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Purification and Characterization of the DNA Polymerase and RNase H Activities in Moloney Murine Sarcoma-Leukemia Virus

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Two RNase H (RNA-DNA hybrid ribonucleotidohydrolase, EC 3.1.4.34) activities separable by Sephadex G-100 gel filtration were identified in lysates of Moloney murine sarcoma-leukemia virus (MSV). The larger enzyme, which we have called RNase H-I, represented about 10% of the RNase H activity in the virion. RNase H-I (i) copurified with RNA-directed DNA polymerase from the virus, (ii) had a sedimentation coefficient of 4.4*S* (corresponds to an apparent mol wt of 70,000), (iii) required Mn^{2+} (2 mM optimum) for activity with a [^{3}H]poly(A)·poly(dT) substrate, (iv) eluted from phosphocellulose at 0.2 M KCl, and (v) degraded [^{3}H]poly(A)·poly(dT) and [^{3}H]poly(C)·poly(dG) at approximately equal rates. The smaller enzyme, designated RNase H-II, which represented the majority of the RNase H activity in the virus preparation, was shown to be different since it (i) had no detectable, associated DNA polymerase activity, (ii) had a sedimentation coefficient of 2.6*S* (corresponds to an apparent mol wt of 30,000), (iii) preferred Mg^{2+} (10 to 15 mM optimum) over Mn^{2+} (5 to 10 mM optimum) 2.5-fold for the degradation of [^{3}H]poly(A)·poly(dT), and (iv) degraded [^{3}H]poly(A)·poly(dT) 6 and 60 times faster than [^{3}H]poly(C)·poly(dG) in the presence of Mn^{2+} and Mg^{2+}, respectively. Moloney MSV DNA polymerase (RNase H-I), purified by Sephadex G-100 gel filtration followed by phosphocellulose, poly(A)·oligo(dT)-cellulose, and DEAE-cellulose chromatography, transcribed heteropolymeric regions of avian myeloblastosis virus 70*S* RNA at a rate comparable to avian myeloblastosis virus DNA polymerase purified by the same procedure.

Several years ago we reported that the RNase H (RNA-DNA hybrid ribonucleotidohydrolase, EC 3.1.4.34) activity in Moloney murine sarcoma-leukemia virus (MSV) was partially separable from the poly(A)·oligo(dT)-directed DNA polymerase activity present by DEAE-cellulose chromatography, but not by phosphocellulose chromatography or glycerol gradient centrifugation (6). We have subsequently quantitatively reexamined the properties of the two MSV enzyme activities purified by different fractionation techniques and in addition have compared the ability of purified MSV and avian myeloblastosis virus (AMV) DNA polymerase to transcribe AMV 70*S* RNA. We report here that MSV contains two distinct RNase H activities, one associated with viral DNA polymerase and the other free of polymerase activity, and that the purified MSV DNA polymerase-RNase H complex can transcribe 70*S* RNA almost as effectively as AMV DNA polymerase purified by an identical procedure.

MATERIALS AND METHODS

Reagents. Calf thymus DNA (type IV) was from Sigma Chemical Co. Calf thymus DNA was activated by the procedure of Aposhian and Kornberg (2). The following is a list of materials and their source: [^{3}H]TTP and [^{3}H]dGTP, New England Nuclear Corp.; Whatman DEAE-cellulose (DE-52), phosphocellulose (P-11), and DEAE-cellulose (DE-81) paper disks, Reeve Angel; deoxyribonucleoside 5'-triphosphates and dithiothreitol, Sigma Chemical Co.; Sephadex G-100, superfine, Pharmacia; synthetic ^{3}H-labeled and unlabeled polyribonucleotides, Miles Laboratories; synthetic polydeoxyribonucleotides and oligo(dT)-cellulose (type 1), P-L Biochemicals; $(dT)_{12-18}$, Collaborative Research, Inc. Adenovirus [^{32}P]DNA was a gift from W. Buettner of this Institute.

Viruses. Moloney MSV produced by the transformed rat cell line 78A-1 was grown and purified as previously described (11). AMV, BAI strain A, was purified as described (11) from frozen plasma virus preparations generously supplied by J. W. and D. Beard.

Preparation of AMV and MSV 70S RNA. Viral

70S RNA was isolated from purified AMV and MSV by sodium dodecyl sulfate-phenol extraction and zonal centrifugation in sucrose gradients (22).

Preparation of RNA-DNA hybrids. Calf thymus DNA-[^{3}H]RNA (7 nmol of RNA nucleotides per ml, 4,600 counts/min per pmol), [^{3}H]poly(A)·poly(dT) {100 μM [^{3}H]poly(A), 50 counts/min per pmol; 400 μM poly(dT}, [^{3}H]poly(U)·poly(dA) {100 μM [^{3}H]poly(U), 80 counts/min per pmol; 400 μM poly(dA)}; and [^{3}H]poly(C)·poly(dG) {100 μM [^{3}H]poly(C), 40 counts/min per pmol; 400 μM poly(dG)} were prepared as described (6). Mixtures of [^{3}H]poly(C) and poly(dG) were heated to 75 C and slow-cooled to facilitate hybrid formation.

Preparation of [^{3}H]poly(dT)·poly(A). [^{3}H]poly(dT)·poly(A) was prepared from poly(A)·$(dT)_{12-18}$ by the polymerization of [^{3}H]TTP in a reaction mixture (2 ml) containing 20 mM Tris-hydrochloride (pH 8.0), 1 mM dithiothreitol, 0.5 mM $MnCl_2$, 48 μM poly(A), 19 μM $(dT)_{12-18}$, 21 μM [^{3}H]TTP (230 counts/min per pmol), and Rauscher murine leukemia virus (MLV) DNA polymerase (27) purified through the DEAE-cellulose step of the procedure described below. After a 1-h incubation at 37 C, the reaction was stopped by the addition of 50 μl of 20% sodium dodecyl sulfate. The product was purified by chromatography on Sephadex G-50 in 0.02 M Tris-hydrochloride (pH 8.0) and 0.1 M NaCl, followed by precipitation with 2 volumes of absolute alcohol after adjustment to 0.3 M NaCl. The precipitate was dissolved in 200 μl of column buffer. Assuming a 70% recovery of poly(A) during the procedure, the concentration of polymers in [^{3}H]poly(dT)·poly(A) was calculated to be 40 and 300 μM, respectively.

DNA polymerase assays. During purification of MSV DNA polymerase, three different templates were used to assay DNA polymerase activity at 37 C by determining polymer product formation on Whatman DEAE-cellulose (DE-81) paper disks (6, 7). All reaction mixtures (0.1 ml) contained 20 mM Tris-hydrochloride (pH 8.0), 2 mM dithiothreitol, and KCl at a final concentration of 50 mM.

Assay I. Reaction mixtures contained 50 μM poly(A), 20 μM $(dT)_{12-18}$, 50 μM [^{3}H]TTP (670 counts/min per pmol), and 0.5 mM $MnCl_2$ or 10 mM $MgCl_2$ when assaying MSV or AMV DNA polymerase, respectively.

Assay II. Activated calf thymus DNA (50 μg/ml) was the template in a reaction mixture containing 60 μM [^{3}H]TTP (560 counts/min per pmol), dATP, dCTP, and dGTP (each 100 μM), and 10 mM $MgCl_2$.

Assay III. Reaction mixtures contained 12 μg of AMV 70S RNA per ml, 10 μM [^{3}H]TTP (34,000 counts/min per pmol), dATP, dCTP, and dGTP (each 100 μM), and 0.5 mM $MnCl_2$ or 10 mM $MgCl_2$ when assaying MSV or AMV DNA polymerase, respectively.

