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DNA-Dependent RNA Polymerase Activity Associated with Subviral Particles of Polyhedral Cytoplasmic Dexoyribovirus

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Polyhedral cytoplasmic deoxyribovirus virions contain a DNA-dependent RNA polymerase which catalyzes the incorporation of ribonucleotides into an acid-precipitable product. Treatment of virions with sodium deoxycholate and dithiothreitol resulted in the formation of subviral particles which could be separated from virions by rate zonal centrifugation in sucrose gradients. Subviral particles were RNA polymerase-positive and more active per unit mass of protein than virions. In vitro enzyme activity associated with subviral particles required addition of ribonucleotides, Mg^{2+}, and exogenous denatured DNA template. Optimal enzyme activity occurred over a broad pH (7.2 to 8.8) and Mg^{2+} concentration (2 to 10 μmol) range. The specific activity of the RNA polymerase was maximal at 37 C. Addition of DNase or actinomycin D to the reaction mixture reduced the incorporation of [^{3}H]UMP into an acid-precipitable product. The product of the reaction was sensitive to degradation by RNase but not to DNase or Pronase. These data suggest that the enzyme copies DNA into RNA.

The frog virus 3 isolate of polyhedral cytoplasmic deoxyribovirus (PCDV) is complex in structure (7). Purified PCDV virions consist of at least 16 different polypeptides (27) including nucleotide phosphohydrolase (29), endodeoxynucleases (12), ribonuclease (12, 20), and protein kinase (9, 23) activities. The nucleotide phosphohydrolase (29) and an acid endodeoxynuclease (12) are associated with subviral particles (SVP) known as cores (2).

Evidence has been reported that PCDV (10), like poxvirus (6), can be non-genetically reactivated. Since nongenetic reactivation is apparently confined to cytoplasmic DNA viruses (6), this group of viruses may have some common component (virion-associated RNA polymerase?) required for replication. The presence of RNA-polymerase activity in poxvirus cores has been demonstrated (13), and Gravell and Cromeans (8) suggested that a virion-associated RNA polymerase supplied by UV-inactivated PCDV may be responsible for nongenetic reactivation of heat-denatured PCDV. Evidence for a PCDV-associated RNA polymerase has not been previously reported.

Data presented in this report favor the interpretation that PCDV contains an enzyme which catalyzes the incorporation of [^{3}H]UTP into an RNA product in vitro. The localization of enzyme activity in SVP suggested that the RNA polymerase probably represents an integral component of the virion rather than simple binding of a contaminant from host cells.

MATERIALS AND METHODS

Chemicals and radioisotopes. The following chemicals and radioisotopes were purchased from the respective companies: calf thymus DNA, bovine serum albumin, dithiothreitol, ATP, GTP, CTP, UTP, and DNase I (pancreatic, RNase free, electrophoretically purified) from Sigma Chemical Co.; RNase (pancreatic, 2× crystallized) from Worthington Biochemicals; actinomycin D, rifampin, α-amanitin, and pronase from Calbiochem; sodium deoxycholate from Fisher Scientific; poly(dA-dT) from P-L Biochemicals, Inc.; Liquifluor, [^{3}H]TdR (20 Ci/mmol), and [^{14}C]TdR (50 mCi/mmol) from New England Nuclear; and [^{3}H]UTP (12.6 Ci/mmol) and sucrose (RNase free) from Schwarz/Mann.

Preparation of purified PCDV virions. A 20× cell-associated virus (CAV) stock was prepared from PCDV-infected fathead minnow cells as previously described (14). The resulting CAV stock was treated with DNase (100 μg/ml of CAV suspension, 37 C, 30 min) and centrifuged (SW27 rotor, 52,000 × *g*, 2 h, 10 C) through 10% (wt/wt) potassium tartrate in 0.01 M Tris buffer (TB) (pH 7.8) (8). The virus pellets were resuspended in 0.05 M sodium phosphate-buffered saline, pH 7.2, supplemented with 0.5% bovine serum albumin to 1/20 the original volume of the 20× CAV

suspension, layered onto linear 10 to 40% (wt/wt) potassium tartrate gradients (32 ml), and centrifuged (SW27 rotor, 52,000 × g, 2 h, 10 C). The virus bands, formed in the middle of the gradients, were recovered from the side of the gradients with a needle and syringe, diluted 50% (vol/vol) with TB, and the virus suspension was centrifuged (SW27 rotor, 52,000 × g, 2 h, 10 C). The pellets containing purified virions were resuspended in phosphate-buffered saline to 1/10 the original volume of the 20× CAV by using ultrasonic vibration (Sonifier Cell Disruptor, Heat Systems, Inc.), and the suspension was dialyzed overnight against TB at 25 C. The purified virion stocks assayed 1.5×10^9 to 5.3×10^9 PFU/ml (1 to 2 mg of protein/ml) and could be stored at −65 C for at least 1 year without appreciable loss of infectivity, as measured by plaque assay (7).

