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Ribonuclease H: a Ubiquitous Activity in Virions of Ribonucleic Acid Tumor Viruses

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Ten ribonucleic acid (RNA) tumor viruses grown in five different host cell species and three non-oncogenic viruses from three different virus groups have been examined for ribonuclease H content. Three different substrates were used to assay ribonuclease H: calf thymus [^{3}H]RNA-deoxyribonucleic acid (DNA) hybrid prepared with denatured calf thymus DNA and *Escherichia coli* DNA-directed RNA polymerase, ^{3}H-polyadenylic acid [^{3}H-poly(A)] complexed to polydeoxythymidylic acid [poly(dT)], and ^{3}H-polyuridylic acid [^{3}H-poly(U)] complexed to polydeoxyadenylic acid [poly(dA)]. All ten RNA tumor viruses contained ribonuclease H activity which degraded the RNA of both the calf thymus hybrid and poly(A)-poly(dT), whereas only the ribonuclease H in the Moloney strain of murine sarcoma-leukemia virus and in RD-feline leukemia virus hydrolyzed the RNA strand of poly(U)-poly(dA). No appreciable ribonuclease H activity was detected in influenza, Sendai, or vesicular stomatitis virus. The ribonuclease H and RNA-directed DNA polymerase activities in Moloney murine sarcoma-leukemia virus were inseparable by phosphocellulose chromatography or glycerol gradient centrifugation, but appeared to be partially separated by diethylaminoethyl-cellulose chromatography.

Although numerous enzymatic activities are associated with the purified virions of ribonucleic acid (RNA) tumor viruses (1, 7, 13, 16–18, 20, 21, 24, 25), only the RNA-directed deoxyribonucleic acid (DNA) polymerase (reverse transcriptase) is considered a biochemical marker characteristic of RNA tumor viruses. The presence of 60 to 70*S* RNA coupled with RNA-directed DNA polymerase activity has been used as a sensitive diagnostic tool to detect RNA tumor virus particles (22). The presence of long polyadenylic acid [poly(A)] sequences in viral RNA may constitute an additional criterion for RNA tumor viruses (8, 10, 14).

It was recently demonstrated that avian myeloblastosis virus (AMV) contained an enzyme, ribonuclease H, which specifically degrades the RNA moiety of RNA-DNA hybrids (19). Ribonuclease H activity in AMV could not be separated from DNA polymerase activity by several fractionation techniques, indicating that it might be a property of the viral polymerase. We have confirmed these results with AMV polymerase purified by diethylaminoethyl (DEAE)-cellulose and phosphocellulose chromatography and glycerol gradient centrifugation (Grandgenett, Gerard, and Green, *unpublished data*). The close association between ribonuclease H and reverse transcriptase in AMV suggested to us that ribonuclease H might be an additional criterion for the identification of RNA tumor viruses. We therefore examined other RNA tumor viruses as well as non-oncogenic RNA viruses for the presence of ribonuclease H activity. Furthermore, we investigated the relationship between ribonuclease H and DNA polymerase in the Moloney strain of murine sarcoma-leukemia virus by attempting to separate the two activities on DEAE-cellulose and phosphocellulose columns and by glycerol gradient centrifugation. The results of these studies are reported here.

MATERIALS AND METHODS

Materials. Whatman DEAE-cellulose (DE-52), phosphocellulose (P-11), and DEAE-cellulose(DE-81) paper discs were purchased from Reeve Angel, New York, N.Y. Nonidet P-40 (NP-40) and Triton N-101 were obtained from Shell Chemical Co. and Research Products International Corp., respectively. ^{3}H-labeled nucleoside triphosphates were from New England Nuclear Corp. Calf thymus DNA and unlabeled nucleoside triphosphates were purchased from Sigma Chemical Co. Poly(A), polyuridylic acid [poly(U)], ^{3}H-poly(A), and ^{3}H-poly(U) were obtained from Miles Laboratories, Inc. Polydeoxyadenylic acid [poly(dA)] and polydeoxythymidylic acid [poly(dT)] were purchased from P-L Biochemicals. Oligo(dA)$_{12-18}$ and oligo(dT)$_{12-18}$ were from Collaborative Research, Inc., Waltham, Mass.

