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Purification, Characterization, and Comparison of the DNA Polymerases from Two Primate RNA Tumor Viruses

JOHN W. ABRELL AND ROBERT C. GALLO

Laboratory of Tumor Cell Biology, National Cancer Institute, Bethesda, Maryland 20014

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The DNA polymerase from the Mason-Pfizer monkey virus (M-PMV), an RNA tumor virus not typical type-C or type-B, has been purified a thousand-fold over the original crude viral suspension. This purified enzyme is compared to a similarly purified DNA polymerase from the primate woolly monkey virus, a type-C virus. The two enzymes have similar template specificities but differ in their requirements for optimum activity. Both DNA polymerases have a pH optimum of 7.3 in Tris buffer. M-PMV enzyme has maximum activity with 5 mM Mg^{2+} and 40 mM potassium chloride, whereas the woolly monkey virus optima are 100 mM potassium chloride with 0.8 mM Mn^{2+}. The apparent molecular weight of the M-PMV enzyme is approximately 110,000, whereas the woolly monkey virus polymerase is approximately 70,000. The biochemical properties of these two enzymes were also compared to a similarly purified enzyme from a type-C virus from a lower mammal (Rauscher murine leukemia virus). The results show that more similarity exists between the DNA polymerases from viruses of the same type (type-C), than between the polymerases from viruses of different types but from closely related species.

Since the original description of an RNA-directed DNA polymerase in RNA tumor viruses (1, 21), most RNA tumor viruses examined, both type-B and type-C, were found to contain this enzyme (20). Avian myeloblastosis virus (AMV) was the source for the first rigorous purification of this enzyme (8). Some recently reported immunological data indicate that important differences exist among some viral DNA polymerases. Specifically, antiserum to the enzyme from type-C murine virus inhibits the polymerases from other mammalian type-C viruses, and there appears to be more relatedness to polymerases from virus of other lower mammals than to those from primates. Moreover, little or no immunological similarity was found between the polymerases from mammalian type-C viruses and polymerases from avian or from type-B mammalian virus (17). It was, then, of interest to determine some detailed biochemical and biophysical properties of purified DNA polymerases from different oncornaviruses, especially those shown to be immunologically different, and to ascertain which properties were common and which were variable. Moreover, our interest in a viral-like RNA-directed DNA polymerase associated with blast cells from some humans with acute leukemia (14; R. C. Gallo, P. S. Sarin, R. G. Smith, S. N. Bobrow, M. G. Sarngadharan, M. S. Reitz, and J. W. Abrell, Proc. Second Steenbock Symp., in press) and the recent demonstration that this human cellular enzyme is closely related immunologically to RNA-directed DNA polymerase from primate type-C viruses (G. J. Todaro and R. C. Gallo, Proc. IV Lepetit Colloq., in press), especially stimulated our interest in a detailed study of the polymerases from two proven primate viruses grown in primate cells (9, 26).

In this report, we describe the biochemical and biophysical properties of the purified DNA polymerases originally isolated from a monkey breast tumor (4, 12), and from the woolley monkey virus (simian sarcoma virus), a type-C particle isolated from a fibrosarcoma of a woolly monkey (22). This latter virus has been shown to have a nontransforming associated virus, which is present in a 10- to 100-fold excess over the sarcoma virus (27). Similarities and differences for the two DNA polymerases from the primate viral preparation are noted and, in turn, compared to the DNA polymerases from type-C virus of a lower mammal (Rauscher

leukemia virus [RLV]) and to the previously described biochemical properties of the polymerase from AMV.

MATERIALS AND METHODS

Viruses. Viruses were obtained from three sources: Mason-Pfizer monkey virus (M-PMV) was supplied by the J. L. Smith Memorial for Cancer Research, Pfizer, Inc. (Maywood, N.J.) from propagation in a suspension culture of cell line NC-37 (9); RLV (grown on JLS-V9 cell-line), a mouse (BALB/c) bone marrow, and woolly monkey virus, were obtained from Electro Nucleonics, Inc. (Bethesda, Md.); the latter virus was grown on the marmoset cell line 71-AP-1 (26); AMV was kindly supplied by Joseph Beard of Duke University, Durham, N.C.

Nucleic acids. The template-primer nucleic acids used in the DNA polymerase assays were obtained from different sources. Homopolymer polydeoxyadenylic acid [poly (dA)] and oligomeric thymidylic acid [$(dT)_{12-18}$], and synthetic duplex polymers of poly (dA)·$(dT)_{12-18}$, polyguanylic acid with oligomeric deoxycytidylic acid [poly (G)·$(dC)_{12-18}$] and polycytidylic acid with oligomeric deoxyguanylic acid [poly (C)·$(dG)_{12-18}$] were obtained from Collaborative Research (Waltham, Mass.). Duplex polythymidylic acid-polyadenylic acid [poly (dT)·poly (A)], the homopolymers, poly (A), polythymidylic acid [poly (dT)], and poly (dA), and the alternating copolymer of deoxyadenylic acid and thymidylic acid [poly d(A-T)] were purchased from Miles Laboratories, Inc. (Elkhart, Ind.). P-L Biochemicals also was a source for the duplexes poly (dA)·$(dT)_{12-18}$ and poly (A)·$(dT)_{12-18}$; Sigma Chemical Co. (St. Louis, Mo.) supplied the native calf thymus DNA; and Calbiochem (La Jolla, Calif.) supplied the salmon sperm DNA, which was subsequently converted to the activated form with DNase I by using the procedure of Schlaback et al. (15). AMV 70*S* RNA was prepared as described previously (13).