RNase H assay. The assay for RNase H activity was carried out at 37 C by using [^{3}H]poly(A)·poly(dT) and monitoring the degradation of [^{3}H]poly(A) to acid-soluble material. The standard reaction mixture (0.1 ml) contained 20 mM Tris-hydrochloride (pH 8.0), 2 mM dithiothreitol, 50 mM KCl, 3 μM [^{3}H]poly(A), 12 μM poly(dT), and either 2 mM $MnCl_2$ or 10 mM $MgCl_2$, except as indicated otherwise.

Preparation of [^{3}H]DNA for hybridization to viral 70S RNA. DNA was synthesized from AMV 70S RNA in a reaction mixture (1.0 ml) containing 20 mM Tris-hydrochloride (pH 8.0), 2 mM dithiothreitol, 50 mM KCl, 0.5 mM $MnCl_2$, 100 μM dATP and dCTP, either 100 μM dGTP or TTP, either 12 μM [^{3}H]TTP (41,000 counts/min per pmol) or 17 μM [^{3}H]dGTP (24,000 counts/min per pmol), 18 μg of AMV 70S RNA per ml, and 200 μl of DEAE-cellulose-purified MSV DNA polymerase. Incubation at 37 C was terminated after 1 h by addition of 100 μl of 0.2 M EDTA. The product was deproteinized and alkali treated as described previously (3).

Nucleic acid hybridization. AMV 70S RNA (540 μg/ml) or MSV 70S RNA (154 μg/ml) was heated at 80 C for 2 min with poly(U) at 2,500 μg/ml. [^{3}H]DNA (150 pg) prepared as already described was added, and the hybridization mixture was brought to 0.72 M NaCl, 0.2 mM EDTA, 0.01 M sodium piperazine-*N*,*N*′-bis(2-ethane sulfonate) (pH 6.7), 0.05% sodium dodecyl sulfate, 250 μg of poly(U) per ml, and either 27 μg of AMV or 12 μg of MSV 70S RNA per ml in a volume of 100 μl. Annealing was at 68 C for 18 h. Hybridization mixtures containing only [^{3}H]DNA and poly(U) were also annealed.

Determination of the extent of nucleic acid hybridization by S_1 nuclease treatment. AMV [^{3}H]DNA prepared and hybridized to viral 70S RNA as described above was treated with *Asperigillus oryzae* S_1 single-strand nuclease as previously described (3) to determine the extent of hybridization. Adenovirus [^{32}P]DNA, native and denatured, was treated with the S_1 nuclease as controls.

Purification of the DNA polymerase and RNase H activities present in Moloney MSV or AMV. Unless otherwise indicated, all steps were carried out at 4 C. Buffer A is 20 mM Tris-hydrochloride (pH 8.0), 1 mM dithiothreitol, 0.1 mM EDTA, 0.2% Nonidet P-40 (NP-40), and 10% glycerol.

Lysis of virus. Purified virus (MSV or AMV), 1.5 ml at 5.7 mg of protein per ml, was dialyzed against 1 liter of 20 mM Tris-hydrochloride (pH 8.0), 1 mM dithiothreitol, 0.1 mM EDTA, 500 mM KCl, and 20% glycerol for 3 to 4 h. Dialyzed MSV was lysed by the addition of 0.2 ml each of 10 M urea and 15% NP-40. AMV was lysed in the absence of urea. After being stirred for 20 min, the lysate was directly applied to a Sephadex G-100 column.

Sephadex G-100 gel filtration. Virus lysates were loaded on a column (1.5 by 90 cm) of Sephadex G-100, superfine, equilibrated with 200 mM KCl in buffer A. The column was developed at a flow rate of 2.8 ml/h with the same buffer. Fractions of 1 ml were collected.

Phosphocellulose chromatography of MSV enzymes. Fractions eluting at 52 to 65 ml from the Sephadex G-100 column (see Fig. 1) were pooled, 1.2 volumes of buffer A was added, and the diluted pool was applied at a flow rate of 0.3 ml/min to a phosphocellulose column (0.9 by 6 cm) equilibrated in buffer A. All detectable DNA polymerase and RNase H activity adsorbed to the column. After a wash with

5 to 10 ml of buffer A with 0.1 M KCl, the column was eluted with a 60-ml continuous gradient of 0.1 to 0.5 M KCl in buffer. Fractions of 1.5 ml were collected. The fractions containing the bulk of enzyme activity were pooled, diluted with 2 volumes of buffer A, and applied to a phosphocellulose column (0.9 by 1.5 cm). Enzyme activity was eluted with 0.5 M KCl in buffer A.

Fractions eluting at 76 to 90 ml from the Sephadex G-100 column (see Fig. 1) were pooled and applied to a phosphocellulose column (0.9 by 6 cm) as described above. RNase H activity was eluted with a 60-ml continuous gradient of 0.2 to 1.0 M KCl in buffer A.

Phosphocellulose chromatography of AMV DNA polymerase. AMV DNA polymerase peak fractions from the Sephadex G-100 column (elution volume was 48 to 65 ml) were pooled, 4 volumes of buffer A was added, and the diluted pool was chromatographed on a phosphocellulose column (0.9 by 6 cm) as described for MSV DNA polymerase. DNA polymerase activity was eluted with a 50-ml gradient of 0.05 to 0.5 M KCl in buffer A. The peak fractions that eluted at 0.22 M KCl (two-subunit enzyme; see ref. 7) were concentrated on a small phosphocellulose column as already described.

DEAE-cellulose chromatography of MSV RNase H. The smaller MSV RNase H preparation free of detectable DNA polymerase activity (RNase H-II), which had been purified on phosphocellulose, was dialyzed for 3 h against 2 liters of buffer A and applied at a flow rate of 0.3 ml/min to a DEAE-cellulose column (0.9 by 6 cm) equilibrated in buffer A. After a wash with 10 ml of buffer A, the column was eluted with a 60-ml gradient of 0 to 0.3 M KCl in buffer A. Fractions of 1.5 ml were collected.

Poly(A)·oligo(dT)-cellulose chromatography of MSV DNA polymerase. Buffer B is 10 mM Tris-hydrochloride (pH 7.4), 2 mM dithiothreitol, 1% NP-40, 1 mM $MnCl_2$, and 10% glycerol. Initially, oligo(dT)-cellulose was washed extensively with 1 M KCl in buffer B. Washed oligo(dT)-cellulose (1 g) was then mixed with 2,140 nmol of poly(A) in 1 M KCl plus buffer B, and the mixture was incubated at 37 C for 30 min. All subsequent steps were performed at 25 C. One-third of the poly(A)·oligo(dT)-cellulose was packed into a column (0.6 cm in diameter) and washed with 5 ml of 1 M KCl followed by 10 ml of 0.1 M KCl in buffer B. The other two-thirds was washed batchwise with 25 and 50 ml, respectively, of 1 and 0.1 M KCl in buffer B. Phosphocellulose-purified and concentrated Moloney MSV DNA polymerase (5 ml) was dialyzed for 1.5 h against three 500-ml batches of 0.1 M KCl in buffer B. The dialyzed preparation was mixed batchwise with the washed poly(A)·oligo(dT)-cellulose, the enzyme was allowed to adsorb for 30 min, and the enzyme-cellulose mixture was loaded into the column at a flow rate of 5 ml/h. The final packed column (0.6 by 15 cm) was washed (5 ml/h) with 10 to 15 ml of 0.1 M KCl in buffer B. The column was eluted (9 ml/h) with a 40-ml gradient of 0.1 to 1 M KCl in buffer B, and fractions of 1 ml were collected and maintained on ice.