Viruses. The Moloney and Harvey strains of murine sarcoma-leukemia virus, MSV-MLV(M) and MSV-MLV(H), were grown and purified as previously described (11). RD-feline leukemia virus (RD-FLV) and RD-114 were grown in the RD-FLV and RD-114 cell lines kindly provided by Robert McAllister, and purified by the procedure of Duesberg and Robinson (6). Feline sarcoma-leukemia virus (Gardner) [FSV-FLV(G)], feline leukemia virus (Rickard) [FLV(R)], Rous sarcoma virus (Schmidt-Ruppin) [RSV(S-R)], and murine leukemia virus (Rauscher) [MLV(R)] were purchased from Electro-Nucleonics Lab., Inc. Mason Pfizer monkey virus was kindly provided by Mumtaz Ahmed of the John L. Smith Memorial for Cancer Research, Pfizer, Inc., Maywood, N.J. Avian myeloblastosis virus (AMV) was generously provided by J. Beard, Duke University, and was purified by the same procedure (6). Purified influenza virus and Sendai virus were gifts from I. Schultze and D. Kingsburg, respectively. Vesicular stomatitis virus (VSV), generously supplied by S. Bose, was propogated in our laboratory in NIH-3T3 mouse fibroblast cells, and the virus was purified by the procedure of Duesberg and Robinson (6).

Preparation of hybrids for ribonuclease H assay. Calf thymus ^{3}H-RNA-DNA hybrid was prepared with denatured calf thymus DNA and *Escherichia coli* DNA-directed RNA polymerase. The reaction mixture (1.0 ml) contained 0.05 M tris(hydroxymethyl) aminomethane (Tris)-hydrochloride, *p*H 7.8; 0.005 M $MgCl_2$; 0.005 M 2-mercaptoethanol; 0.002 M each of adenosine triphosphate (ATP), guanosine triphosphate, and cytidine triphosphate; 3 μM ^{3}H-uridine triphosphate (UTP) (1.1×10^4 counts per min per pmole); 160 μg of calf thymus DNA denatured by heating at 100 C for 10 min; 0.05 M NaCl, and 100 μg of *E. coli* RNA polymerase (glycerol gradient enzyme prepared as described by Burgess [5]). The reaction was terminated after 15 min at 37 C by the addition of sodium dodecyl sulfate to 0.5%. The RNA-DNA hybrid was purified by chromatography on a G-50 Sephadex column (1.2 by 50 cm), and was concentrated by ethanol precipitation in the presence of 180 μg of purified yeast RNA. The hybrid was dissolved in 0.1 M NaCl-0.01 M Tris-hydrochloride, *p*H 7.8, and dialyzed extensively against the same buffer, and the concentration was adjusted to 2 nmoles of RNA nucleotides/ml (2,800 counts per min per pmole). The hybrid was 85% resistant to ribonuclease A (5 μg/ml, 10 min at 37 C), but was completely sensitive after heat denaturation. The ribonuclease A was heated at 80 C for 10 min before use, and the assay was carried out under the conditions described below for assaying ribonuclease. When denatured calf thymus ^{3}H-RNA-DNA was required, the hybrid was heated at 100 C for 10 min before use.

^{3}H-poly(U)-poly(dA) hybrid was prepared by incubating ^{3}H-poly(U) (35 counts per min per pmole, 170 μM) and poly(dA) (700 μM) in 0.1 M NaCl-0.02 M Tris-hydrochloride, *p*H 7.4, for 24 hr at 25 C. ^{3}H-poly(A)-poly(dT) hybrid was prepared in a similar manner with ^{3}H-poly(A) (10 counts per min per pmole, 100 μM) and poly(dT) (400 μM). ^{3}H-poly(U)-poly(dA) (6,000 counts/min) was 97% resistant to ribonuclease A (5 μg/ml) during a 10-min incubation at 37 C under the conditions described below for assaying ribonuclease. Under the same assay conditions, all of ^{3}H-poly(U) at 3 nmoles/ml was solubilized. ^{3}H-Poly(A)-poly(dT) (4,000 counts/min) was 96% resistant to ribonuclease T_2 (Sankyo, Co., Tokyo, Japan) at 20 units/ml during a 10-min incubation at 37 C in 0.01 M Tris-hydrochloride, *p*H 0.03 M Tris-hydrochloride (*p*H 7.8), 0.1 M $(NH_4)_2SO_4$, 7.4 and 0.002 M ethylenediaminetetraacetatic acid (EDTA). ^{3}H-Poly(A) at 5 nmoles/ml was 54% solubilized under the same conditions.

