced with ribonucleo-

TABLE 2. *Characteristics of ^{3}H-UMP incorporation by phage $\phi 6$*

Additions or omissions	^{3}H-UMP incorporated[a]	% of the control
Complete system	592	100
-ATP	65	11
-GTP	30	5
-CTP	347	59
-ATP, GTP, CTP	30	5
-Phage $\phi 6$	0	0
-Heat treatment of phage $\phi 6$	21	3.5
-Manganese acetate	13	2.2
-NH_4Cl	249	42
+α-amanitin (10 μg)	623	105
+Actinomycin D (10 μg)	548	93
+Rifamycin SV (10 μg)	564	95
+Rifampicin (10 μg)	564	95
+Chloramphenicol (10 μg)	524	88

[a] Picomoles incorporated per milligram of phage protein per 10 min.

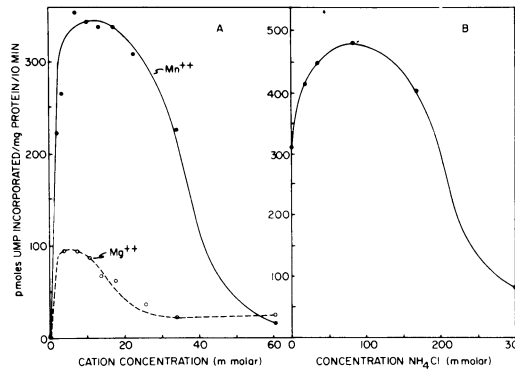

FIG. 1. *The effect of manganese acetate and magnesium acetate (A) and NH_4Cl concentrations (B) on ^{3}H-UMP incorporation by phage $\phi 6$.*

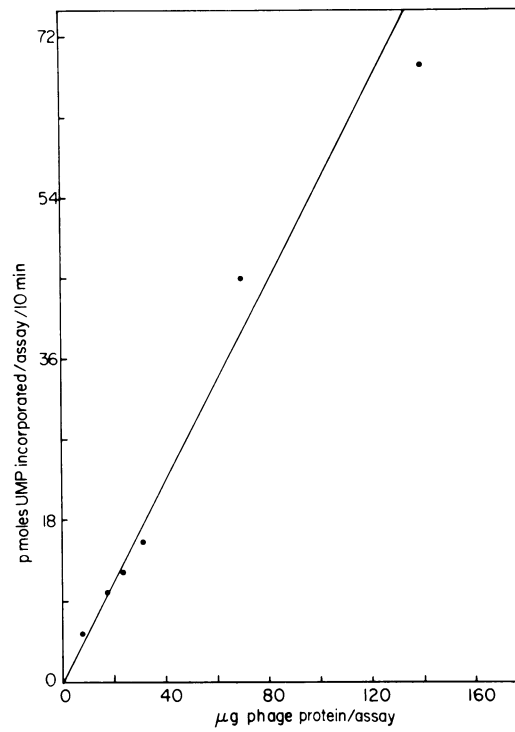

FIG. 2. *Effect of increasing concentrations of phage $\phi 6$ on ^{3}H-UMP incorporation.*

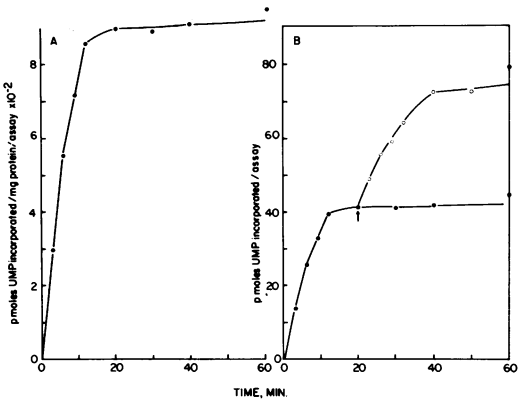

FIG. 3. *Time course of incorporation of ^{3}H-UMP into RNA. A, Standard reaction; B, an additional 0.3 A_{260} of heat treated-phage ϕ6 was added at 20 min* (↑).

TABLE 3. *Effect of RNase and phosphate on ^{3}H-UMP incorporation by phage ϕ6*

Conditions	^{3}H-UMP incorporated[a]
Control	793
5 μg per assay of RNase added at 0 time[b]	186
5 μg per assay of RNase added at 20 min[b, c]	804
+5 mM orthophosphage	844
+25 mM orthophosphage	660
+5 mM pyrophosphate	243
+10 mM pyrophosphate	19
+25 mM pyrophosphate	10

[a] Picomoles incorporated per milligram of phage protein per 10 min.

[b] RNase added directly to the reaction mixture.