Poly(A)·oligo(dT)-cellulose chromatography of AMV DNA polymerase. Poly(A)·oligo(dT)-cellulose chromatography of AMV DNA polymerase was carried out as described for MSV DNA polymerase except that in buffer B, 10 mM potassium phosphate (pH 7.5) and 5 mM $MgCl_2$ were substituted for Tris-hydrochloride and $MnCl_2$, respectively.

Glycerol gradient centrifugation. A sample (200 μl) from the peak tube of the poly(A)·oligo(dT)-cellulose-purified MSV DNA polymerase or from the pool of peak tubes of the DEAE-cellulose-purified RNase H-II was layered on a 4.8-ml 20 to 40% glycerol gradient in 50 mM Tris-hydrochloride (pH 8.0), 5 mM dithiothreitol, 0.1 mM EDTA, 0.2% NP-40, and 0.35 M KCl. The gradients were centrifuged at 46,000 rpm at 3 C in a Spinco SW50.1 rotor for 29 or 42 h. Fractions were collected from the bottom of the centrifuge tube. Hemoglobin was run in a separate tube as a sedimentation marker.

DNA-cellulose chromatography. Calf thymus DNA-cellulose was prepared according to the procedure of Litman (16). The peak fractions from the poly(A)·oligo(dT)-cellulose column of MSV DNA polymerase were pooled (5 ml) and dialyzed as already described before poly(A)·oligo(dT)-cellulose chromatography. The enzyme was adsorbed and eluted from DNA-cellulose (700 mg) as already described for poly(A)·oligo(dT)-cellulose chromatography.

DEAE-cellulose chromatography of MSV DNA polymerase. The peak fractions from the poly(A)·oligo(dT)-cellulose column of MSV DNA polymerase were pooled (KCl concentration was 0.25 M), and 1.5 ml of the pool was applied to a DEAE-cellulose column (0.9 by 1 cm) equilibrated with buffer A. Fractions of 1 ml were collected, and the first two fractions in the column flow-through containing the bulk of the DNA polymerase activity were used for subsequent experiments.

Sodium dodecyl sulfate-polyacrylamide disc gel electrophoresis. During several different purifications, pooled, peak fractions from (i) poly(A)·oligo(dT)-cellulose columns of MSV or AMV DNA polymerase, (ii) three glycerol gradients of poly(A)·oligo(dT)-cellulose-purified MSV polymerase, or (iii) a DNA-cellulose column of poly(A)·oligo(dT)-cellulose-purified MSV DNA polymerase were dialyzed for 48 h against 6 liters of water at 4 C, dialyzed for 6 h against 2 liters of 0.1% sodium dodecyl sulfate-0.1% 2-mercaptoethanol, and then lyophilized. The lyophilized material was subjected to sodium dodecyl sulfate-polyacrylamide disc gel electrophoresis as already described (7).

RESULTS

RNase H and DNA polymerase activity in purified avian myeloblastosis and Moloney murine sarcoma leukemia virions. Table 1 compares, on a unit protein basis, the RNase H and DNA polymserase activities present in lysed preparations of purified MSV and AMV as assayed with either $MnCl_2$ or $MgCl_2$ at concentrations optimal for AMV DNA polymerase (G. Gerard and D. Grandgenett, unpub-

TABLE 1. *Comparison of the DNA polymerase and RNase H activities in avian myeloblastosis and Moloney murine sarcoma-leukemia virions*[a]

Virus	Metal ion concn (mM)	[^{3}H]TTP incorporated or [^{3}H]poly(A) solubilized (pmol/μg of protein per 20 min)			
		Poly(A)·(dT)$_{12-18}$	Activated calf thymus DNA	AMV 70S RNA	[^{3}H]poly(A)·poly(dT)
Avian myeloblastosis	Mg^{2+} (10)	44.1	6.16	0.27	6.14
	Mn^{2+} (0.5)	60.8		0.38	
	Mn^{2+} (5)				18.6
Moloney murine sarcoma-leukemia	Mg^{2+} (10)	20.7	10.5	0.01	39.2
	Mn^{2+} (0.5)	133		0.15	
	Mn^{2+} (5)				41.2

[a] Virus was lysed at a protein concentration of 570 μg/ml in 20 mM Tris-hydrochloride (pH 8.0), 100 mM NaCl, 10 mM dithiothreitol, 0.1 mM EDTA, 10% glycerol, and 0.5% NP-40 at 4 C for 10 min. Samples, 5 and 10 μl, were then assayed in the reaction mixtures described in Materials and Methods. The reaction velocities in all four types of reaction mixtures were proportional to protein concentration with both virus preparations. The DNA polymerase and RNase H assays contained 50 and 20 mM KCl, respectively.

lished data). Several important conclusions can be drawn. First, it is clear for MSV DNA polymerase with either poly(A)·(dT)$_{12-18}$ or AMV 70S RNA as template that Mn^{2+} is the preferred metal ion; in fact, little detectable DNA was synthesized from 70S RNA in the presence of 10 mM $MgCl_2$. Similar results were obtained when the divalent metal ion requirements of phosphocellulose-purified MSV DNA polymerase with poly(A)·(dT)$_{12-18}$ and AMV 70S RNA were determined (data not shown). Second, with the metal ions used to assay MSV (Mn^{2+}) and AMV (Mg^{2+}) poly(A)·(dT)$_{12-18}$-directed DNA polymerase activity in subsequent experiments, MSV DNA polymerase had three times more activity than the AMV enzyme. Last, both AMV and MSV contained RNase H activity that responded to either $MnCl_2$ or $MgCl_2$, but with either metal ion, MSV contained appreciably more RNase H activity than did AMV.

Purification of MSV DNA polymerase. Because of a lack of large quantities of murine sarcoma-leukemia virions, we have attempted to develope a purification procedure that allows the use of small amounts of starting material (5 to 10 mg of protein) with minimum inactivation of enzyme activity during purification. To stabilize DNA polymerase and RNase H activity, nonionic detergent (20) and glycerol are added to all buffers and KCl is maintained at a minimum concentration of 0.1 M.

(i) Sephadex G-100 gel filtration. Initially, MSV lysed in 0.5 M KCl, 1 M urea, 20% glycerol, and 1.5% NP-40 was subjected to Sephadex G-100 gel filtration (Fig. 1). DNA polymerase activity responding to poly(A)·(dT)$_{12-18}$, AMV 70S RNA, and activated calf thymus DNA (data not shown) co-eluted as a single peak from Sephadex G-100 with an elution volume corresponding to a mol wt of about 100,000. Two RNase H activities separable by

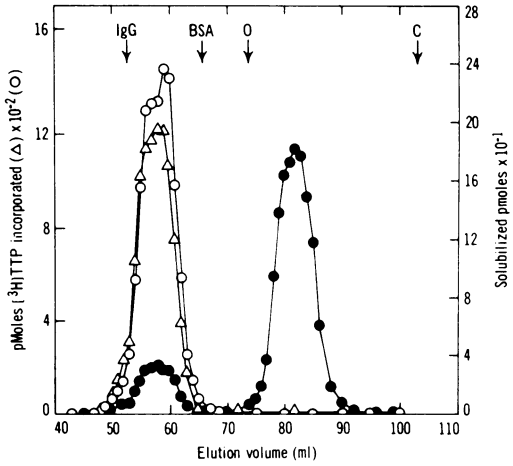

FIG. 1. *Gel filtration in Sephadex G-100 of the DNA polymerase and RNase H activities of lysed Moloney MSV. Lysed MSV (8.6 mg of protein) was chromatographed in Sephadex G-100 as described in Materials and Methods. Selected fractions were assayed for activity as described in Materials and Methods with poly(A)·(dT)$_{12-18}$ (O), 10 μl for 20 min; AMV 70S RNA (Δ), 25 μl for 40 min; and [^{3}H]poly(A)·poly(dT) (●), fractions 40 to 70, 20 μl for 20 min with 1 mM $MnCl_2$, and fractions 70 to 100, 10 μl for 20 min with 10 mM $MgCl_2$. Marker proteins, detected by absorbance at 280 nm, used to calibrate the Sephadex G-100 column were gamma globulin (IgG), bovine serum albumin (BSA), ovalbumin (O), and cytochrome c (C).*

gel filtration were also present in lysates of MSV (Fig. 1). The larger, which we have called RNase H-I, co-migrated with the DNA polymerase activity and represented only about 10% of the RNase H activity associated with the MSV preparation. The smaller RNase H, which we have called RNase H-II, was free of detectable DNA polymerase activity and eluted at a volume corresponding to a mol wt of 30,000. We have also observed two RNase H activities with similar properties and in the same relative proportion in lysates of Harvey MSV grown in NIH-Swiss mouse embryo culture cells (10; G. Gerard, unpublished data).