Ribonuclease assays. The reaction mixture (0.1 ml) for assaying ribonuclease H activity (19) contained 0.03 M Tris-hydrochloride (*p*H 7.8), 0.1 M $(NH_4)_2SO_4$, 0.002 M $MnCl_2$, and one of the following substrates: calf thymus ^{3}H-RNA-DNA (11,500 counts/min), ^{3}H-poly(A)-poly(dT) (4,000 counts/min), or ^{3}H-poly(U)-poly(dA) (6,000 counts/min). The reaction mixture for assaying single-stranded ribonuclease was the same except that 3 μM ^{3}H-poly(U) (11,000 counts/min) was used as substrate. Reactions were terminated after 10 min of incubation at 37 C by the addition of cold 10% trichloroacetic acid (90 μliters) and calf thymus DNA (30 μg) as carrier. Radioactivity of acid-soluble material in the supernatant fluid was determined after centrifugation at 8,000 rev/min for 15 min.

RNA-directed DNA polymerase assay. The reaction mixture (0.1 ml) for assaying RNA-directed DNA polymerase activity contained 0.02 M Tris-hydrochloride (*p*H 8.3), 0.05 M NaCl, and either (i) 0.002 M $MgCl_2$, 0.1 mM ^{3}H-thymidine triphosphate (126 counts per min per pmole), 10 μM poly(A), and 10 μM oligo$(dT)_{12\text{-}18}$, or (ii) 0.001 M $MnCl_2$, 0.1 mM ^{3}H-dATP (82 counts per min per pmole), 10 μM poly(U), and 10 μM oligo$(dA)_{12\text{-}18}$. The reaction was terminated after a 10-min incubation at 37 C by the addition of 10 μliters of 0.2 M EDTA. The amount of polymer product formed was determined by assay on Whatman DEAE-cellulose (DE-81) paper discs (3).

DEAE-cellulose chromatography. A sample of MSV-MLV(M) containing 30 mg of protein was lysed with 1% NP-40 and applied to a 1 by 23 cm DEAE-cellulose column. The column was washed with three column volumes of 0.01 M potassium phosphate (*p*H 8.0), 2 mM dithiothreitol, 0.1 mM EDTA, and 10% glycerol and developed with a 180-ml continuous gradient of 0.01 to 0.5 M potassium phosphate, *p*H 8.0, containing the same constituents. Fractions of 1.9 ml were collected, and samples were assayed for DNA polymerase and ribonuclease H. The potassium phosphate concentration in each fraction was determined conductimetrically. The peak fractions were pooled and diluted to reduce the potassium phosphate concentration to 0.01 M.

Phosphocellulose chromatography. The pooled, diluted peak fractions from the DEAE-cellulose column were applied to a 1 by 15 cm phosphocellulose column. The column was washed and developed as described for DEAE-cellulose except that 2.0-ml fractions were collected. Samples from various fractions were assayed for DNA polymerase and ribonu-

clease H. The peak fractions were pooled for further purification.

Glycerol gradient centrifugation. The pooled peak fractions from the phosphocellulose column were concentrated by cyclic dialysis against 50 and 10% glycerol in 0.2 M potassium phosphate buffer (*p*H 8.0), 0.1 mM EDTA, and 0.002 M dithiothreitol. This procedure reduces the sample volume by 50 to 60% during each cycle. Prior to centrifugation, the enzyme preparation was dialyzed against 10% glycerol in 0.05 M Tris-hydrochloride (*p*H 8.0), 0.35 M KCl, 0.2% NP-40, 0.1 mM EDTA, and 0.003 M dithiothreitol. The final dialyzed sample (0.3 ml) was layered on a 4.6-ml 20 to 40% linear glycerol gradient in the same buffer and was centrifuged at 46,000 rev/min at 2 C for 22 hr in a Spinco SW50.1 rotor. Fractions were collected from the bottom of the centrifuge tube. Hemoglobin and ^{125}I-gamma globulin were run in a separate tube as sedimentation markers.