Proteins. DNase I (bovine pancreas) was purchased from both Calbiochem and Sigma Chemical Co. Bovine pancreatic RNase A was a product of Worthington Biochemical Corp. (Freehold, N.J.). Armour Pharmaceutical Co. (Chicago, Ill.) supplied the bovine serum albumin. Other purified proteins, catalase, ovalbumin, and 7*S* gamma globulin (human immunoglobulin G [IgG]) were obtained from Schwarz-Mann (Orangeburg, N.Y.).

Labeled compounds. Tritiated nucleoside triphosphates were obtained from Schwarz-Mann and New England Nuclear (Boston, Mass.). Specific activities were given with the experimental data.

Template-primer duplex preparation. The commercial synthetic polynucleotide duplex preparations contained equimolar amounts of the two complementary homopolymers or homopolymer/oligomer. Other duplexes were prepared by annealing the two complementary homopolymers or homopolymer/oligomer at 1 mg/ml in 0.01 M Tris (pH 7.5), 0.1 M sodium chloride at 30 C for 1 h. Duplexes containing 4:1 template-primer molar ratios had no advantage over equimolar template-primer duplexes. All data given were obtained using 1:1 molar duplexes.

DNA polymerase assays. Reaction mixtures of 0.05 ml consisted of 50 mM Tris-hydrochloride (pH 7.3), 50 mM potassium chloride, 5 mM magnesium chloride, 5 mM dithiothreitol (DTT), 80 μM each of dATP, dCTP, dGTP, and 5.6 μM ^{3}H-dTTP (4×10^4 counts per min per pmol), unless otherwise stated. The template concentration was 20 μg/ml for poly (dT)·poly (A) and oligo (dT)·poly (A), but 60 μg/ml for poly d(A-T) and activated DNA. The reactions were terminated by the addition of 50 μg of carrier tRNA and 2 ml of 10% trichloroacetic acid containing 0.02 M sodium pyrophosphate. After 10 min at 0 C, the acid-insoluble material was collected by filtration on nitrocellulose filters. After drying, the filters were counted in Liquifluor solution by a liquid scintillation counter. The only variations to the above assay conditions were the replacement of magnesium chloride with 1 mM manganese chloride for DNA polymerase from the two type-C viruses, and increase of potassium chloride to 80 mM for the woolly monkey virus enzyme.

Preparation of M-PMV DNA polymerase: virus preparation. The virus suspension was subjected to centrifugation at $100,000 \times g$ for 1 h to obtain a viral pellet which was then used for the polymerase extraction. No variation of the extraction procedure was necessary to accommodate the differences in virus homogeneity between the pelleted suspension or preparations which were purified further before disruption. The methods employed for further purification of the commercial virus before collection of a $100,000 \times g$ viral pellet were either to centrifuge through a 20% glycerol column onto a 100% glycerol cushion, or to reband the virus on a continuous glycerol gradient of 20 to 80%. After either of these two procedures, the virus was then collected as a $100,000 \times g$ pellet.

DNA polymerase extraction. Extraction of the DNA polymerase activity from 10 mg of frozen virus pellet commenced with the addition of 10 ml of virus disruption buffer (0.05 M Tris [pH 7.9], 0.05 M NaCl, 0.25% Triton X-100, 0.02 M DTT, 0.001 M EDTA, 0.5 M KCl). After stirring for 3 h at 0 C in the buffer, the suspension was subjected to centrifugation at $30,000 \times g$ for 30 min. The supernatant fluid was decanted, and the pellet was resuspended in 5 ml of the virus disruption buffer. The second suspension was subjected to sonic treatment for four 15-s bursts at full power with a micro-tip using a Branson model W185D sonifier. The sonic extract was centrifuged at $30,000 \times g$ for 30 min. The two $30,000 \times g$ supernatant extracts were combined and dialyzed against three portions of buffer A (0.05 M Tris [pH 7.9], 0.001 M EDTA, 0.001 M DDT, 5% glycerol), 1 liter each, for 1 h each.

DEAE-cellulose chromatography. The dialyzed extract was applied to a DEAE-cellulose column (25 cm^3) which was equilibrated with buffer A. This column was then washed with 75 ml of buffer A containing 0.05 M sodium chloride followed by 125 ml of buffer A containing 0.3 M sodium chloride. Fractions (5 ml) were collected by a drop counting fraction collector. Optical density scan at 280 nm was made directly on the column effluent by an LKB Uvicord II.