(ii) Phosphocellulose chromatography. The Sephadex G-100 peak fractions of DNA polymerase (RNase H-I) were pooled and chromatographed on a phosphocellulose column (Fig. 2A). DNA polymerase activity, with either poly(A)·$(dT)_{12-18}$ or AMV 70S RNA as template, and RNase H activity (RNase H-I) co-eluted from phosphocellulose at 0.2 M KCl.

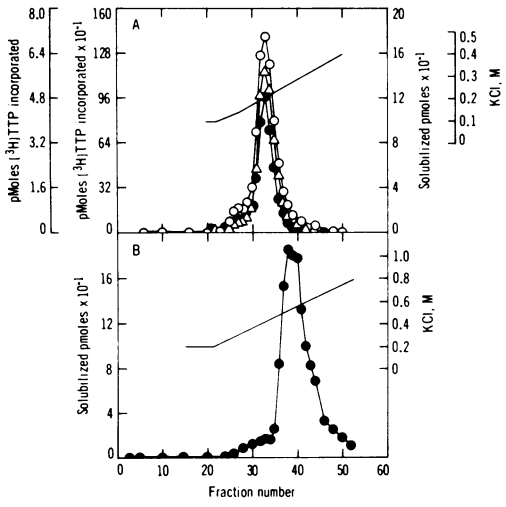

FIG. 2. *Chromatography of DNA polymerase (RNase H-I) and RNase H-II from Moloney MSV on phosphocellulose. (A) Sephadex G-100 pool (fractions 52 to 65) from Moloney MSV was chromatographed on phosphocellulose as described in Materials and Methods. Samples from each fraction were assayed for activity as described in Materials and Methods with poly(A)·$(dT)_{12-18}$ (○), 10 μl for 20 min; AMV 70S RNA (△), 25 μl for 40 min; and [³H]poly(A)·poly(dT) (●), 25 μl for 40 min with 1 mM $MnCl_2$. (B) Sephadex G-100 pool (fractions 76 to 90) from Moloney MSV was chromatographed on phosphocellulose as described in Materials and Methods. A sample (10 μl for 20 min) from each fraction was assayed for RNase H activity as described in Materials and Methods with [³H]poly(A)·poly(dT) (●) and 10 mM $MgCl_2$. Salt concentrations were determined conductimetrically.*

(iii) Poly(A)·oligo(dT)-cellulose chromatography of MSV DNA polymerase (RNase H-I). Gerwin et al. (4, 5) first used affinity chromatography with oligo(dT)-cellulose to purify viral RNA-directed DNA polymerase. The affinity of this matrix for viral RNA-directed DNA polymerase can be substantially increased by hybridizing poly(A) to the oligo(dT)-cellulose, and details concerning the use of poly(A)·oligo(dT)-cellulose to purify RNA-directed DNA polymerase and the basis for its selective affinity for the viral enzyme have been described (9). A profile of phosphocellulose-purified MSV DNA polymerase subjected to poly(A)·oligo(dT)-cellulose chromatography is shown in Fig. 3. The majority of the DNA polymerase and RNase H-I activity was adsorbed by the column at 0.1 M KCl, and both activities co-eluted in a linear KCl gradient at 0.27 M KCl. Variable amounts of activity (the range was 1 to 20% of that applied to the column in four different experiments) did not bind to the column in the presence of 0.1 M KCl, due perhaps to leaching of enzyme bound to poorly hybridized poly(A) or poly(A)·oligo(dT) cleaved from the cellulose matrix by nucleases present in the enzyme preparations. In addition, the peak fractions from the column that were pooled and used in subsequent experiments were found to contain poly(A) at a concentration in the 10^{-7} M range and oligo(dT) at a substantially lower concentration. These concentrations were determined by assaying the poly(A)·oligo(dT)-cellulose pool in reaction mixtures containing either 50 μM poly(A) or 20 μM oligo(dT) alone, and [³H]TTP (29,000 counts/min per pmol) plus $MnCl_2$. All detecta-

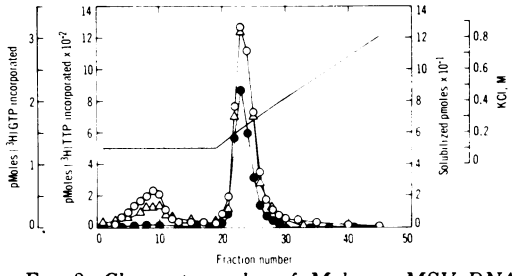

FIG. 3. *Chromatography of Moloney MSV DNA polymerase on poly(A)·oligo(dT)-cellulose. Concentrated phosphocellulose eluate of Moloney MSV DNA polymerase was chromatographed on poly(A)·oligo(dT)-cellulose as described in Materials and Methods. Samples from each fraction were assayed for activity as described in Materials and Methods with poly(A)·$(dT)_{12-18}$ (○), 10 μl for 20 min; AMV 70S RNA (△), 25 μl for 40 min with [³H]dGTP (18,500 counts/min per pmol) instead of [³H]TTP; and [³H]poly(A)·poly(dT) (●), 25 μl for 40 min with 2 mM $MnCl_2$.*

ble traces of poly(A) and oligo(dT) were removed by passing the poly(A)·oligo(dT)-cellulose enzyme preparation over a small DEAE-cellulose column (see Materials and Methods for details). At the KCl concentration in the pool (0.25 M), DNA polymerase and RNase H-I activity passed directly through the column, whereas poly(A), oligo(dT), and any residual contaminating nucleic acids originating from the virus were retained on the column. It should be noted that under the conditions we used to assay MSV DNA polymerase activity with AMV 70*S* RNA as template, $(dT)_{12-18}$ at a minimum concentration of 3×10^{-7} M was required to produce any stimulation of the DNA synthetic rate (G. Gerard, unpublished data). We estimate the contaminating oligo(dT) concentration in poly(A)·oligo(dT)-cellulose-purified MSV DNA polymerase to be one to two orders of magnitude less than 3×10^{-7} M.

(iv) Glycerol gradient centrifugation of MSV DNA polymerase. A sample from the peak fraction of poly(A)·oligo(dT)-cellulose-purified MSV DNA polymerase was analyzed by glycerol gradient centrifugation in high salt (0.35 M KCl) (Fig. 4). DNA polymerase activity responding to AMV 70*S* RNA and poly(A)·$(dT)_{12-18}$ and RNase H activity co-sedimented as a single peak with a sedimentation coefficient ($s_{20,w}$) of 4.4*S* relative to a hemoglobin marker (4.3*S*). Using the relationship $SW_1/SW_2 = (MW_1/MW_2)^{2/3}$ (18) (hemoglobin = 66,000), the molecular weight of MSV DNA polymerase (RNase H-I) was calculated to be 70,000.