RESULTS

Ribonuclease H activity in the virions of RNA tumor viruses. The most sensitive assay for ribonuclease H involves the use of a ^{3}H-RNA-DNA hybrid prepared with *E. coli* DNA-directed RNA polymerase, heat-denatured calf thymus DNA, ^{3}H-UTP of high specific radioactivity, and the three other common ribonucleoside triphosphates (12, 23). RNA tumor virus preparations were disrupted with NP-40 and tested for ability to digest the calf thymus ^{3}H-RNA-DNA as shown in Table 1 (column 1). Most virus preparations degraded from 25 to 50% of the RNA in the hybrid during 10 min of incubation. The nuclease activity is specific for an RNA-DNA hybrid and is indeed ribonuclease H (23) since denaturing the hybrid reduced the amount of RNA degraded 80 to 90% in most cases (Table 1, column 2). The small amount of ^{3}H-RNA of the denatured hybrid that was solubilized probably reflects the presence in RNA tumor virus preparations of a ribonuclease which degrades single-stranded RNA (15, 20). The ^{3}H-RNA of the denatured calf thymus hybrid was protected from more extensive single-stranded ribonuclease degradation by the presence of excess yeast RNA which was added during hybrid preparation (see Materials and Methods). The presence of single-stranded ribonuclease in these RNA tumor virus preparations was verified using ^{3}H-poly(U) as the substrate (Table 1, column 5).

Absence of ribonuclease H activity in the virions of non-oncogenic RNA viruses. Three non-

TABLE 1. *Ribonuclease H activity in the virions of RNA viruses*

Virus	RNA solubilized per 0.1 ml per 10 min[a, b, c]									
	Calf thymus native hybrid		Calf thymus denatured hybrid		^{3}H-poly(A)-poly(dT)		^{3}H-poly(U)-poly(dA)		^{3}H-poly(U)	
	pmoles	%	pmoles	%	pmoles	%	pmoles	%	pmoles	%
AMV	11.2	27	0.8	2	180	5	0	0	740	24
RSV(S-R)	17.8	43	2.0	5	940	23	0	0	170	5
MSV-MLV(M)	23.0	56	3.7	9	1,990	50	100	6	730	23
MSV-MLV(H)	17.5	42	1.5	4	260	6	0	0	130	4
MLV(R)	12.8	31	2.7	7	120	3	0	0	290	9
FSV-FLV(G)	18.2	44	2.4	6	470	12	0	0	600	19
FLV(R)	13.1	32	0.8	2	560	14	0	0	40	1
RD-FLV	21.5	52	3.1	8	1,890	48	420	24	1,130	42
RD-114	10.3	25	2.4	6	170	4	0	0	170	5
Mason-Pfizer Monkey	5.6	14	2.2	5	25	1	0	0	ND	
VSV	3.9	9	4.8	12	ND		ND		ND	
Influenza	0.5	1	ND[d]		ND		ND		ND	
Sendai	1.4	3	2.3	6	ND		ND		ND	

[a] Virus preparations were suspended in 10% glycerol, 0.01 M Tris-hydrochloride (*p*H 7.8), 0.1 M NaCl, 0.0001 M EDTA, and 0.05 M dithiothreitol and were lysed with 0.5% NP-40 or with 1% Triton N-101 for influenza and VSV. Ten microliters (3 to 7 μg of protein) of each lysate was assayed for nuclease activity as described in Materials and Methods.

[b] The average of duplicate determinations which varied by less than ± 10%.

[c] The concentration and total amount of RNA present in each hybrid in a 0.1-ml reaction mixture were the following: 0.04 nmole/ml (11,500 counts/min) of RNA nucleotides in ^{3}H-labeled native and denatured calf thymus hybrid; 4 nmoles/ml (4,000 counts/min) ^{3}H-poly(A) in ^{3}H-poly(A)-poly(dT); and 1.7 nmoles/ml (6,000 counts/min) ^{3}H-poly(U) in ^{3}H-poly(U)-poly(dA). ^{3}H-poly(U) was at 3 nmoles/ml (11,000 counts/min).