The fractions were assayed for DNA polymerase

activity to define the elution profile of the enzyme(s). Generally, the enzyme activity eluted with the buffer containing 0.05 M sodium chloride. On occasion, some DNA polymerase activity eluted with the 0.3 M salt, but this was always less than 10% of the total enzyme activity. Elutions with higher salt concentrations, 0.5 M or 0.7 M, yielded no further DNA polymerase activity. The nucleic acid fractions which eluted at the higher salt concentrations contain DNA which could serve as template for the enzyme.

The enzyme activity which eluted with the 0.05 M salt was pooled and dialyzed against two 1-liter portions of buffer B (0.05 M Tris [pH 7.9], 0.05 M NaCl, 0.001 M EDTA, 0.001 M DDT, 20% glycerol). Any DNA polymerase which eluted with the 0.3 M salt was treated in a similar fashion.

Phosphocellulose chromatography and enzyme concentration. The dialyzed DEAE-cellulose enzyme was applied to a 10-ml Whatman P-11 phosphocellulose column (1.4 by 7 cm) which had been equilibrated with buffer B. The column was developed with a 200-ml linear sodium chloride gradient from 0.1 to 0.5 M in buffer B. Gradients continued to higher sodium chloride concentrations produced no additional DNA polymerases. The fractions constituting each peak of enzymatic activity were pooled and dialyzed against buffer C (30% polyethylene glycol, 0.05 M Tris [pH 7.9], 0.05 M NaCl, 0.001 M DDT, 0.001 M EDTA, 20% glycerol). This latter procedure concentrated the enzymatic activity and adjusted the sodium chloride concentrations to 0.05 M. Unless otherwise stated, this concentrated enzyme pool was used in the experiments cited for characterization.

Preparation of other DNA polymerases. The RLV and woolly monkey virus were extracted by the same procedure given for M-PMV. The DNA polymerase activities of these two type-C viruses behaved on both DEAE-cellulose and phosphocellulose the same as the DNA polymerase activity from M-PMV.

Estimations of molecular weights of the DNA polymerases: sucrose gradient. Density gradient centrifugation was done by the procedure of Martin and Ames (11) on preformed 5 to 20% (wt/vol) sucrose gradients in 0.05 M Tris (pH 7.9), 0.35 M NaCl, 0.001 M DTT, and 0.001 M EDTA. One milliliter of the concentrated enzyme pool was further concentrated to approximately 200 μliters by dialysis versus buffer C without glycerol and then layered on a 3-ml gradient and centrifuged at 150,000 × *g* for 16 h at 4 C. Fractions of 15 drops were collected from the bottom and then assayed to determine the DNA polymerase activity profile. The positions of the protein markers on parallel gradients were detected by their A_{280}. The markers used were catalase, bovine serum albumin, ovalbumin, and 7*S* gamma globulin (human IgG).

Disk gel electrophoresis. Polyacrylamide gel electrophoreses were done in the presence of sodium dodecyl sulfate (SDS) by the method of Weber and Osborn (24). The primate viral DNA polymerases were subjected to this procedure. Before applying the enzyme solution in the gel, a further 10-fold concentration of the protein was necessary to obtain a protein level which could be visualized by the Coomassie blue staining procedure (5). The concentration was achieved by dialysis against buffer C. Markers used for the disk gel analysis were bovine serum albumin and ovalbumin.

Protein determinations were done by a micro adaptation of the procedure of Lowry et al. (10).

RESULTS

Purification of M-PMV DNA polymerase. Endogenous ribonuclease A-sensitive DNA synthesis (no added template-primer) is associated with the M-PMV DNA polymerase until the DEAE-cellulose chromatography step. Since much of the viral protein, including a major portion of the DNA polymerase activity, elutes from DEAE-cellulose with the 0.05 M sodium chloride buffer, this step is useful primarily for removal of nucleic acids. The subsequent phosphocellulose column chromatography (Fig. 1) produces the DNA polymerase activity at approximately 0.15 M sodium chloride. The phosphocellulose step achieves the major purification, approximately 100-fold over the DEAE-cellulose step. Table 1 summarizes the progression of the M-PMV DNA polymerase through the

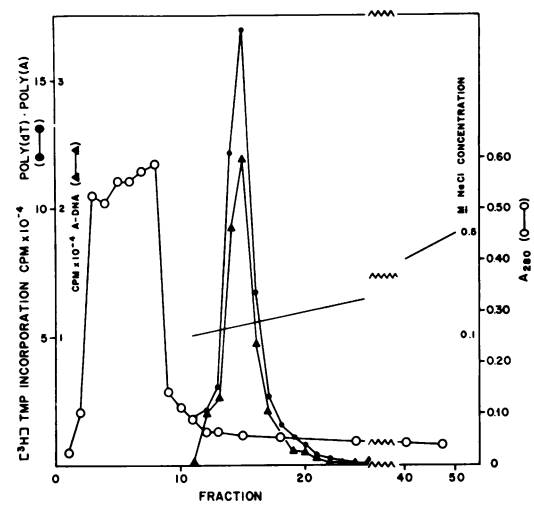

FIG. 1. *Chromatography on phosphocellulose of the M-PMV DNA polymerase. The DEAE-cellulose fractions containing the polymerase activity were pooled and dialyzed before adsorption on a column (Whatman P-11) (1.4 by 7 cm) equilibrated in buffer B. After a 5-ml wash with the same buffer containing 0.1 M NaCl, the 200-ml linear gradient of NaCl between 0.1 M and 0.5 M was attached. Fractions of approximately 4 ml were collected and assayed as described in Materials and Methods. The fractions were assayed with poly (A)·poly (dT) (●) template illustrated on the 0 to 15 × 10⁴ counts/min scale, and activated DNA (A-DNA) (▲) template given on the 0 to 3 × 10⁴ counts/min scale. The NaCl gradient is represented by a solid line from 0.1 M to 0.5 M. ○ represents the optical density of the fractions at 280 nm.*