Preparation of SVP. SVP were prepared from purified PCDV virions according to the following procedure. Purified virions (approximately 3.0×10^9 PFU/ml and 1.5 mg of protein/ml) were subjected to ultrasonic vibration to dissociate virion aggregates, treated with 0.5% sodium deoxycholate and 0.03 M dithiothreitol (DTT) (37 C, 15 min), and then rapidly cooled to 0 C. SVP released from the treated virions were centrifuged (Type 40 rotor, 85,000 × g, 2 h, 10 C) through 1.7 ml of 30% (wt/wt) sucrose in TB, and the SVP pellets were resuspended in 1.0 ml of sterile phosphate-buffered saline by ultrasonic vibration.

Following centrifugation, results showed that detectable RNA polymerase activity was associated with the SVP pellet fraction but not with the 30% sucrose-TB supernatant fraction. Subviral suspensions were free of contamination by bacteria or molds detectable by inoculation of blood agar plates. Mycoplasma were not detectable in purified virion stocks when tested by L. Hayflick (Stanford University; personal communication). Subviral preparations could be stored at −65 C for about 4 months without significant loss of RNA polymerase activity.

Prelminary experiments using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (25) showed that six to seven polypeptides were removed following treatment of virions with sodium deoxycholate and DTT to produce SVP (N. S. Gaby and L. S. Kucera, manuscript in preparation).

Labeling of PCDV virions or SVP. To label PCDV virions or SVP with [^{3}H]TdR or [^{14}C]TdR, fathead minnow cell monolayers were infected with PCDV as previously described (14). Five hours after infection, by which time host-cell DNA synthesis was completely blocked by virus infection (7), the medium was supplemented with either 1 μCi of [^{3}H]TdR or 0.1 μCi of [^{14}C]TdR per ml. The cell monolayers were harvested 24 to 36 h after infection, and purified PCDV was prepared as described above.

Rate zonal centrifugation. Purified virions and SVP labeled with ^{3}H or ^{14}C were layered onto 17-ml linear gradients of 20 to 63% (wt/wt) sucrose in 0.01 M TB (pH 7.8) and 0.001 M EDTA. After centrifugation (SW27 rotor, 100,000 × g, 2 h, 10 C), 1.0-ml fractions were collected from the top of the gradient by displacing the gradient from the bottom with 80% (wt/vol) sucrose in TB. Refractive indexes and virus infectivity of various fractions were measured with an Abbe refractometer at 25 C and assayed by plaque count, respectively.

To measure acid-precipitable radioactivity, each fraction received 0.1 ml of calf serum (diluted 1–50 in water) as carrier protein and 5% trichloroacetic acid (final concentration). The acid precipitates were collected and washed on glass fiber filters (Reeve-Angel) with 5% trichloroacetic acid. The radioactivity was measured by liquid scintillation spectrometry using toluene and Liquifluor.

Standard assay for in vitro RNA polymerase activity. The PCDV virion or SVP suspension was rapidly thawed, disrupted by ultrasonic vibration, treated with 0.03 M DTT (15 min, 37 C), and immediately cooled to 0 C. The standard assay mixture contained in a final volume of 0.3 ml: 12 μmol of TB (pH 7.8); 45 nmol each of ATP, GTP, and CTP; 4.5 nmol (10^6 counts/min) of [^{3}H]UTP, unless otherwise stated; 2 μmol of $MgCl_2$; 25 μg of exogenous heat-denatured calf thymus DNA; and 8 to 25 μg of PCDV virion or SVP (equivalent to 10^7 to 4×10^7 PFU) protein. The final pH of the standard assay mixture was 7.8. The reaction mixture was incubated at 28 C for 30 min unless stated otherwise. The enzyme reaction was terminated by the addition of 0.1 ml of ice-cold 0.1 M EDTA. After 5 min at 0 C, 3 ml of 8% trichloroacetic acid (wt/vol) in 0.1 M $NaPP_i$ were added to each reaction mixture. Following an additional 20 min at 0 C, the trichloroacetic acid-insoluble precipitates were collected on glass fiber filters and washed six times with 5-ml volumes of 8% trichloroacetic acid (wt/vol) in 0.1 M $NaPP_i$ and one time with 3 ml of 95% ethanol. The filters were dried, and radioactivity was measured by liquid scintillation spectrometry using toluene and Liquifluor. One unit of enzyme activity represented incorporation of 1 pmol of [^{3}H]UMP (220 counts per min per pmol) into an acid-precipitable product under standard assay conditions. Specific activity was expressed as picomoles per milligram of protein per 30 min.