[d] Not determined.

oncogenic RNA viruses, influenza virus, VSV, and Sendai virus, two of which, influenza virus and VSV, carry their own RNA-directed RNA polymerase, were also assayed for ribonuclease H activity with the calf thymus hybrid (Table 1). Little or no ribonuclease H activity was detected in influenza or Sendai virus. VSV solubilized 9% of the native calf thymus RNA-DNA in 10 min. This degradation was probably not due to the action of ribonuclease H since denatured hybrid was a better substrate than native hybrid for VSV. The possibility cannot be excluded, however, that VSV contains ribonuclease H activity less active than the ribonuclease H in RNA tumor viruses.

Poly(A)-poly(dT) and poly(U)-poly(dA) as substrates for ribonuclease H. In additional experiments, we tested the ability of the ribonuclease H in each RNA tumor virus to degrade the RNA of the homopolymer hybrid duplexes, ^{3}H-poly(A)-poly(dT) and ^{3}H-poly(U)-poly(dA). The ribonuclease H activity in all 10 RNA tumor viruses degraded the ^{3}H-poly(A) of ^{3}H-poly(A)-poly(dT) (Table 1, column 3). But only the ribonuclease H in MSV-MLV(M) and RD-FLV solubilized detectable amounts of ^{3}H-poly(U)-poly(dA) during 10 min of incubation (Table 1, column 4). It is of interest that ^{3}H-poly(U)-poly(dA) is a good substrate for the ribonuclease H found in calf thymus cells (12, 23).

Baltimore and Smoler (2) have reported that poly(A)-oligo(dT) is a good template for the DNA polymerase of both AMV and MLV(M), whereas only MLV(M) polymerase can use poly(U)-oligo(dA) as template. In light of the fact that ribonuclease H in both MSV-MLV(M) and AMV can degrade poly(A)-poly(dT), while only MSV-MLV(M) ribonuclease H is able to degrade poly(U)-poly(dA) (see Table 1), the possibility existed that a qualitative correlation could be made between the template specificity of RNA-directed DNA polymerase and the substrate specificity of ribonuclease H in a given virus. RNA-directed DNA polymerase activity in all 10 RNA tumor viruses was therefore assayed with poly(A)-oligo(dT) and poly(U)-oligo(dA) as templates (Table 2). A comparison of the results shown in Table 1 and 2 is presented in Table 3. The correlation between ribonuclease H substrate activity and DNA polymerase template activity seems to hold quite well for AMV, RSV(S-R), MSV-MLV(M), RD-114, and Mason Pfizer, less well for MSV-MLV(H), MLV(R), and RD-FLV, and not at all for FSV-FLV(G) and FLV(R). In the absence of a correlation between template and substrate specificity of reverse transcriptase and ribonuclease H in every RNA tumor virus, very little can be implied from these data at this time regarding the relationship between the two enzyme activities in the virus.

TABLE 2. *RNA-directed DNA polymerase activity in RNA tumor viruses*

Virus	^{3}H-nucleotide incorporated (pmoles per 0.1 ml per 10 min)[a]	
	Poly(A)-oligo(dT)$_{12-18}$	Poly(U)-oligo(dA)$_{12-18}$
AMV	490	0
RSV(S-R)	260	0
MSV-MLV(M)	1,580	280
MSV-MLV(H)	585	39
MLV(R)	300	25
FSV-FLV(G)	1,400	190
FLV(R)	240	90
RD-FLV	280	42
RD-114	185	0
Mason-Pfizer Monkey	75	0

[a] Samples (10 μliters) of the same virus lysates described in Table 1 were assayed for RNA-directed DNA polymerase as described in Materials and Methods.