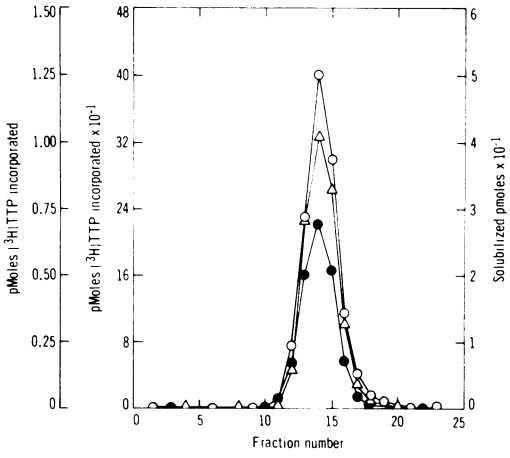

FIG. 4. *Glycerol gradient centrifugation of MSV DNA polymerase. A sample (200 μl) from the peak fraction of a poly(A)·oligo(dT)-cellulose column of MSV DNA polymerase was analyzed by glycerol gradient centrifugation as described in Materials and Methods. Centrifugation was for 29 h. Samples from each fraction were assayed for activity as described in Materials and Methods with poly(A)·*$(dT)_{12-18}$ (○), *10 μl for 20 min; AMV 70S RNA* (△), *25 μl for 40 min; and [3H]poly(A)·poly(dT)* (●), *25 μl for 40 min with 2 mM* $MnCl_2$. *The peak of a hemoglobin marker sedimented at fraction 14.*

(v) DNA-cellulose chromatography of MSV DNA polymerase. The pool of poly(A)·oligo(dT)-cellulose-purified MSV DNA polymerase was dialyzed to reduce the KCl concentration to 0.1 M and then subjected to DNA-cellulose chromatography (see Materials and Methods). Greater than 90% of the DNA polymerase activity, with 70*S* RNA or poly(A)·$(dT)_{12-18}$ as template, and RNase H activity was adsorbed by the column. Both activities co-eluted as a single peak in a linear KCl gradient at 0.44 M KCl (data not shown).

(vi) Summary of purification of MSV DNA polymerase (RNase H-I). Quantitative determination of the DNA polymerase and RNase H activities present during purification of MSV DNA polymerase were made and are summarized in Table 2. For comparison, an assessment was also made of the same enzyme activities present during purification of DNA polymerase from an equivalent amount of AMV protein. The same basic steps of Sephadex G-100, phosphocellulose, and poly(A)·oligo(dT)-cellulose column chromatography were used. DNA polymerase activity was monitored with poly(A)·$(dT)_{12-18}$, activated calf thymus DNA, and AMV 70*S* RNA, and RNase H activity was monitored with [^{3}H]poly(A)·poly(dT). Enzyme activity is expressed in terms of initial rate of [^{3}H]TTP incorporated or [^{3}H]poly(A) solubilized per unit volume of enzyme, since the protein concentration in all fractions was too low to determine accurately. Ratios of reaction rates were calculated by setting the rate with poly(A)·$(dT)_{12-18}$ at unity and expressing the rates determined with the other templates and substrate as fractions of 1.0. Enzyme activity with poly(A)·oligo(dT)$_{12-18}$ was selected as the reference, since it was the most stable activity during purification. The numbers in the ratios were multiplied by 1,000 and 250 for MSV and AMV, respectively, so that comparisons could be made between the ratios obtained for the two DNA polymerases. On the average, in an equivalent amount of lysed virus (Table 1), as well as in the various purification pools (Table 2), MSV DNA polymerase had 3.75 times more activity with poly(A)·$(dT)_{12-18}$ than AMV DNA polymerase.

Of primary interest in Table 2 are the ratios of activities obtained for the two viral enzyme

TABLE 2. *Summary of the DNA polymerase and RNase H activities present during purification of MSV and AMV DNA polymerase*[a]

Fraction[c]	[^{3}H]TTP incorporated or [^{3}H]poly(A) solubilized[b] (pmol/μl of enzyme per 20 min)								Ratio of activities	
	Poly(A)·(dT)$_{12-18}$		Activated calf thymus DNA		AMV 70S RNA		[^{3}H]poly(A)·poly(dT)			
	MSV	AMV	MSV	AMV	MSV	AMV	MSV	AMV	MSV	AMV
Sephadex G-100 pool	77.4	20.1	4.60	1.40	0.072 (0.009)[d]	0.092	2.08	1.22	1,000/59/0.9/34	250/18/1.2/15
Phosphocellulose pool concentrate (dialyzed)	92.4	17.2	5.70	1.23	0.058 (0.002)[d]	0.080	3.28	1.19	1,000/62/0.6/13	250/18/1.2/17
Poly(A)·oligo(dT)-cellulose pool	85.6	21.5	2.50	1.19	0.059	0.085	1.64	1.19	1,000/29/0.7/14	250/14/1.0/14
DEAE-cellulose pool	21.3		0.68		0.012 (0.0)[d]		0.36		1,000/32/0.6/17	
DNA-cellulose pool	18.0		0.42		0.007		0.38		1,000/23/0.4/21	
Glycerol gradient peak fraction	39.8				0.021		0.55		1,000/—/0.5/14	

[a] DNA polymerase assays contained the constituents described in Materials and Methods. The components used in the RNase H assays are also described in Materials and Methods; KCl was at 20 mM, and 10 mM $MgCl_2$ and 2 mM $MnCl_2$ were present in the assay mixtures for AMV and MSV RNase H, respectively. Proportionality between reaction velocity and enzyme concentration was determined for each fraction with each template or substrate by assay at three different enzyme concentrations.

[b] The results through the poly(A)·oligo(dT)-cellulose step presented for MSV were quite reproducible and are the average of determinations made during three different purifications. Determinations for AMV polymerase were from a single purification.

[c] The total volumes of the Sephadex G-100, phosphocellulose concentrate, poly(A)·oligo(dT)-cellulose, DEAE-cellulose, and DNA-cellulose pools were 17, 6, 6, 4, and 5 ml, respectively.

[d] The values in parentheses represent the amount of endogenous incorporation obtained in a complete reaction mixture without exogenously added 70S RNA.

preparations. In the case of MSV DNA polymerase using poly(A)·(dT)$_{12-18}$-directed activity as a reference, there was a preferential loss of DNA-directed and 70S RNA-directed DNA polymerase activity and RNase H activity during purification, so that in the final and most highly purified enzyme pools the relative amount of each of these activities decreased to approximately one-half the original value. In contrast, the relative activities of AMV DNA polymerase-RNase H did not change appreciably during purification. In spite of the preferential loss of these activities during purification of MSV DNA polymerase (RNase H-I), the purified murine enzyme still had DNA-directed DNA polymerase and RNase H activity comparable with or better than that of AMV DNA polymerase. The relative 70S RNA-directed activity of purified MSV DNA polymerase was approximately one-half that of the AMV enzyme.