Preparation of PCDV-DNA. Preparations of PCDV-DNA were obtained from PCDV-infected baby hamster kidney cells as previously described (14).

Protein determination. Protein was measured by the method of Lowry et al. (18) using crystalline bovine serum albumin as a standard.

Heat denaturation of DNA. Preparations of DNA were dissolved in sterile 0.01 M TB (pH 7.8) (1 mg/ml, final concentration), melted at 100 C (15 min), and rapidly cooled to 0 C.

RESULTS

Isolation, characterization, and localization of RNA polymerase activity in SVP. Poxvirus in vitro DNA-dependent RNA polymerase is associated with viral cores (13). To determine the location of in vitro RNA polymerase activity in PCDV virions, we treated [^{14}C]TdR-labeled virus with sodium deoxycholate and DTT to solubilize the outer capsid and release SVP, as outlined in Materials and

Methods. Data showed that when ^3H-labeled virions and ^{14}C-labeled SVP were subjected to rate zonal centrifugation through sucrose density gradients (Fig. 1A) virions banded at a density of 1.185 g/cm^3, whereas SVP banded at a density of 1.230 g/cm^3. RNA polymerase activity, which originally cosedimented with virions, was now found to cosediment with SVP at a density of 1.230 g/cm^3 in sucrose density gradients. Enzyme activity was not found elsewhere in the gradients (Fig. 1B). SVP had about 30% more RNA polymerase activity (specific activity: 1,282 pmol per mg per 30 min) than virions (specific activity: 915 pmol per mg per 30 min). Therefore, subsequent experiments were done with SVP.

In other experiments, we observed that [^3H]TdR-labeled DNA in virions was resistant to degradation by exogenous DNase (100 μg/ml, 37 C, 1 h). After conversion of virions to SVP by sodium deoxycholate and DTT treatment (see Materials and Methods), approximately 50% of the DNA in the SVP was degradable by exogenous DNase (100 μg/ml, 37 C, 1 h) (data not shown). There was no detectable infectivity associated with SVP (data not shown).

Conditions for in vitro RNA polymerase activity in SVP. Under standard assay conditions (see Materials and Methods), RNA polymerase activity increased as a function of protein concentration ranging from 4 to 20 μg of protein (Fig. 2) and, with time, for at least 45 min of incubation at 28 C (Fig. 3).

Addition of the detergent Sarkosyl (Ciba-Geigy Corp., Ardsley, N.Y.) to the reaction mixture reduced the specific activity of the enzyme by about 50%, whereas the addition of NP-40 had no effect on RNA polymerase activity (Table 1). Ammonium sulfate (100 μmol/reaction mixture) resulted in 92% inhibition of enzyme activity (Table 1).

Addition of heat-denatured DNA was an abolute requirement for RNA polymerase activity (Table 1). In other experiments it was found that increased percent hyperchromic shift due to heat denaturation of DNA resulted in enhanced specific activity of the enzyme; stimulation of the polymerase activity could be increased by as much as sevenfold (Table 2). This increased activity may be attributed to the fact that larger numbers of attachment sites on the DNA template are made available to the polymerase.

RNA polymerase activity appears to require all four ribonucleotides; maximal activity occurred in the presence of 45 nmol each of ATP, GTP, and CTP and 4.5 nmol of [^3H]UTP (Table 3). Reducing the concentration of ATP, GTP, and CTP to 4.5 nmol each resulted in 88% loss of specific activity (Table 3). Enzyme activity showed a dependence upon Mg^{2+}; maximal activity occurred over a range of 2 to 5 μmol of