Fractionation of MSV-MLV(M) ribonuclease H and RNA-directed DNA polymerase. As mentioned before, ribonuclease H found in AMV cannot be separated from AMV polymerase by a number of fractionation procedures. To determine if the two activities are closely associated in an RNA tumor virus from another host species, we purified the MSV-MLV(M) enzymes. Figure 1A shows the profiles of ribonuclease H and DNA polymerase activity obtained after fractionation of detergent-lysed virus on DEAE-cellulose. The peak fractions of both activities eluted at 0.084 M potassium phosphate; however, there was proportionately more ribonuclease H than DNA polymerase activity which eluted at higher salt concentrations. To improve resolution on DEAE-cellulose, a second batch (4 mg) of detergent-lysed MSV-MLV(M) was fractionated on a 1 by 12 cm DEAE-cellulose column developed with a 100-ml continuous gradient of 0.01 to 0.2 M potassium phosphate buffer. The activity profiles obtained (data not shown) were similar to those in Fig. 1A except that the DNA polymerase peak fraction eluted at 0.078 M potassium phosphate and the ribonuclease H peak at 0.090 M. This represented a separation of two column fractions. When the peak fractions, numbers 32 to 44, from the DEAE-cellulose column (Fig. 1A) were pooled and fractionated with a linear salt gradient on phosphocellulose, the enzyme activities chromatographed completely together (Fig. 1B). After concentration of the pooled phosphocellulose peak fractions, numbers 43 to

TABLE 3. *Comparison of the template and substrate specificities of the DNA polymerase and ribonuclease H activities in RNA tumor viruses*[a]

Virus	Relative DNA polymerase activity		Relative ribonuclease H activity	
	Poly(A)-oligo(dT)	Poly(U)-oligo(dA)	^{3}H-poly(A)-poly(dT)	^{3}H-poly(U)-poly(dA)
AMV	++	−	+	−
RSV(S-R)	+	−	+++	−
MSV-MLV(M)	++++	+	++++	+
MSV-MLV(H)	++	±	+	−
MLV(R)	+	±	+	−
FSV-FLV(G)	++++	+	++	−
FLV(R)	+	+	+	−
RD-FLV	+	±	+++	+++
RD-114	+	−	+	−
Mason-Pfizer Monkey	+	−	±	−

[a] Relative activities were estimated from the data in Table 1 and 2 after normalization to a constant amount of viral protein. The degree of activity is expressed on a scale of ± to ++++, and inactive polymers are designated as −.

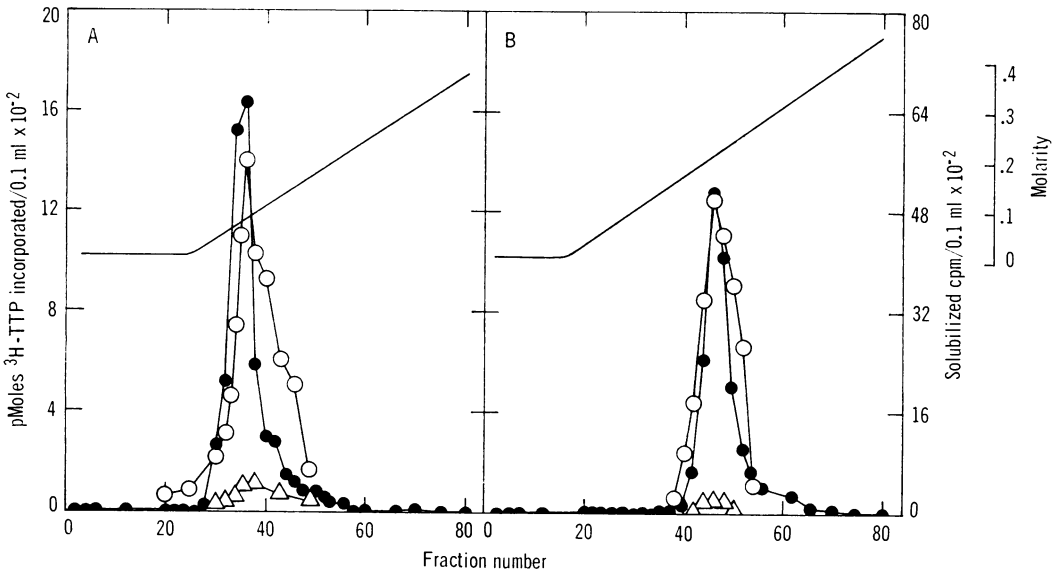

FIG. 1. *Purification of MSV-MLV(M) enzyme activities by DEAE-cellulose and phosphocellulose column chromatography. A, Thirty milligrams of purified MSV-MLV(M) protein was fractionated on a DEAE-cellulose column as described in Materials and Methods. Samples (5 μliters) of various fractions were assayed for DNA polymerase with poly(A)-oligo(dT)* (●) *and for ribonuclease H with native* (○) *and denatured* (△) *calf thymus ^{3}H-RNA-DNA as described in Materials and Methods. B, Pooled peak fractions from the DEAE-cellulose column were fractionated on a phosphocellulose column as described in Materials and Methods. Samples (5 μliters) of various fractions were assayed for DNA polymerase* (●) *and ribonuclease H* (○ *or* △) *as described above.*