[c] The reaction was measured an additional 30 min after the addition of RNase.

side diphosphates. The phage retained some enzyme activity, even after repeated heat treatments and freezings (Table 4).

Association of the enzyme activity with the phage particle. Confirmation of the association of the RNA polymerase with phage ϕ6 presents a special problem since it has a lipid envelope which is essential for infection (24); it is possible that the RNA polymerase activity is a host enzyme which is associated with this envelope. To test this possibility, unheated phage was recentrifuged to equilibrium in CsCl, the gradient was fractionated, and the samples were assayed for infectivity, enzyme activity after heat treatment, and A_{260}. The region of maximum A_{260}, infectivity, and enzyme activity in the gradient were identical (Fig. 4A). However, enzyme activity was also detected in less dense regions of the gradient. This enzyme activity may be due to the partial disruption of the phage envelope by the CsCl and subsequent release of the enzyme, because we have noted previously that CsCl causes a decrease in infectivity of the phage (24). The CsCl-purified phage was also centrifuged in a linear 10 to 35% (wt/vol) sucrose density gradient; the region of maximum A_{260}, infectivity, and enzyme activity were identical (Fig. 4b). Experiments to determine if the radioactivity incorporated in the assay mixture was associated with the phage particle could not be conducted since the short heat treatment used to stimulate enzyme activity caused the virion to dissociate into many fragments which sedimented slowly in a sucrose density gradient (Fig. 5A and B).

The effect of several concentrations of phage ϕ6 antiserum on RNA polymerase activity is reported in Table 5. If antiserum was added prior to heat treatment of the phage, it severely inhibited enzyme activity. If phage were subjected to the heat treatment before addition of antiserum, there was no inhibition of enzyme activity. These results can be explained if the antiserum only reacted with the surface antigens of the phage and if it prevented the dissociation of the phage during the heat treatment. In a separate experiment, the phage was incubated with antiserum, subjected to the heat treatment, incubated under assay conditions, and analyzed on sucrose density gradients. After this series of treatments, the virion did not dissociate (Fig. 5C), whereas it did dissociate after the heat treatment without antiserum (Fig. 5B). In contrast, if the enzyme was present inside the virion, the heat treatment would dissociate the phage and allow the enzyme to function in the presence of the antiserum.

Phage ϕ6 purified by two consecutive centrifugations on sucrose density gradients (24) also

TABLE 4. *Effect of repeated heat and freezing treatments on phage ϕ6 RNA polymerase activity*

Treatment[a]	^{3}H-UMP incorporated[b]
None	7.4
Heat, fast cool	518
Heat, slow cool	458
Heat, freeze	325
Heat, freeze, heat, fast cool	518
Heat, freeze (4 times); last step was a heat treatment	333

[a] Heat treatments were at 60 C for 10 s; fast cooling consisted of puting the tube in ice; freezing was done in liquid N_2.

[b] Picomoles incorporated per milligram of phage protein per 10 min.

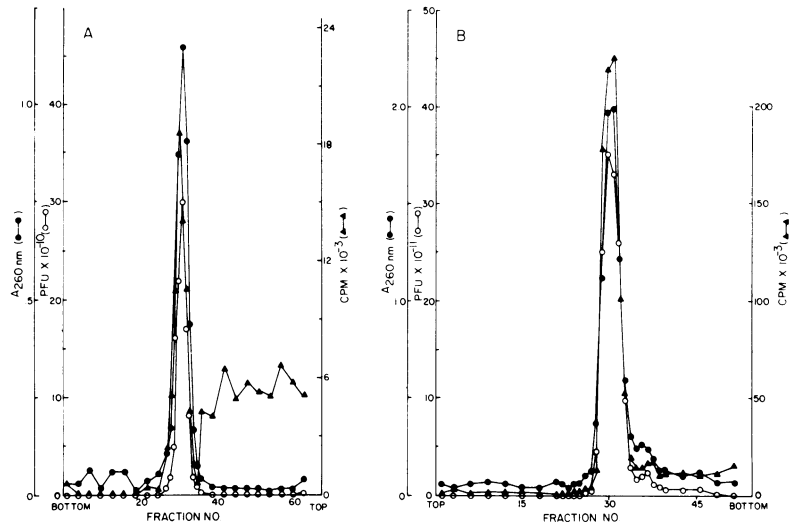

FIG. 4. *A, Equilibrium centrifugation of phage ϕ6 on a CsCl gradient; B, rate sedimation of phage ϕ6 in a 10 to 35% linear sucrose gradient. After fractionation, the gradients were assayed for infectivity* (○), A_{260} (●), *and the incorporation of* ^{3}*H-UMP into RNA* (▲) *as described in Materials and Methods.*

contained replicase activity. Therefore, these observations would suggest that the enzyme is enclosed inside the phage.