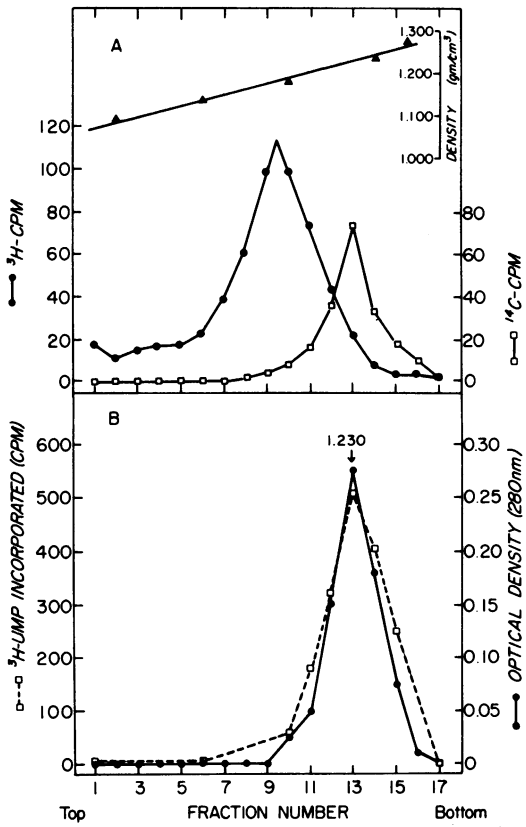

FIG. 1. *(A) Sucrose density gradient centrifugation of [^3H]TdR-labeled PCDV virions and [^{14}C]TdR-labeled SVP. Virions or SVP (0.1 ml) were layered onto 20 to 63% (wt/wt) linear sucrose density gradients in 0.01 M TB, pH 7.8, and 0.001 M EDTA. After centrifugation (SW27 rotor, 100,000 × g, 2 h, 10 C), fractions (1.0 ml) were collected, and refractive indexes were measured. Radioactivity in acid-precipitable material from each fraction was measured by liquid scintillation spectrometry. Data from duplicate sucrose gradients containing either ^3H virions (●) or ^{14}C SVP (□) were superimposed in the same figure. (▲) = Density of sucrose in selected fractions. (B) PCDV-RNA polymerase activity in sucrose density gradients of SVP. Unlabeled SVP (0.2 ml in phosphate-buffered saline and 1.7 mg of protein per ml) were centrifuged in a sucrose density gradient (same conditions as described in [A]). Fractions (1.0 ml) were collected, and OD_{260} (●) and refractive indexes were measured. Each fraction was treated with 0.03 M DTT (15 min, 37 C) and then cooled on ice. RNA polymerase activity (□) in 225-μliter samples was measured by the standard assay procedure (see Materials and Methods). These data are representative of duplicate gradients.*

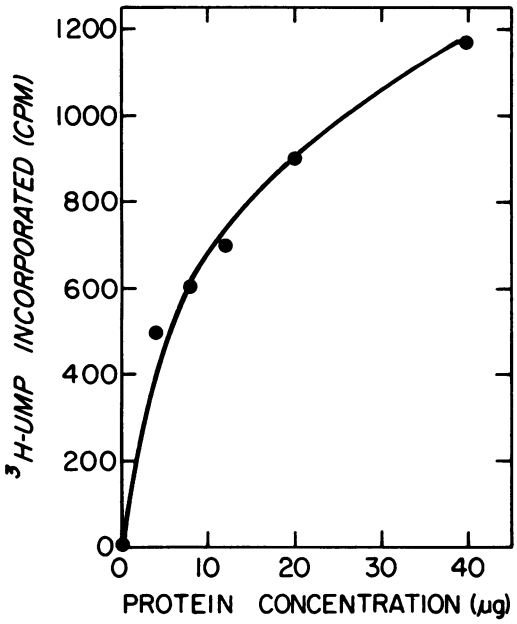

FIG 2. *Dependence of [^{3}H]UMP incorporation into an acid-precipitable product as a function of protein concentration. Standard assay conditions (see Materials and Methods) were employed, except in each reaction mixture the concentration of [^{3}H]UTP was 45 nmol (10^6 counts/min; specific activity, 22 counts per min per pmol). Points (●) are averages of triplicate determinations.*

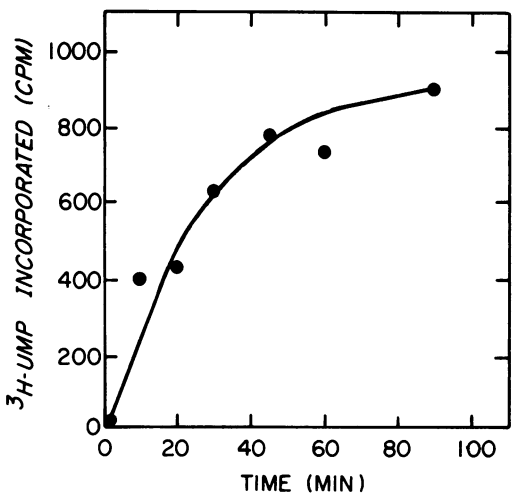

FIG. 3. *Effect of incubation time on [^{3}H]UMP incorporation into an acid-precipitable product. Duplicate assays for measuring PCDV-RNA polymerase activity were carried out by standard procedure (see Materials and Methods), except that the concentration of [^{3}H]UTP was 45 nmol (10^6 counts/min; specific activity, 22 counts per min per pmol). The concentration of subviral protein was 20 μg per enzyme reaction.*