TABLE 1. *Purification of DNA polymerase from M-PMV*

Source of protein	Protein (mg)	Total activity[a]		Specific actiity[a]	
		Activated DNA[b]	Poly (dT) · poly (A)	Activated DNA[b]	Poly (dT) · poly (A)
Virus	10	1.6×10^3	5.5×10^3	0.16	0.55
30,000 × *g* supernatant fluid	4	3.3×10^3	14.6×10^3	0.82	3.8
30,000 × *g* pellet	6	0.3	1		
DEAE pool	1	3.9×10^3	16.6×10^3	3.9	16
Phosphocellulose pool	<0.01	4.0×10^3	8.2×10^3	>400	>820

[a] Total activity is the total picomoles of labeled nucleotide incorporated into acid-precipitable polymer in 30 min. Specific activity is the picomoles of labeled nucleotide incorporated into acid-precipitable polymer in 30 min per microgram of protein.

[b] Salmon sperm DNA has been activated by a limited DNase I digestion (15).

entire purification. The DNA polymerases from RLV and woolly monkey virus are purified by the same procedure.

Estimations of molecular weights of the viral polymerases. The apparent molecular weight of the M-PMV DNA polymerase, assuming a globular shape, is approximately 110,000, whereas the woolly monkey virus and RLV enzymes are approximately 70,000. Figure 2 shows the sucrose gradient analyses for the three viral DNA polymerases. Losses of 60 to 80% of DNA polymerase are sustained during the sucrose gradient sizing procedure. The apparent molecular weights are given in Table 2. By this procedure, the M-PMV DNA polymerase has an apparent molecular weight of 110,000, which is heavier than the value previously reported (23). The two DNA polymerases from the type-C viruses, RLV and woolly monkey virus, have an apparent molecular weight of 70,000.

Disk gel electrophoreses were performed to determine the purity of the M-PMV and woolly monkey virus enzyme preparations and to further verify the molecular size estimates made by the previous method. The results of SDS disk gel electrophoresis of the preparations from various steps in the purification of the M-PMV and woolly monkey virus DNA polymerases are shown in Fig. 3. The apparent molecular weight of the major protein in the phosphocellulose fraction of the M-PMV enzyme purification is approximately 110,000, as determined by comparison of the R_f to that of the markers. The major protein from phosphocellulose with the woolly monkey virus is approximately 70,000 daltons. Although it is not possible to conclude that these bands are DNA polymerases, the value is similar to those determined by sucrose gradient centrifugation.

Divalent cation requirements. Table 3 summarizes the preference of the two primate viral DNA polymerases for Mg^{2+} or Mn^{2+} and compares their response to that of RLV DNA

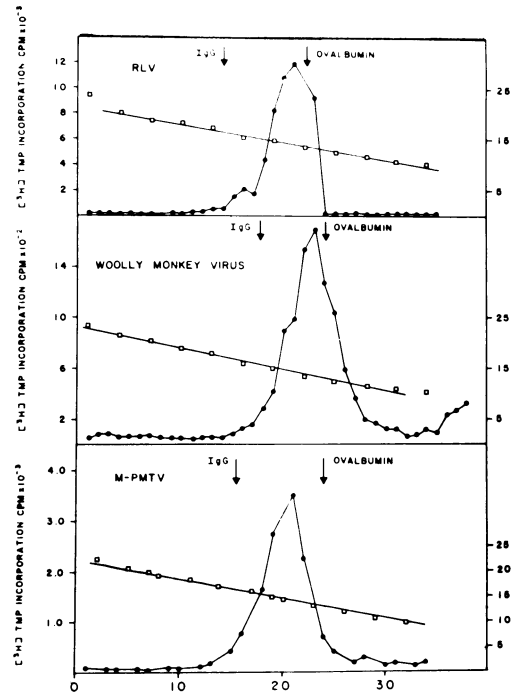

FIG. 2. *Velocity sedimentation of the three viral DNA polymerases on a sucrose gradient. A 1-ml amount of each concentrated enzyme pool was further concentrated to 0.2 ml by dialysis against buffer C without glycerol in order to detect DNA polymerase activity on the gradients. This concentrate was layered on continuous sucrose gradient (5–20%) in 0.5 M Tris (pH 7.9), 0.001 M EDTA, 0.001 M DTT, and 0.35 M NaCl, and centrifuged for 16 h at 40,000 rpm in an SW50.1 rotor at 5 C. Ovalbumin and IgG were simultaneously sedimented as external standards. About 34 equal fractions were collected from the bottom of the tube for each gradient. The marker proteins were detected by the UV absorbance at 280 nm. The DNA polymerase activity (●) from each gradient was detected by the standard assay except no potassium chloride was added due to the high salt concentration of the buffer. The sucrose concentration was measured optically (□). Source of each DNA polymerase is given with gradient profile.*