51, the enzyme preparation contained only four major protein subunits as determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (data not shown). The concentrated phosphocellulose peak was subjected to rate-zonal centrifugation on a glycerol gradient (Fig. 2). No separation of the two activities was obtained under conditions which separated hemoglobin (4.3*S*) and ^{125}I-gamma globulin (6.5*S*) by four fractions.

DISCUSSION

These studies indicate that, in addition to reverse transcriptase, ribonuclease H is a second enzyme activity characteristic of RNA tumor viruses. Ten RNA tumor viruses grown in

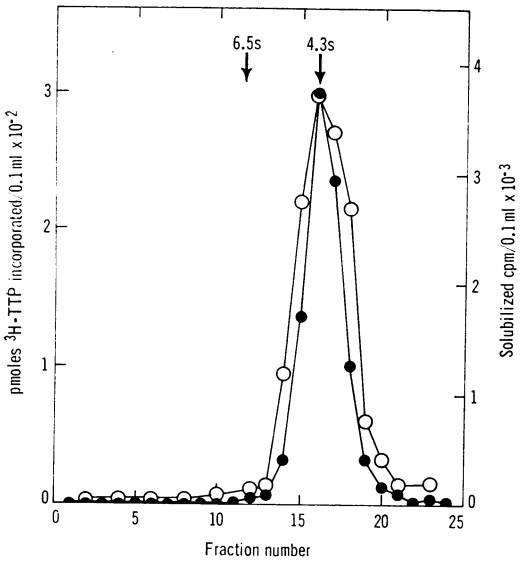

FIG. 2. *Glycerol gradient centrifugation of MSV-MLV(M) DNA polymerase and ribonuclease H activities. Pooled peak fractions from the phosphocellulose column were concentrated and analyzed by glycerol gradient centrifugation as described in Materials and Methods. Samples (2 μliters) of various fractions were assayed for DNA polymerase with poly(A)-oligo(dT)* (●) *and for ribonuclease H with native calf thymus* ^{3}*H-RNA-DNA* (○).

chicken, rat, mouse, cat, and human cell lines all contained ribonuclease H activity whereas non-oncogenic viruses representative of the myxo-, paramyxo-, and rhabdovirus groups had little or no detectable ribonuclease H activity.

The possibility exists that in some cases the ribonuclease H associated with a RNA tumor virus is a host-specific enzyme acquired during virus maturation. This possibility is difficult to exclude at present, since very little is known about the ribonuclease H content of host cell lines themselves. In the case of AMV, ribonuclease H (19) as well as DNA polymerase (4) is associated with the viral core, suggesting that both enzyme components are viral specific. The fact that three non-oncogenic RNA viruses which mature by budding from the host cell plasma membrane do not contain appreciable ribonuclease H activity argues against the ribonuclease H in RNA tumor viruses being a host-cell contaminant.

MSV-MLV(M) ribonuclease H and RNA-directed DNA polymerase appear to be partially separable by DEAE-cellulose chromatography. However, no separation of the two activities was obtained during subsequent steps of phosphocellulose chromatography and glycerol gradient centrifugation. These preliminary results can be explained in several different ways. The two enzyme activities may reside on different protein molecules which are very similar in size, conformation, and charge distribution and are therefore difficult to separate. Perhaps MSV-MLV(M) ribonuclease H and DNA polymerase are tightly associated components of a multienzyme complex which is partially dissociated by DEAE-cellulose chromatography. Numerous examples of multienzyme complexes which are aggregates of different, functionally related enzymes occur throughout nature (9). Attempts have been made in several laboratories (19; D. Baltimore and D. Smoler, *personal communication*; Grandgenett, Gerard, and Green, *in manuscript*) to separate AMV ribonuclease H and DNA polymerase with procedures similar to those described in this report. Thus far no separation has been achieved. Moreover, we have obtained direct evidence through the use of non-dissociating and sodium dodecyl sulfate disc-gel electrophoresis that AMV ribonuclease H and DNA polymerase reside on the same protein molecule. Disc-gel electrophoresis was not used to attempt to separate the MSV-MLV(M) enzymes because of a lack of sufficient amounts of the purified MSV-MLV(M) proteins.