the divalent cation per enzyme reaction mixture (Fig. 4). Some enzyme activity was detected without addition of exogenous Mg^{2+}; this activity may have been due to residual Mg^{2+} in the SVP preparation. Data presented in Fig. 5 indicate that the enzyme had a broad pH optimum from 7.4 to 8.7 in 0.01 M Tris buffers.

Effect of various chemical inhibitors and degradative enzymes on RNA polymerase activity. The association of enzyme activity with purified virions and the localization of the enzyme in SVP are consistent with the idea that a structural component of PCDV is mediating the incorporation of [^{3}H]UMP into an acid-precipitable product. The next experiment was

TABLE 1. *Conditions for in vitro RNA polymerase activity*

Assay mixture	Sp act[a] (pmol per mg per 30 min)
Complete assay mixture[b]	1,133
+ Sarkosyl (0.5%)	566
+ NP_{40} (0.5%)	1,130
+ $(NH_4)_2SO_4$ (100 μmol)	91
− DNA (denatured calf thymus)	0
− DNA (denatured calf thymus) + DNA (native calf thymus)	0

[a] 220 counts per min per pmol.

[b] The assay was carried out by using 12 μg of subviral protein, as described in Materials and Methods. The data represent the average of two experiment done with triplicate points. Symbols: +, added to assay mixture; −, deleted from assay mixture.

TABLE 2. *Effect of percent hyperchromic shift of heat-denatured calf thymus DNA on PCDV-RNA polymerase activity*[a]

DNA template[b]	Ratio (280:260)	% Hyperchromic shift[c]	Sp act[d] (pmol per mg per 30 min)
Calf thymus DNA	0.53	18	224
Calf thymus DNA	0.52	24	361
Calf thymus DNA	0.53	30	1,575

[a] Standard assays (see Materials and Methods) were performed by using 10 μg of subviral protein per enzyme reaction. DNA was denatured as described in Materials and Methods. The data represent the average of three experiments done with triplicate points.

[b] Calf thymus DNA preparations were of different lot numbers.

[c] Hyperchromic shift = [(OD_{260} before heat denaturation)/(OD_{260} after heat denaturation)] × 100. OD_{260}, optical density at 260 nm.

[d] 220 counts per min per pmol.

TABLE 3. *Effect of ribonucleotide concentration on in vitro subviral RNA polymerase activity*

Expt	Concn of ribonucleotides per reaction mixture[a] (nmol)		Sp act of [^{3}H]UTP per reaction (counts per min per pmol)	[^{3}H]UMP incorporated (count/min)	Sp act of polymerase (pmol per mg per 30 min)
	ATP, GTP, CTP	[^{3}H]UTP			
1	45.0	2.25	444.4	3,234	728
	45.0	4.5	222.2	3,696	1,660
	45.0	9.0	111.1	1,709	1,538
	45.0	45.0	22.2	233	1,050
2	45.0	4.5	222.2	3,437	1,547
	9.0	4.5	222.2	1,213	546
	4.5	4.5	222.2	431	194

[a] The enzyme assays were done by standard procedure (see Materials and Methods), except that the concentration of each ribonucleotide and the specific activity of [^{3}H]UTP was varied as shown in the table. Subviral protein concentration in each reaction was 10 μg.

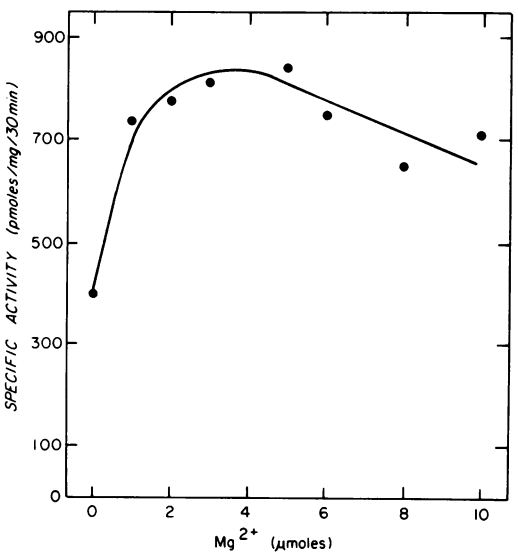

FIG. 4. *Effect of Mg^{2+} concentration upon PCDV-RNA polymerase activity. Duplicate assays were done by standard procedure (see Materials and Methods), except that the concentration of exogenous Mg^{2+} per enzyme reaction mixture was varied as shown in the figure. The concentration of subviral protein was 8 μg per enzyme reaction.*