Purified MSV DNA polymerase transcribes 70S RNA into heteropolymeric DNA. To demonstrate that DNA synthesized from viral 70S RNA by purified MSV DNA polymerase is transcribed from heteropolymeric regions of that RNA, [^{3}H]DNA product synthesized by DEAE-cellulose-purified MSV DNA polymerase (RNase H-I) (free of all detectable contaminating nucleic acids) from AMV 70S RNA with [^{3}H]TTP or [^{3}H]dGTP was annealed with 70S RNA in the presence of an excess of poly(U). The extent of hybridization was determined with *A. oryzae* S_1 nuclease (15) (Table 3). We found that 20% of the DNA labeled with either [^{3}H]TMP or [^{3}H]dGMP was resistant to digestion with S_1 nuclease without annealing to RNA. This material represents rapidly renaturable double-stranded DNA that is probably hairpin or cross-linked (G. Gerard, unpublished data). The synthesis of rapidly renaturable double-stranded DNA in these reaction mixtures is not the result of transcription by MSV DNA polymerase of contaminating DNA, since pretreatment of AMV 70S RNA with heated RNase A (100 μg/ml) completely inhibited the reaction (G. Gerard, unpublished data). If the radioactivity represented by this DNA was subtracted from the total acid-insoluble radioactivity, 65 and 95% of the remaining DNA labeled with [^{3}H]TMP and [^{3}H]dGMP, respectively, hybridized to AMV 70S RNA. The fact that the majority of the single-stranded DNA labeled with either [^{3}H]TMP or [^{3}H]dGMP hybridized to RNA in the presence of excess

TABLE 3. *Demonstration of the complementarity between [^{3}H]DNA synthesized from AMV 70S RNA by DEAE-cellulose-purified MSV DNA polymerase and viral 70S RNA as determined by S_1 nuclease*[a]

Labeled DNA	70S RNA annealed	Nuclease	Cl_3CCOOH insoluble counts/min	% of total radioactivity resistant to nuclease	% of single-stranded DNA resistant to nuclease
Native adenovirus [^{32}P]DNA[b]	None	−	25,260		
		+	25,317	100	
Denatured adenovirus [^{32}P]DNA[b]	None	−	24,259		
		+	984	4	
[^{3}H]dTMP-labeled AMV DNA	None	−	1,197		
		+	241	20	0
[^{3}H]dTMP-labeled AMV DNA	MSV	−	1,375		
		+	377	27	9
[^{3}H]dTMP-labeled AMV DNA	AMV	−	1,591		
		+	1,142	72	65
[^{3}H]dGMP-labeled AMV DNA	None	−	631		
		+	126	20	0
[^{3}H]dGMP-labeled AMV DNA	MSV	−	758		
		+	233	31	13
[^{3}H]dGMP-labeled AMV DNA	AMV	−	751		
		+	720	96	95

[a] AMV [^{3}H]DNA was synthesized and hybridizations were performed and analyzed with S_1 nuclease as described in Materials and Methods.

[b] These samples were not annealed before nuclease treatment.

poly(U) demonstrates that the DNA product is a complementary heteropolymeric copy of the RNA template.

Subunit structure of purified MSV DNA polymerase (RNase H-I). Sodium dodecyl sulfate-polyacrylamide gels were run of MSV DNA polymerase purified through the poly(A)·oligo(dT)-cellulose, DNA cellulose, and glycerol gradient stages of purification. A densitometric tracing of a sodium dodecyl sulfate-disc gel of poly(A)·oligo(dT)-cellulose-purified MSV DNA polymerase is shown in Fig. 5A. The MSV DNA polymerase had three prominent polypeptides of mol wt 82,000, 68,000, and 60,000 relative to the marker proteins adenovirus hexon, bovine serum albumin, gamma globulin, ovalbumin, and chymotrypsinogen (markers not shown). These three polypeptides were also the primary proteins present in preparations of the MSV enzyme purified through additional steps of DNA-cellulose chromatography and glycerol gradient centrifugation (data not shown). The ratio of the amounts of the three proteins as determined from the areas of the peaks was different for enzyme purified through each step. This observation plus the fact that the ratios of enzymatic activities present in these three purification fractions were relatively constant (see Table 2) suggests that all three proteins are probably not responsible for enzymatic activity. They are, however, closely related in molecular properties and are

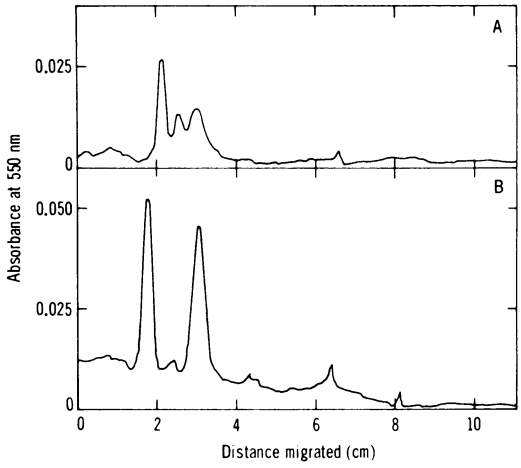

FIG. 5. *Densitometric tracings of sodium dodecyl sulfate-polyacrylamide disc gels of MSV (A) and AMV (B) DNA polymerase purified through poly(A)·oligo(dT)-cellulose. Samples were prepared and electrophoresis was carried out as described in Materials and Methods. Electrophoresis times were 7 h in (A) and 7.5 h in (B). Gels were stained with Coomassie blue, and tracings at 550 nm were made with a Beckman Acta V equipped with a linear transport.*

difficult to fractionate. AMV DNA polymerase purified through poly(A)·oligo(dT)-cellulose had only two prominent subunits with mol wt of 65,000 and 100,000 that correspond to α and β of $\alpha\beta$ AMV DNA polymerase (7). The ratio of the

areas of α and β was not 1.0 to 1.6 as was reported for the AMV two-subunit enzyme purified by different procedures (7), but in this case was 1.0 to 1.0.

Purification of MSV RNase H-II. (i) Phosphocellulose chromatography. The Sephadex G-100 peak fractions of the small (30,000 mol wt) RNase H activity (RNase H-II) from MSV that was free of DNA polymerase activity (see Fig. 1) were pooled and chromatographed on a phosphocellulose column (Fig. 2B). RNase H-II eluted as a single peak from phosphocellulose at 0.5 M KCl (Fig. 2B).

(ii) DEAE-cellulose chromatography and glycerol gradient centrifugation. The pool of phosphocellulose-purified RNase H-II activity was dialyzed to reduce the KCl concentration to 0.01 M and applied to a DEAE-cellulose column (Fig. 6). A major peak of activity was reproducibly observed eluting at 0.055 M KCl. Variable quantities of small peaks were usually also obtained that eluted at higher and lower KCl concentrations. A sample from the pool of peak tubes eluting at 0.055 M KCl was used to determine the sedimentation coefficient of RNase H-II on a 20 to 40% glycerol gradient in 0.35 M KCl (see Materials and Methods) spun at 46,000 rpm for 42 h (data not shown). Relative to a hemoglobin marker, the sedimentation coefficient was 2.6S. Using the equation relating sedimentation coefficient to molecular weight already described (18), the molecular weight was calculated to be 30,000, in agreement with the molecular weight estimated for RNase H-II (Fig. 1) from Sephadex G-100 gel filtration.

Biochemical characterization of RNase H-I and RNase H-II. To ascertain whether RNase H-II and DNA polymerase associated RNase H-I from MSV differ in properties other than molecular size, the divalent metal ion optima and substrate specificities of the two enzymes were determined. The metal ion optima for RNase H-II were 10 to 15 mM Mg^{2+} and 5 to 10 mM Mn^{2+}, and at these optimal concentrations, Mg^{2+} was preferred over Mn^{2+} by 2.5-fold (Fig. 7A). Most significantly, RNase H-I could not use Mg^{2+} to catalyze the degradation of [^{3}H]poly(A), and its Mn^{2+} optimum was very sharp at 2 mM (Fig. 7B).

RNase H-I and RNase H-II specifically degraded the poly(A) portion of poly(A)·poly(dT), and not poly(dT) of the hybrid or poly(A) alone, and were therefore true RNase H activities (Table 4). There was some DNase and single-stranded RNase activity present in Sephadex G-100 and phosphocellulose-purified RNase H-I, but the bulk of these contaminating activities was removed by poly(A)·oligo(dT)-cellulose chromatography.