Analysis of the RNA polymerase product. To determine if ^{3}H-UMP was incorporated into ss- or dsRNA, the radioactive RNA was isolated and incubated with 10 μg of RNase/ml in 2 × SSC for 30 min at 37 C. The radioactive RNA as well as ϕ6 dsRNA was unaffected, whereas, TRV ssRNA was degraded by this treatment (Fig. 6). If the ^{3}H-RNA was incubated with RNase in 0.1 × SSC, both the A_{260} and the radioactivity were degraded. Therefore, it was concluded that ^{3}H-UMP was incorporated into a dsRNA.

As reported previously the phage ϕ6 genome consists of three distinct dsRNA components with molecular weights of 2.2×10^6, 2.8×10^6, and 4.5×10^6 (Semancik, Vidaver, and Van Etten, Abstr. Annu. Meet. Amer. Soc. Microbiol., p. 220, 1972; Semancik, Vidaver, and Van Etten, J. Mol. Biol. in press). The data in Fig. 6 suggest that most of the ^{3}H-UMP incorporated by the enzyme was associated with the two smaller RNA segments. This observation was verified by co-electrophoresis of the ^{3}H-RNA together with unlabeled ϕ6 dsRNA on polyacrylamide gels (Fig. 7).

To determine if incorporation from each of the four ribonucleoside triphosphates was predominantly into the two smaller RNA components or if this were a property peculiar to UTP, radioactive RNAs were synthesized under the normal conditions except that ^{14}C-ATP, ^{14}C-GTP, ^{14}C-CTP, or ^{14}C-UTP was substituted for ^{3}H-UTP. Each of the four ribonucleoside triphosphates was incorporated into RNA by the phage enzyme (Table 6). However, the degree of incorporation varied depending upon the ribonucleoside triphosphate; CMP incorporation was highest followed by GMP, UMP, and AMP. The incorporation of CMP was 5- to 10-fold higher than AMP incorporation. Maximum incorporation of each ribonucleoside monophosphate was dependent on the simultaneous presence of the other three ribonucleoside triphosphates. In certain instances, however, one of the three ribonucleoside triphosphates could be omitted without completely inhibiting the reaction. For example, the absence of CTP from the reaction mixture reduced AMP incorporation into RNA by only 25% or UMP incorporation by 63%; the absence of ATP reduced GMP incorporation by 37% (Table 6). At present we have no explanation for this observation although a similar phenomenon was reported in RNA polymerase (replicase) studies with the large-particle fraction of reovirus-infected L cells (26).

Electrophoresis of the ^{14}C-RNAs synthesized from each of the ^{14}C-ribonucleoside triphosphates on polyacrylamide gels produced a surprising result. The incorporation of CMP (Fig. 8D) and AMP (Fig. 8B) were distributed among the three RNA segments at about the same ratio as the A_{260} of the three segments. In contrast, incorporation of GMP (Fig. 8C) was predominantly into the largest RNA component, and, as mentioned previously, the incorporation of UMP (Fig. 8A) was predominantly into the two smaller RNA components. When each of the

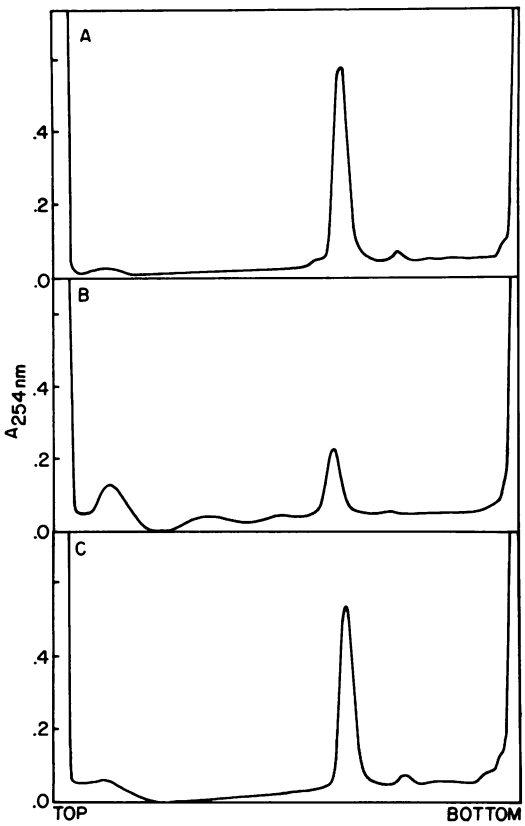

FIG. 5. *Centrifugation of 150 μg of phage φ6 on 10 to 35% (wt/vol) linear sucrose gradients for 75 min at 70,000 × g at 4 C in an SW27 rotor. The phage was heated in the presence and absence of phage φ6 antiserum for 30 s at 50 C and quickly cooled prior to layering on the gradients. A, Untreated φ6; B, heated φ6; C, φ6 added to φ6 antiserum (diluted 1:50) prior to the heat treatment. If the φ6 antiserum was added to φ6 after the heat treatment, the profile was identical to B.*