designed to determine the effect of various chemical inhibitors of DNA-dependent RNA polymerases and degradative enzymes on RNA polymerase activity. Results showed (Table 4) that [^{3}H]UMP incorporation was inhibited in the presence of added actinomycin D (inhibits DNA template activity) or rifampin (inhibits RNA polymerase activity), but not by added α-amanitin (inhibits mammalian cell RNA polymerase II activity). Enzyme activity was significantly inhibited when either DNase or RNase was added to the reaction mixture. These data further support the conclusion that

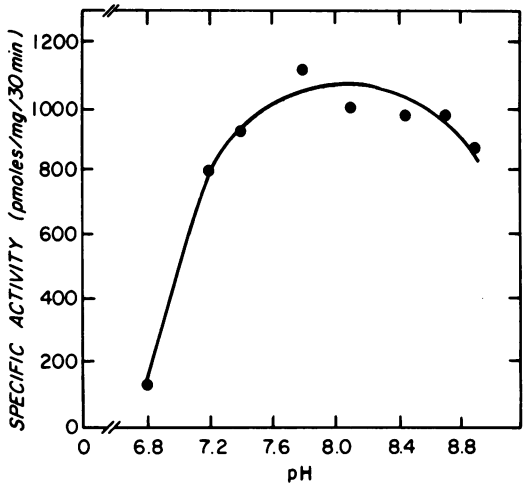

FIG. 5. *Effect of pH on PCDV-RNA polymerase activity. Standard assays (see Materials and Methods) were carried out at the pH values noted in the figure by using 12 μg of subviral protein per enzyme reaction. Tris buffers at a final concentration of 0.01 M were used for each assay. The pH values, determined after addition of all assay components, remained constant during incubation of the enzyme reaction. Points (●) are averages of triplicate determinations.*

TABLE 4. *Effect of chemical inhibitors and degradative enzymes[a] on RNA polymerase activity*

Assay mixture	% Inhibition
Complete assay mixture[b]	(0)
+ Actinomycin D (50 μg)	53.0
+ Rifampin (25 μg)	68.8
+ α-Amanitin (5 μg)	0.8
+ DNase (200 μg)	67.2
+ RNase (200 μg)	74.5

[a] All chemical inhibitors and enzymes were dissolved in 0.01 M Tris buffer (pH 7.8). DNase and RNase solutions contained 0.01 M $MgCl_2$.

[b] The standard assay procedure (see Materials and Methods) was performed by using 10 μg of subviral protein per enzyme reaction. The specific activity of the control reaction was 1,575 pmol per mg per 30 min (220 counts per min per pmol). The data represent the average of three experiments done with triplicate points. Symbol: +, added to the reaction mixture.

the RNA polymerase activity associated with SVP requires a DNA template. Loss of RNA polymerase activity correlates with inhibition of RNA synthesis.

Properties of the RNA polymerase product. To ascertain some properties of the ^{3}H-labeled product synthesized by SVP in vitro, experiments were done to determine the effect of degradative enzymes on the product of the RNA polymerase reaction. Data (Table 5) showed that the product of the reaction was RNA, since it was degradable by exogenous RNase but not by DNase or Pronase.

Effect of incubation temperature on RNA polymerase activity. Initiation, replication, and encapsidation of PCDV-DNA is temperature sensitive during infection of baby hamster kidney cells at 37 C (14). However, small amounts of virus-specific RNA synthesis were detected in infected cells at this temperature (8). It has been hypothesized that parental PCDV-DNA is transcribed by an enzyme (RNA polymerase?) presumably brought into the cell as a component of the infecting virion (8). The next experiments were designed to determine whether PCDV-RNA polymerase is active at temperatures nonpermissive for virus replication. Results showed that optimal enzyme activity was measured at 37 C as compared with 28 C. However, when the enzyme reaction was incubated at 45 or 56 C, the specific activity of the RNA polymerase was markedly reduced as compared with similar reactions incubated at 28 or 37 C (Fig. 6).