TABLE 2. *Molecular weight determinations by sucrose gradient*

DNA polymerase	Mol wt[a]	Reported values for mol wt
Mason-Pfizer monkey virus	110,000	
Rauscher leukemia virus	70,000	
Woolly monkey virus	70,000	
Avian myeloblastosis virus	Not determined	170,000[b] 160,000 (7) 110,000 (8)

[a] Molecular weight estimations were made by the technique described in Materials and Methods. The DNA polymerase activity was detected in the fractionated gradient by the standard assay using poly (dT)·poly (A) as the template-primer.

[b] Avian myeloblastosis virus DNA polymerase data are unpublished data from M. R. Robert-Guroff in our laboratory.

polymerase. The two DNA polymerases isolated from the type-C particles (RLV and woolly monkey virus) use Mn^{2+} with greater efficiency than Mg^{2+}. In fact, the enzyme from the woolly monkey virus is partially inhibited with 5 mM Mg^{2+}. On the other hand, the RLV DNA polymerase appears to be unaffected by the presence of Mg^{2+}. The M-PMV enzyme displays a strong preference for Mg^{2+} as the divalent cation and appears to be potently inhibited by Mn^{2+} at a concentration of 1 mM. The dependence of the M-PMV and woolly monkey virus DNA polymerases on the concentration of divalent cations is illustrated in Fig. 4. The M-PMV enzyme shows maximum activity between 4 to 8 mM Mg^{2+} (Fig. 4A), whereas the woolly monkey DNA polymerase prefers Mn^{2+} within the narrow range of 0.8 to 1.5 mM (Fig. 4B). The concentrations used in the standard assay are 5 mM Mg^{2+} and 1 mM Mn^{2+} for the M-PMV and woolly monkey virus enzyme, respectively.

Template specificities. The three viral DNA polymerases have template specificities as previously described (2, 6, 13) for other RNA tumor virus DNA polymerases. The response of the purified enzymes to the various natural DNA and synthetic templates is shown in Table 4. As noted elsewhere, the viral DNA polymerases have a strong preference for some synthetic RNA-DNA hybrids and relatively less response to activated DNA. Of the RNA-DNA duplexes, poly (dT)·poly (A) was the template best transcribed by both primate viral DNA polymerases. These two DNA polymerases also utilize $(dT)_{12-18}$·poly (A) and $(dG)_{12-18}$·poly (C) with excellent efficiency. The synthetic DNA duplexes and native DNA are relatively poor template-primers for the viral DNA polymerases.

The ratio of reaction with the template-primers poly (A)·$(dT)_{12-18}$ over poly (dA)·$(dT)_{12-18}$ is 60-100 to 1 with the three viral DNA polymerases in the presence of the divalent cation of choice. However, that ratio is reduced to 10-20 to 1 when the divalent cation is changed to Mn^{2+} in the case of M-PMV enzyme or Mg^{2+} for the enzymes from the two C-type particles. Similarly, the ratio of poly (A)·$(dT)_{12-18}$ to activated DNA changes from 30-55 to 1 with the divalent cation of choice to 1-3 to 1 with the other divalent cation.

Association constants for the M-PMV DNA polymerase with the template-primers, poly (dT)·poly (A), $(dt)_{12-18}$·poly (A), and activated

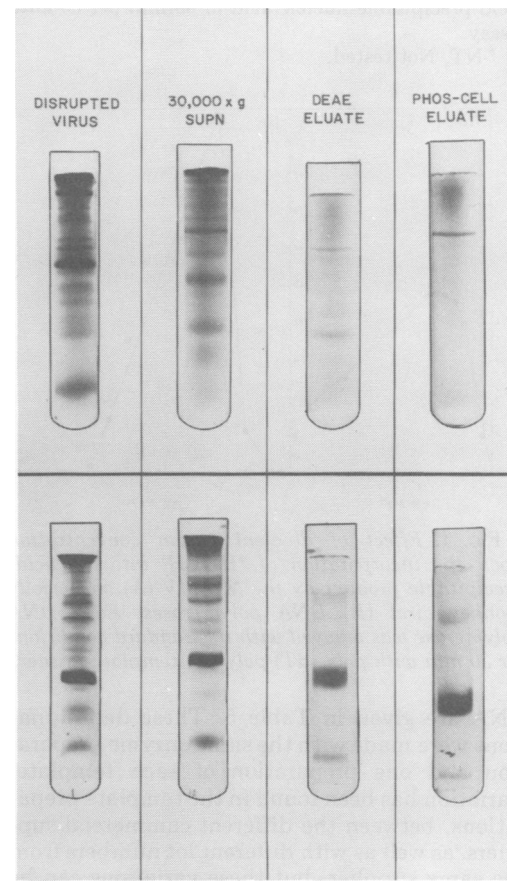

FIG. 3. *SDS disk gel electrophoresis of the viral DNA polymerase preparations from the steps during the purification of the enzymes from M-PMV (top) and woolly monkey virus (bottom). SDS disk gel was run by the procedure of Weber and Osborn (24). The gels were stained with Coomassie blue dye. Marker proteins, ovalbumin, and bovine serum albumin have R_f values of 0.85 and 0.68, respectively.*