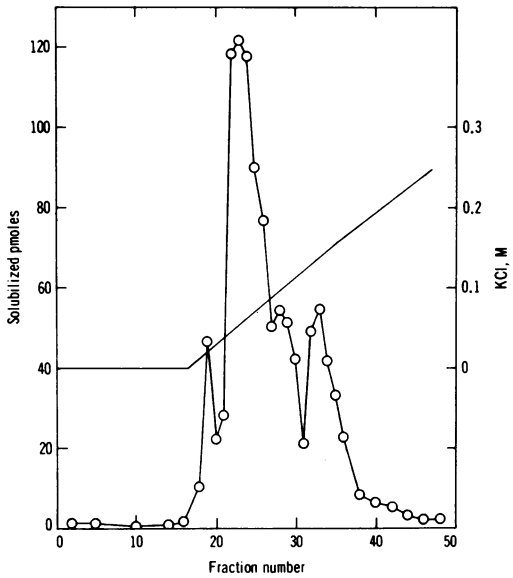

FIG. 6. *Chromatography of MSV RNase H-II on DEAE-cellulose. The dialyzed phosphocellulose pool of RNase H-II was chromatographed on DEAE-cellulose as described in Materials and Methods. Samples (10 µl for 20 min) from various fractions were assayed for RNase H activity as described in Materials and Methods with [^{3}H]poly(A)·poly(dT) and 15 mM $MgCl_2$.*

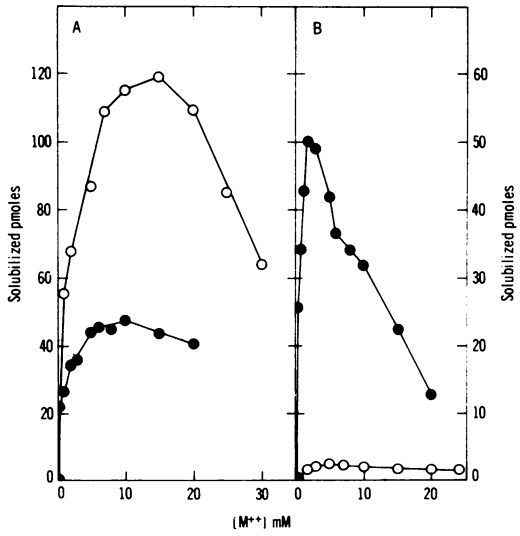

FIG. 7. *Metal ion optimum of RNase H-I and RNase H-II from Moloney MSV. Phosphocellulose-purified RNase H-I (B) (20 µl for 40 min) and RNase H-II (A) (10 µl for 20 min) were assayed for RNase H activity with [^{3}H]poly(A)·poly(dT) at the $MgCl_2$ (○) and $MnCl_2$ (●) concentrations indicated.*

The specificities of RNase H-I and RNase H-II for various [^{3}H]RNA-DNA hybrids were determined (Table 5) at the concentrations of divalent metal ion optimal for activity with [^{3}H]poly(A)·poly(dT) as substrate (see Fig. 7). Under these assay conditions, poly(A)·poly(dT) was the best substrate for both enzymes. Both RNase H-I and RNase H-II could use poly(U)·poly(dA) as substrate, but both enzymes preferred Mn^{2+} to Mg^{2+} as the divalent metal ion for the degradation of poly(U). The one outstanding difference in substrate specificity between these enzymes was the inefficiency with which RNase H-II degraded poly(C) in poly(C)·poly(dG) relative to the RNA in the other homopolymer hybrids. Both enzymes preferred Mg^{2+} for the degradation of the labeled RNA in the calf thymus DNA-RNA hybrid.

DISCUSSION

In our initial experiments on the purification of Moloney MSV DNA polymerase (6), we found that the RNase H activity present in MSV was partially separable from the DNA polymerase activity by DEAE-cellulose chromatography. Subsequent phosphocellulose chromatography revealed only one peak of RNase H activity that co-eluted with DNA polymerase activity. However, the gradient used to develop the phosphocellulose column terminated at 0.45 M potassium phosphate and therefore probably did not elute RNase H-II activity, which we have shown in this report elutes from phosphocellulose at 0.5 M KCl (see Fig. 2B).

The two RNase H activities we observe in MSV appear to represent distinctly different enzymes since (i) RNase H-I requires Mn^{2+} (2 mM) for activity with [^{3}H]poly(A)·poly(dT), whereas RNase H-II prefers Mg^{2+} (10 to 15 mM), (ii) the two enzymes have different substrate specificities, (iii) RNase H-I has an apparent mol wt of 70,000, whereas RNase H-II is much smaller (30,000 mol wt), and (iv) the two enzymes elute from phosphocellulose at different KCl concentrations. In addition, the

TABLE 4. *Nuclease activities present in MSV RNase H-I and RNase H-II during purification*[a]

Enzyme fraction	[^{3}H]poly(A) or [^{3}H]poly(dT) solubilized (pmol/μl of enzyme per unit incubation)		
	[^{3}H]poly(A)·poly(dT)	[^{3}H]poly(dT)·poly(A)	[^{3}H]poly(A)
Sephadex G-100 pools			
RNase H-I[b]	1.53	0.31	0.12
RNase H-II[c]	27.3	0.04	<0.01
Phosphocellulose pools			
RNase H-I[b]	1.28	0.10	0.06
RNase H-II[c]	34.6	0.02	<0.01
Poly(A)·oligo(dT)-cellulose pool			
RNase H-I[b]	0.44	<0.01	<0.01

[a] Assays were carried out as described for RNase H in Materials and Methods. [^{3}H]poly(A) was at 3 μM, and [^{3}H]poly(dT)·poly(A) were at 0.8 and 6 μM, respectively.
[b] Reaction mixtures contained 2 mM $MnCl_2$ and were incubated for 20 min.
[c] Reaction mixtures contained 10 mM $MgCl_2$ and were incubated for 10 min.

TABLE 5. *Substrate specificities of RNase H activities isolated from Moloney murine sarcoma-leukemia virus*[a]

Enzyme	Metal ion concn (mM)	RNA solubilized (pmol/μl of enzyme per 20 min)			Calf thymus [^{3}H]RNA-DNA[b]
		[^{3}H]poly(A)·poly(dT)	[^{3}H]poly(U)·poly(dA)	[^{3}H]poly(C)·poly(dG)	
RNase H-I[c]	Mn^{2+} (2)	0.58	0.31	0.50	0.02
	Mg^{2+} (10)		0.08		0.16
RNase H-II	Mn^{2+} (10)	5.13	3.45	0.82	0.28
	Mg^{2+} (10)	13.0	0.11	0.21	0.48

[a] Assay mixtures contained the constituents described in Materials and Methods. KCl was at 50 and 20 mM, respectively, in the assay mixtures for poly(A)·oligo(dT)-cellulose-purified RNase H-I and DEAE-cellulose-purified RNase H-II. The concentrations of the RNA and DNA in each synthetic hybrid were 3 and 12 μM, respectively; [^{3}H]RNA in the calf thymus [^{3}H]RNA-DNA was 0.2 μM.
[b] Five-minute instead of 20-min incubations were used.
[c] Samples assayed, 5 and 10 μl, contained 20 and 40 nmol of $MnCl_2$.

two enzymes, upon limited digestion (5% solubilization) of [^{3}H]poly(A) in [^{3}H]poly(A)·poly(dT), produce oligonucleotide products of different sizes separable from the larger polynucleotide substrate by electrophoresis on 7 M urea-15% polyacrylamide gels (8). RNase H-I produces two species of oligonucleotides, of approximately 10 and 40 nucleotides, similar to α and $\alpha\beta$ AMV RNase H (8), whereas RNase H-II produces only one species of approximately 10 nucleotides (Grandgenett and Gerard, unpublished data). Detectable amounts of polynucleotides, intermediate in length between these oligonucleotides and the polynucleotide substrate, are not produced by either enzyme upon limited digestion of poly(A). At present we do not known whether RNase H-I or RNase H-II requires a hybrid substrate with free termini for activity, and therefore whether these enzymes are exo- or endoribonucleases (13, 14). Furthermore, the question of the origin of RNase H-I (see below) and RNase H-II has not been settled.