^{14}C-RNAs were treated with RNase in 0.1 × SSC or 2 × SSC and then analyzed on sucrose density gradients, the results indicated that all four radioactive RNAs were resistant to RNase in high salt but susceptible in low salt. Interconversion of the ribonucleoside triphosphates by the phage did not occur since paper electrophoresis of alkaline hydrolysates of each of the ^{14}C-RNAs resulted in more than 95% of the radioactive counts appearing in the appropriate ^{14}C-ribonucleoside monophosphate precursor regions.

DISCUSSION

This report presents evidence that an enzyme is associated with bacteriophage φ6 which mediates the incorporation of ribonucleoside triphosphates into dsRNA. In many respects the enzyme resembles the RNA replicase associated with the large-particle fraction obtained from reovirus-infected L cells (16–18, 28). These similarities include: (i) the kinetics of the reaction, (ii) the stimulation of the reaction by manganese ion much more than by magnesium ion, (iii) the incorporation of ribonucleoside monophosphate into dsRNA, and (iv) the lack of stimulation of the reaction by the addition of free ss- or dsRNA. As a result of these similarities, we have tentatively assigned the name RNA replicase to the enzyme.

Analogies drawn from evidence available on the replication of reovirus dsRNA (1, 16, 17, 19) would suggest that the enzyme is completing one complementary strand of the dsRNA. The existence of a small single-stranded region (tail) in φ6 dsRNA is supported by two experiments. First, if RNase was added to the assay mixture prior to the start of the reaction, the amount of ribonucleoside monophosphate otherwise incorporated was severely reduced (Table 3). Second, incubation of ^{32}P-dsRNA (prepared from lysates grown in the presence of $H_3{}^{32}PO_4$) with RNase A and RNase T_1, prior to layering on linear log sucrose density gradients, resulted in the release of approximately 2 to 3% of the ^{32}P counts; thus a total φ6 RNA extract contains an RNase-susceptible region in 2 × SSC of 2 to 3%, possibly as single-stranded tails (manuscript in preparation). However, it is not known if the value of 2 to 3% is valid for all φ6 dsRNA molecules or if some molecules are completely double stranded and others have a correspondingly larger single-stranded tail. It is curious that the pattern of radioactivity incorporated into the three dsRNA components is dependent on the ribonucleoside monophosphate incorporated. If the assumption that the enzyme is

TABLE 5. *Effect of phage φ6 antiserum on φ6 RNA polymerase activity*

Treatment	Antiserum dilution	^{3}H-UMP incorporated[a]
Control		746
+Antiserum before the heat treatment	1:50	44
	1:200	36
	1:500	269
	1:1000	243
+Antiserum after the heat treatment	1:50	801
	1:200	689
	1:1000	828

[a] Picomoles incorporated per milligram of phage protein per 10 min.

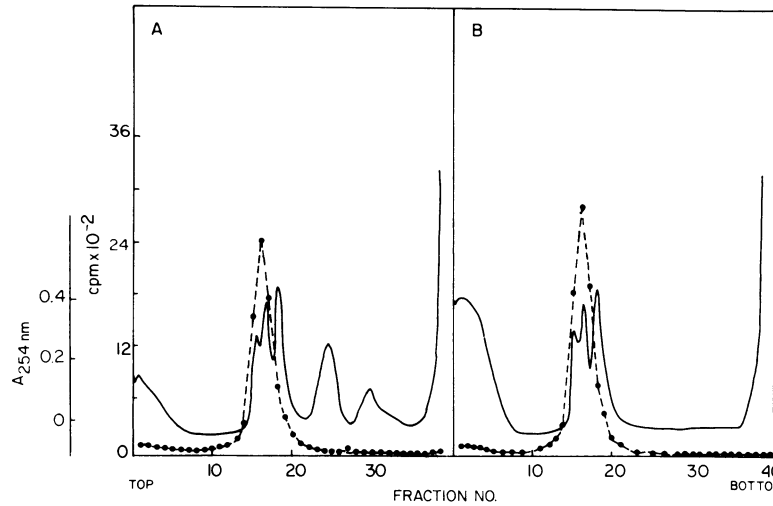

FIG. 6. *Co-sedimentation of ^{3}H-RNA synthesized by phage ϕ6 from ^{3}H-UTP together with ϕ6 dsRNA (components at fractions 12 to 20) and TRV ssRNA (components at fractions 24 and 30) in linear log sucrose density gradients equilibrated with 2 × SSC. A, Untreated; B, sample incubated with 10 μg of RNase per ml in 2 × SSC prior to placing on the gradient. The A_{254} is represented by the solid line and the counts per minute by the dashed line.*