TABLE 3. *Divalent cation preference of viral DNA polymerases*

Metal	DNA polymerases[a]		
	Mason-Pfizer monkey virus	Rauscher leukemia virus	Woolly monkey virus
5 mM Mg^{2+}	54	10	2
1 mM Mn^{2+}	5	69	160
5 mM Mg^{2+} + 1 mM Mn^{2+}	5	69	25
5 mM Mg^{2+} + 5 mM Mn^{2+}	2	NT	NT
1 mM Mg^{2+} + 1 mM Mn^{2+}	NT[b]	NT	120

[a] DNA polymerase activity was measured as described in Materials and Methods by using poly (dT)·poly (A) as template. The divalent cation concentrations are as given with each entry. Activity is recorded as picomoles of ^{3}H-TMP incorporated into acid-precipitable nucleic acid in 30 min per 50-μliter assay.

[b] NT, Not tested.

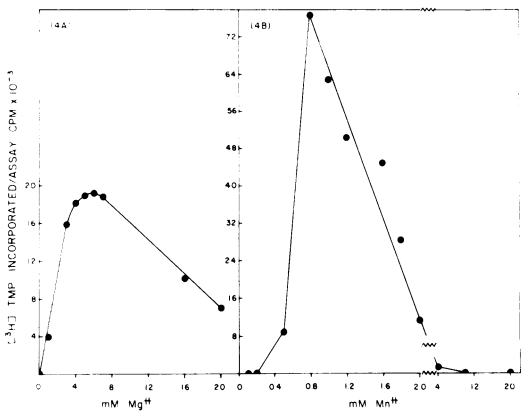

FIG. 4. *Effect of divalent cation concentration upon the incorporation of ^{3}H-TMP into the acid-precipitable product by the M-PMV (A) and woolly monkey viral (B) DNA polymerases. Each DNA polymerase was assayed with its standard conditions for 30 min with poly (dT)·poly (A) template-primer.*

DNA are given in Table 5. These determinations were made with the same enzyme preparation and one preparation of each template. Variation has been found in the template preparations, between the different commercial suppliers, as well as with different lot numbers from the same supplier, but these variations can be minimized when the template concentration is kept well above the calculated K_m.

Transcription of AMV 70S RNA by the M-PMV and woolly monkey virus DNA polymerases was achieved only in the presence of added primer such as $(dT)_{12-18}$. Table 6 contains the results observed with the natural RNA templates. The results with the AMV DNA polymerase are included to demonstrate that the RNA used can be transcribed by viral DNA polymerase. With the $(dT)_{12-18}$-primed AMV 70S RNA template, both primate viral enzymes catalyze the incorporation of all four deoxyribonucleotides. However, with M-PMV enzyme TMP incorporation into product occurs in approximately fivefold excess over any one of the other

TABLE 4. *Template activities of viral DNA polymerase*

Template	Enzyme[a]		
	Mason-Pfizer monkey virus	Woolly monkey virus	Rauscher leukemia virus
Activated DNA	32	50	8
Native DNA	3.7	8	1.1
Poly [d(A-T)]	2.5	20	1.6
Poly (dA)·$(dT)_{12-18}$	1.6	1.9	2.4
Poly (A)·Poly (dT)	252	573	119
Poly (A)·$(dT)_{12-18}$	175	200	146
Poly (C)·$(dG)_{12-18}$	142	167	24
Poly (G)·$(dC)_{12-18}$	0.6	0.9	0.3

[a] The standard assay for each enzyme was run for 30 min (see Materials and Methods). The Mason-Pfizer monkey viral enzyme was incubated at 37 C, while the other two were incubated at 30 C. Within an enzyme template set, the amount of polymerase added was constant. All the assays were done as described in Materials and Methods with the most pure enzyme fraction. The DNA duplex concentrations (synthetic and natural) in the assays were 60 μg/ml, while the RNA-DNA duplex concentrations were 20 μg/ml. ^{3}H-TMP was the label used for all the templates except poly (C)·$(dG)_{12-18}$ and poly (G)·$(dC)_{12-18}$. ^{3}H-dGMP was incorporated into product with poly (C)·$(dG)_{12-18}$ as template and ^{3}H-dCMP for the poly (G)·$(dC)_{12-18}$ template. The enzyme was measured in picomoles of labeled deoxynucleotide incorporated per microgram of protein.