TABLE 5. *Effect of various degradative enzymes upon the product of the RNA polymerase reaction*

Treatment	[^{3}H]UMP in acid-precipitable product after treatment (counts/min)[a]
None[b]	2,705
+ DNase (200 μg)[c]	2,280
+ RNase (200 μg)[c]	715
+ Pronase (100 μg)[c]	2,500

[a] 220 counts per min per pmol.

[b] Assays for RNA polymerase activity (SVP, 12 μg of protein per assay) were done in standard reaction mixtures (see Materials and Methods). Following the 30 min of incubation at 28 C, the reactants were boiled for 5 min and cooled on ice, and DNase, RNase, or Pronase was then added to the product formed in each reaction mixture. After additional incubation at 37 C for 30 min, acid-precipitable radioactivity was counted by liquid scintillation spectrometry. The data are representative of three experiments done with triplicate points.

[c] Enzymes were dissolved in 0.01 M Tris buffer (pH 7.8). DNase and RNase solutions contained 0.01 M $MgCl_2$. Symbol: +, added to the product of the enzyme reaction.

Stability of RNA polymerase activity to increased temperatures. To determine the stability of SVP-RNA polymerase activity to increased temperature, the capacity of heated (56 C) PCDV-SVP to incorporate [^{3}H]UMP into an acid-precipitable product at 28 C was measured. Results of these experiments showed that heating PCDV-SVP at 56 C, for as long as 60 min, caused a 30% reduction in subsequent RNA polymerase activity when assayed at 28 C (Fig. 7). In conclusion, PCDV-RNA polymerase is active and stable to temperatures nonpermissive (37 C) for virus replication (7).

Specificity of DNA template. The template specificity of PCDV-RNA polymerase activity was tested with highly purified DNA templates. Results showed that specific activity of the enzyme was significantly increased in the presence of denatured PCDV-DNA as compared with denatured calf thymus DNA (Table 6).

DISCUSSION

Data obtained during this investigation showed that frog PCDV has in vitro DNA-dependent RNA polymerase activity which appears to be associated with a structural component of SVP. The RNA polymerase requires the addition of an exogenous denatured DNA template, ribonucleotides, and Mg^{2+} for maximal

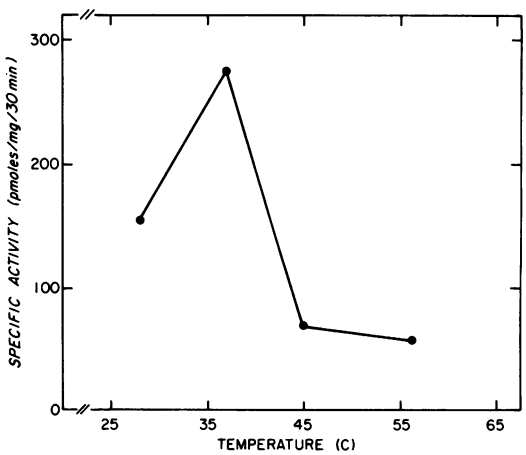

FIG. 6. *Effect of incubation temperature on PCDV-RNA polymerase reaction. Triplicate assays were done by the standard procedure (see Materials and Methods) using 12.5 μg of subviral protein per enzyme reaction, except that the incubation temperature was changed as noted in the figure.*

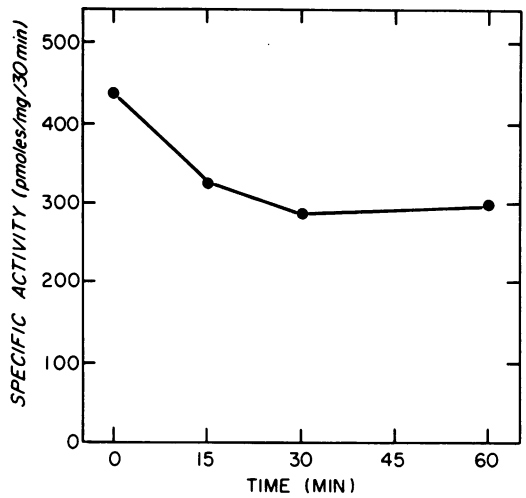

FIG. 7. *Temperature stability of PCDV-RNA polymerase activity. SVP (12.5 μg of protein per 10 μliters) were incubated at 56 C. At the indicated times, samples (10 μliters) were removed and assayed for residual RNA polymerase activity by the standard procedure (see Materials and Methods). Points (●) are averages of triplicate determinations.*

activity (Tabels 1 and 3). The enzyme is active (Fig. 6) at temperatures permissive (28 C) and nonpermissive (37 C) for virus replication (7). The product of the RNA polymerase reaction is degradable by the addition of exogenous RNase but not DNase or pronase (Table 5). The data suggest that the viral enzyme copied DNA into RNA.