RNase H-II did not have associated DNA polymerase activity and appeared to behave as a single enzyme species during Sephadex G-100 and phosphocellulose chromatography. The majority of the enzyme activity in RNase H-II eluted as a single peak from DEAE-cellulose at 0.055 M KCl. Smaller and variable amounts of activity eluted at higher and lower salt concentrations from DEAE-cellulose, however, indicating the influence of contaminating nucleic acids or perhaps the presence of other minor species of enzyme.

RNase H-I copurified with MSV DNA polymerase. All attempts to separate the two activities failed; in addition, there was a good correspondence between RNase H and DNA polymerase activity during each fractionation procedure. MSV RNase H-I and DNA polymerase cosedimented with a sedimentation coefficient of 4.4*S*, which corresponds to an apparent mol wt of 70,000. This is in agreement with the molecular weight determined for Rauscher MLV DNA polymerase (1, 17, 21). It would therefore appear that both MSV activities reside on a protein subunit(s) of 70,000 mol wt. However, since sodium dodecyl sulfate-gel analysis showed that all of our most highly purified MSV DNA polymerase preparations contained three polypeptides with mol wt of 82,000, 68,000, and 60,000, we cannot draw any definitive conclusions regarding the structural relationship of the two enzyme activities. Similar to our results, Weimann et al. (26) found that the RNase H and DNA polymerase activities in Friend leukemia virus were not separable by standard fractionation procedures, their most highly purified enzyme preparation containing two components of mol wt 51,000 and 67,000 on sodium dodecyl sulfate-gels. The sedimentation coefficient (5.7*S*) determined for the Friend leukemia virus enzymes corresponded to a mol wt of 123,000 (26).

Wu et al. (27) have also found multiple RNase H activities in murine RNA tumor viruses (Rauscher MLV and Kirsten MSV) separable by phosphocellulose chromatography. The bulk of the virion RNase H activity in Rauscher MLV and Kirsten MSV was not bound by phosphocellulose at 0.2 M KCl and was devoid of associated DNA polymerase activity. The MLV and MSV DNA polymerases eluted at 0.4 M KCl from phosphocellulose, along with some RNase H activity that could not be separated from the DNA polymerase activity by subsequent DEAE-cellulose chromatography. The ratio between RNase H and DNA polymerase activity in these purified enzyme preparations was four- to eightfold lower than the ratio found for the AMV enzymes. In addition, [^{3}H]poly(A)·poly(dT) substrate of high specific radioactivity (about 16,000 counts/min per pmol) was required to detect the DNA polymerase-associated RNase H activity. These results are in contrast to those reported here for Moloney MSV, where the bulk of the RNase H activity, RNase H-II (free of DNA polymerase), eluted from phosphocellulose at 0.5 M KCl (see Fig. 2B). More importantly, the RNase H activity (RNase H-I) in Moloney MSV associated with the purified DNA polymerase eluted from phosphocellulose at 0.2 M KCl and could be easily detected with [^{3}H]poly(A)·poly(dT) of low specific radioactivity (50 counts/min per pmol). RNase H-I was present in amounts comparable with that found in purified AMV DNA polymerase (see Table 2).

By treatment of Rauscher murine leukemia virions with nonionic detergent, Wu et al. (27) were able to demonstrate endogenous RNA-directed DNA polymerase activity free of any detectable RNase H activity that had a density of 1.21 to 1.26 g/ml on sucrose gradients. These results indicate that in Rauscher MLV the RNase H activity does not reside in the virus core, where the DNA polymerase activity is located. We have not been able to confirm or contradict this finding with Moloney MSV, since repeated attempts to generate cores have not as yet been successful.

It is clear from the results reported here as well as elsewhere (1, 12, 19, 26, 27) that Mn^{2+} is greatly preferred over Mg^{2+} as the divalent metal ion for assaying poly(A)·$(dT)_{12-18}$-

directed and 70S RNA-directed DNA polymerase activity and DNA polymerase-associated RNase H activity {[^{3}H]poly(A)·poly(dT) degradation} of mammalian type C RNA tumor viruses. In fact, in our hands the 70S RNA-directed DNA polymerase activity and RNase H-I activity (DNA polymerase associated) of MSV could barely be detected in the presence of Mg^{2+} at a concentration that is optimal for AMV DNA polymerase-RNase H. Therefore, analyses (25) that attempt to compare the enzyme activities in avian versus mammalian RNA tumor viruses using Mg^{2+} as the only divalent metal ion for assay are inconclusive.

Reports from several other laboratories (1, 24) indicate that purified RNA-directed DNA polymerases from mammalian RNA tumor viruses have reduced ability to read viral 70S RNA relative to AMV DNA polymerase. We have demonstrated that purified Moloney MSV DNA polymerase preparations containing three polypeptides, RNase H activity, and no detectable contaminating nucleic acids can efficiently transcribe heteropolymeric regions of viral 70S RNA. The purified MSV enzyme copied 70S RNA relative to poly(A)·$(dT)_{12-18}$ about one-half as well as AMV DNA polymerase. It is interesting that there was a selective loss of activated calf thymus DNA-directed and 70S RNA-directed DNA polymerase and RNase H activity relative to poly(A)·$(dT)_{12-18}$-directed polymerase activity during purification of Moloney MSV DNA polymerase, but not AMV DNA polymerase (see Table 2). Selective inactivation of a greater magnitude than obtained here might explain the loss of 70S RNA-transcribing activity observed during purification of woolly monkey and Mason Pfizer monkey virus DNA polymerase (1) and Rauscher MLV DNA polymerase (24, 25). In the case of hamster leukemia virus (24), where no endogenous reaction directed by the virion 70S RNA could be demonstrated and where most of the viral particles were noninfectious, the viral enzymes might be structurally defective.

The role that RNase H might play during viral infection and transformation of cells has not been defined. There is good evidence in avian RNA tumor viruses that RNA-directed DNA polymerase and RNase H activity reside on the same polypeptide subunit (7, 23), and several mechanisms for RNase H involvement in proviral DNA synthesis have been suggested (for review see ref. 10). It does not follow a priori in mammalian RNA tumor viruses that the two activities reside on the same polypeptide subunit. It is important, however, to isolate and characterize any RNase H activity present in a particular virus, since (i) that enzyme might play a role in vivo in viral genome transcription and integration, and (ii) comparisons with known cellular enzymes could then be made.

Substantial evidence has accumulated indicating that virion-associated RNA-directed DNA polymerase is encoded by viral RNA and is required for proviral DNA synthesis (10, 23). Therefore, purified RNA tumor virus DNA polymerase should be able to transcribe viral 70S RNA in vitro, unless (i) certain catalytic capabilities of the enzyme are selectively inactivated during purification, (ii) in vitro conditions used for assay are not suitable for demonstrating activity with a particular polymerase, (iii) factors necessary for activity located in the virion are removed during purification, (iv) the DNA polymerase in the majority of the particles in a virus population is structurally defective, or (v) a cellular or viral-coded protein(s) necessary for reverse transcription is not contained in purified virions. Moloney MSV DNA polymerase purified by the procedure described here has the ability to effectively transcribe heteropolymeric regions of viral 70S RNA.

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