TABLE 5. *K_m constants for the M-PMV DNA polymerase[a]*

Template	K_m (μg/ml)
Activated DNA	10.0
$(dT)_{12-18}$·poly (A)	0.5
Poly (dT)·poly (A)	0.1

[a] Miles Laboratories supplied the poly (dT)·poly (A), Collaborative Research supplied the $(dT)_{12-18}$·poly (A) and activated DNA was prepared as described in Materials and Methods. The standard M-PMV DNA polymerase assay was used except that the template concentration was varied. K_m constants were determined from a standard Lineweaver-Burke plot.

TABLE 6. *Transcription of natural RNAs by the primate viral DNA polymerases*

Template primer[a]	Picomoles of ^{3}H-TMP incorporated in 30 min/50-μliter assay		
	M-PMV	Woolly monkey virus	AMV[b]
AMV 70S RNA	<0.01	<0.01	1.0
AMV 70S RNA + $(dT)_{12-18}$	24	2.5	17.0
$(dT)_{12-18}$	<0.001	<0.001	<0.001

[a] The standard DNA polymerase assay was used except the concentrations of the nucleic acid template were AMV 70S RNA (25 μg/ml) and $(dT)_{12-18}$ (50 μg/ml).

[b] AMV DNA polymerase was purified by the procedure described in Materials and Methods.

TABLE 7. *Effect on polymerization by M-PMV DNA polymerase by deletion of each deoxyribonucleoside triphosphate*

Triphosphate deleted	Activity remaining[a] (%)
None	100
dATP	34
dCTP	54
dGTP	46
TTP[b]	25

[a] Activity measured by incorporation of ^{3}H-TMP into an acid-insoluble nucleic acid product. Activated DNA (60 μg/ml) was the template.

[b] Activity measured by incorporation of ^{3}H-dCMP.

three deoxyribonucleotides. Under conditions resulting in the incorporation of 1.6 pmol of TMP, 0.04, 0.28, and 0.10 pmol of dCMP, dGMP, and dAMP, respectively, were polymerized. In contrast, the woolly monkey virus enzyme catalyzes the incorporation of similar amounts of all four deoxyribonucleotides into product with the AMV 70S RNA template and $(dT)_{12-18}$ primer. Conditions utilizing AMV 70S RNA $(dT)_{12-18}$ primer and incorporating 0.18 pmol of TMP into product also would result in the incorporation of 0.09, 0.26, and 0.31 pmol of dCMP, dGMP, and dAMP, respectively. The AMV 70S RNA concentration in the assay is generally 25 μg/ml. However, increasing the concentration to 50 μg/ml did not increase the formation of product.

Evidence that the enzyme isolated from the M-PMV is not a terminal addition enzyme is provided with experiments deleting the individual deoxyribonucleoside triphosphates from the assay in which activated DNA is the template. As shown in Table 7, omission of any one of the four deoxyribonucleoside triphosphates markedly reduces synthesis of DNA.

pH and salt optima. The dependence of DNA polymerase activity on pH for both primate viral enzymes was examined in three buffer systems—Tris (pH 7.3–8.3), phosphate (pH 6.6–8.0), and imidazole (pH 6.6–7.7) (Fig. 5). The lower ranges of the Tris buffer appear to be the areas of maximal activity for both polymerases.

Each of the two primate viral DNA polymerases display different sensitivities to the potassium chloride concentration. The M-PMV enzyme has a potassium chloride optimum between 20 and 60 mM (Fig. 6A), while the optimum for the woolly monkey viral enzyme is between 80 and 110 mM (Fig. 6B). Both enzymes work less effectively with sodium chloride, although the M-PMV enzyme will tolerate a wide concentration range of this salt (5 mM–200 mM). However, the woolly monkey

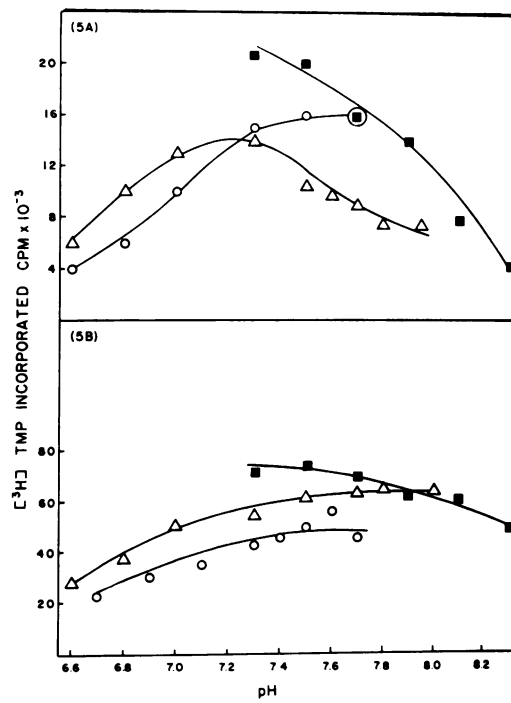

FIG. 5. *pH dependence of the two primate viral DNA polymerases. M-PMV (A) and woolly monkey viral (B) enzymes were assayed with poly (A)·$(dT)_{12-18}$ template primer by the standard procedures for 20 min. The buffer concentrations for the assay were: Tris, 0.05 M (■); phosphate, 0.02 M (△); and imidazole, 0.02 M (○).*