Evidence supporting the conclusion that PCDV virions contain RNA polymerase activity comes from other reports showing that protein-denatured PCDV can be non-genetically reactivated by UV-irradiated virus (10). The UV-irradiated virus probably supplies the functional RNA polymerizing enzyme. Additionally, PCDV-specific RNA is synthesized in cells blocked in their capacity for de novo protein synthesis or during incubation of infected cells at temperatures nonpermissive for virus replication (8).

RNA polymerase activity in poxvirus cores can mediate synthesis of virus-specific RNA in vitro in the presence of rifampin (inhibitor of RNA polymerase) and without addition of exogenous DNA (13, 19). The newly synthesized RNA is released into the medium from a site in which the DNA template is totally resistant to the action of DNase. In contrast, RNA polymerase activity in PCDV-SVP is inhibited by rifampin (Table 4); it requires the addition of an exogenous denatured DNA template (Table 1); viral DNA in SVP is partially degradable after the addition of DNase. Previous work showed that rifampin did not selectively inhibit PCDV-induced RNA synthesis during infection of baby hamster kidney cells (15). Since in vitro RNA polymerase activity in SVP is inhibited in the presence of rifampin, this discrepancy may be due to solubilization of viral capsids and increased accessiblity of the enzyme to the drug in vitro as compared with infected cells. Solubilization of viral capsids may also explain why viral genomes in SVP are sensitive to added DNase. The requirement for an exogenous DNA template is not completely understood. It is possible that SVP genomes are not capable of acting as template for DNA transcription into RNA because extensive uncoating of the virion may be necessary to allow further changes in the configuration of SVP-DNA before it can be transcribed.

Mammalian cells contain at least two transcriptases (RNA polymerases). RNA polymerase I is probably localized in the nucleolus, is active in vitro over a wide Mg^{2+} concentration range, exhibits optimal activity in the presence of less than 0.04 M $(NH_4)_2SO_4$, and is not affected by the inhibitor α-amanitin (22). RNA polymerase II is found in the nucleoplasm of mammalian cells, and its activity is inhibited by α-amanitin (17). The RNA polymerase activity associated with SVP of PCDV was demonstrated over a wide Mg^{2+} concentration (Fig. 4) and was inhibited in the presence of moderate concentrations of $(NH_4)_2SO_4$ (Table 1) but not in the presence of α-amanitin (Table 4). Furthermore, other investigators reported that a structural component(s) of purified PCDV (1) is

TABLE 6. *Specificity of DNA templates on PCDV-RNA polymerase activity*[a]

DNA template	Ratio (280:260)	% Hyperchromic shift[b]	Sp act[c] (pmol per mg per 30 min)
Calf thymus DNA	0.53	30	1,575
PCDV-DNA[d]	0.53	30	4,200

[a] Standard assay procedure (see Materials and Methods) was performed by using 10 μg of subviral protein per enzyme reaction. DNA was denatured as described in Materials and Methods. The data represent an average of several experiments.

[b] % Hyperchromic shift = [(OD_{260} before heat denaturation)/(OD_{260} after heat denaturation)] × 100. OD_{260}, optical density at 260 nm.

[c] 220 counts per min per pmol.

[d] Preparation of PCDV-DNA was described in Materials and Methods.

a potent inhibitor of host-cell RNA polymerase II (5). These data suggest that PCDV-RNA polymerase has properties in common with RNA polymerase I of mammalian cells. The use of PCDV mutants should be used to establish if the RNA polymerase is virus specific.

The significance of PCDV-RNA polymerase during virus infection is speculative. The enzyme does not appear to play an important role in virus uncoating, since parental genomes are uncoated to a DNase-sensitive state by constitutive cellular enzymes (14). However, PCDV-RNA polymerase may function in transcribing "early" viral messages needed for translation into proteins required for viral DNA replication.

Many lytic and tumorigenic viruses contain nucleic acid-polymerizing enzymes in their virions (3, 4, 11, 16, 24, 26, 28). Little information is available concerning regulatory mechanisms of virion-associated enzyme activities during virus replication and tumorigenesis. The temperature sensitivity of PCDV replication (14) and the wide host range of this virus (7) may provide a useful model system for investigating regulation and control of transcription with a virion-associated RNA polymerase.

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