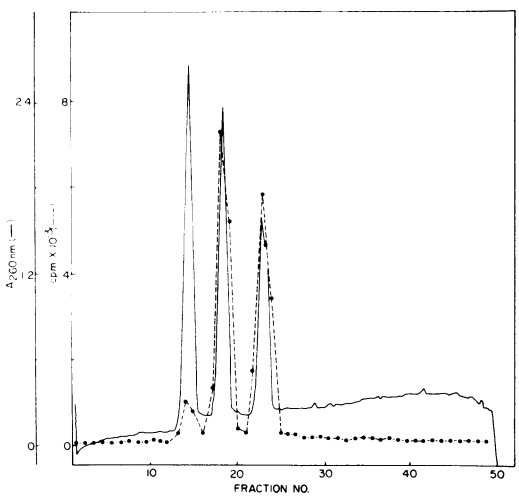

FIG. 7. *Polyacrylamide gel co-electrophoresis of the ^{3}H-RNA synthesized from ^{3}H-UTP by phage ϕ6 RNA polymerase together with ϕ6 dsRNA. Solid line is A_{260}, dashed line is counts per minute. Migration proceeded from left to right.*

TABLE 6. *Incorporation of ^{14}C-ribonucleoside monophosphates into RNA by phage ϕ6 RNA polymerase*

Assay mixture	^{14}C-ribonucleoside monophosphate incorporated[a]			
	UTP	ATP	GTP	CTP
Complete	319	219	503	1,555
-UTP		78	136	411
-ATP	37		316	373
-GTP	51	129		265
-CTP	117	165	88	
-3 ribonucleoside triphosphates	37	34	47	32

[a] Picomoles incorporated per milligram of phage protein per 10 min.

completing a complementary strand is valid and if the direction of synthesis is from 5′ to 3′, these results would suggest that the 5′ end(s) of the three RNA segments have different base sequences.

The biological significance, if any, of the enzyme in the phage particle is not known. The enzyme could either be an integral part of the phage and consequently be required for the next cycle of infection or it might have completed its biological functions by the time the phage is released from the host. With regard to the former possibility the enzyme may have to complete short single-stranded "tail" regions of the dsRNA before successful phage infection can occur. Experiments to determine if replicase activity is essential for phage infection have been inconclusive. For example, we tried to obtain conditions which resulted in a loss in replicase activity without producing a corresponding loss in infectivity. However, conditions such as incubation of the phage at various temperatures for various periods of time always resulted in a more rapid decrease in infectivity than in enzyme activity.

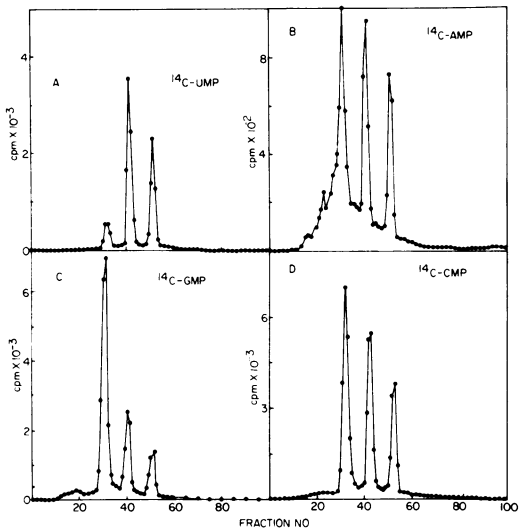

FIG. 8. *Polyacrylamide gel electrophoresis of the ^{14}C-RNA synthesized from ^{14}C-UTP (A), ^{14}C-ATP (B), ^{14}C-GTP (C), and ^{14}C-CTP (D) by phage ϕ6 RNA polymerase. Migration proceeded from left to right.*

In summary, an enzyme which mediates the incorporation of ribonucleoside monophosphates into dsRNA is associated with phage ϕ6. The location of the enzyme in the phage and the biological function of the enzyme are unknown.

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