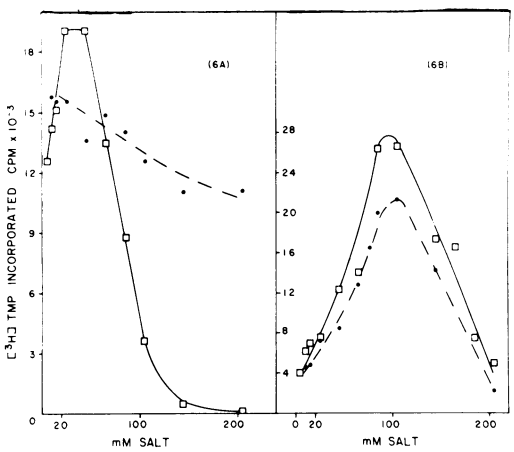

FIG. 6. *The salt optimum for the DNA polymerases from two primate viruses. The standard assay conditions were used for both enzymes (M-PMV [A] and woolly monkey virus [B]) with poly (A)·poly (dT) template-primer for 20 min. Potassium chloride (□——□) and sodium chloride (●——●) concentrations are given in millimolar units.*

viral DNA polymerase exhibits a sodium chloride concentration dependence similar, but of lower magnitude, to that found for potassium chloride.

DISCUSSION

M-PMV was initially found in a spontaneous mammary tumor of a rhesus monkey (4) and was shown to contain an RNA-directed DNA polymerase (16, 20). The classification of this virus as either type-C or type-B was difficult because of its mixed properties; e. g., its budding process resembles that of the murine mammary tumor virus (type-B), but the smooth viral envelope is similar to that of murine leukemia virus (type-C) (9). In addition, unlike known type-B or type-C particles, the M-PMV has been described as unusually fragile, a condition which has been attributed to an inherent tendency of the supercoiled nucleocapsid to unwind when pressure is applied to the virion envelope (9).

The other primate virus studied for this report was the woolly monkey virus, which originated from a naturally occurring fibrosarcoma in a woolly monkey. The classification of this virus as type-C was based upon morphological criteria (22). Subsequent investigations have shown that this sarcoma virus has a nontransforming associated virus (27).

These two proven primate RNA viruses, as well as RLV, were used as sources for viral DNA polymerases. Some biophysical and biochemical properties of these enzymes were compared, including their molecular size, template specificities, divalent cation preference, monovalent cation optimum, and pH optimum. The three mammalian viral DNA polymerases isolated for this study display some common template specificities which allow them to be distinguished from the major cellular DNA polymerases. Three purified DNA polymerases from normal human lymphocytes, stimulated with phytohemagglutinin, have a strong preference for the primer-template $(dT)_{12-18}$·poly (dA) over $(dT)_{12-18}$·poly (A) (3, 19). These results with cellular DNA polymerases are in agreement with previous reports on similar systems (6, 13). Conversely, as previously reported (2, 6, 13), and reconfirmed here, the RNA tumor virus DNA polymerases have a strong preference for primer-template $(dT)_{12-18}$·poly (A) over $(dT)_{12-18}$·poly (dA).

The M-PMV enzyme has some properties in common with a poly (dT) polymerase which has been found in intracisternal type-A particles (25). This enzyme is very efficient at poly (dT) synthesis with the synthetic template-primer poly (A)·$(dT)_{12-18}$ with Mg^{2+} as the divalent cation of choice (25). With Mn^{2+} or with a DNA template, natural or synthetic, this enzyme from A-particles has little or no activity (25). The M-PMV DNA polymerase is also very efficient at poly (dT) synthesis and prefers Mg^{2+}. However, in contrast to the type-A particle enzyme, the M-PMV DNA polymerase is almost as efficient at poly (dG) synthesis (see Table 6). In addition, M-PMV DNA polymerase repair activated DNA, incorporating all four deoxyribonucleotides into the product.

The similarities among the RNA tumor virus DNA polymerases appear to reside mostly in their template specificities and their properties to adsorb to ion exchange celluloses. All three mammalian viral DNA polymerases were extracted and purified by the same procedure. The three enzymes exhibited similar elution patterns on both DEAE-cellulose and phosphocellulose. In addition, the template specificities are almost identical. In fact, the two primate viral DNA polymerases have the same order of preference for the series of template-primers tested.

The differences among the viral DNA polymerases emerged primarily from the molecular weight determinations and divalent cation requirements for polymerization activity. Other response to differences for optimal activity were found in salt requirements and the three buffer systems tested.

The differences in the biochemical and biophysical behavior of the three mammalian viral DNA polymerases are probably not a function

of the isolation procedure. The two primate viral enzymes differ strikingly in their requirements for maximal activity; more similarity exists between the two type-C viral polymerases then between the two primate enzymes. These biochemical results indicate, therefore, that not all RNA tumor virus DNA polymerases are identical. This is in keeping with the immunological data showing differences in antisera response between type-C and other RNA tumor viruses and also between type-C viruses originating in primates and those derived from lower-order mammals (17, 18).

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