These results indicate that the structural relationship between DNA polymerase and ribonuclease H may vary from one virus to another, and that a more extensive investigation of a number of RNA tumor viruses will be required to establish definitively the structural relationship between the two enzyme activities. The ubiquitous presence of ribonuclease H in RNA tumor viruses and its close association with DNA polymerase suggest that ribonuclease H may play a role in the replication of virus-specific DNA, but at present our conception of this role remains only speculative.

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LITERATURE CITED

1. Baltimore, D. 1970. Viral RNA-dependent DNA polymerase. Nature (London) 226:1209–1211.
2. Baltimore, D., and D. Smoler. 1971. Primer requirement and template specificity of the DNA polymerase of RNA tumor viruses. Proc. Nat. Acad. Sci. U.S.A. 68:1507–1511.
3. Blatti, S. P., C. J. Ingles, T. J. Lindell, P. W. Morris, R. F. Weaver, F. Weinberg, and W. J. Rutter. 1970. Structure and

regulatory properties of eukaryotic RNA polymerase. Cold Spring Harbor Symp. Quant. Biol. 35:649–657.
4. Bolognesi, D. P., H. Gelderblom, H. Bauer, K. Mölling, and G. Hüpen. 1972. Polypeptides of avian RNA tumor viruses. V. Analysis of the core. Virology 47:567–578.
5. Burgess, R. R. 1969. A new method for the large scale purification of *Escherichia coli* DNA-dependent RNA polymerase. J. Biol. Chem. 244:6160–6167.
6. Duesberg, P. H., and N. S. Robinson. 1966. Nucleic acid and proteins isolated from the Rauscher mouse leukemia virus (MLV). Proc. Nat. Acad. Sci. U.S.A. 55:219–227.
7. Gantt, R. R., K. J. Stromberg, and F. Montes de Oca. 1971. Specific RNA methylase associated with avian myeloblastosis virus. Nature (London) 234:35–37.
8. Gillespie, D., S. Marshall, and R. C. Gallo. 1972. RNA of RNA tumor viruses contains poly A. Nature N. Biol. 236: 227–231.
9. Ginsburg, A., and E. R. Stadtman. 1970. Multienzyme systems. Annu. Rev. Biochem. 39:429–472.
10. Green, H., and M. Cartas. 1972. The genome of RNA tumor viruses contains polyadenylic acid sequences. Proc. Nat. Acad. Sci. U.S.A. 69:791–794.
11. Green, M., M. Rokutanda, K. Fujinaga, R. K. Ray, H. Rokutanda, and C. Gurgo. 1970. Mechanism of carcinogenesis by RNA tumor viruses. I. An RNA-dependent DNA polymerase in murine sarcoma viruses. Proc. Nat. Acad. Sci. U.S.A. 67:385–393.
12. Hausen, P., and H. Stein. 1970. Ribonuclease H. An enzyme degrading the RNA moiety of DNA-RNA hybrids. Eur. J. Biochem. 14:278–283.
13. Hurwitz, J., and J. P. Leis. 1972. RNA-dependent DNA polymerase activity of RNA tumor viruses. I. Directing influence of DNA in the reaction. J. Virol. 9:116–129.
14. Lai, M. C., and P. H. Duesberg. 1972. Adenylic acid-rich sequence in RNAs of Rous sarcoma virus and Rauscher mouse leukemia virus. Nature (London) 235:383–386.
15. Leis, J. P., and J. Hurwitz. 1972. RNA-dependent DNA polymerase activity of RNA tumor viruses. II. Directing influence of RNA in the reaction. J. Virol. 9:130–142.
16. Miller, L. K., and R. D. Wells. 1971. Nucleoside diphosphokinase activity associated with DNA polymerases. Proc. Nat. Acad. Sci. U.S.A. 68:2298–2302.
17. Mizutani, S., O. Boettiger, and H. M. Temin. 1970. A DNA-dependent DNA polymerase and a DNA endonuclease in virions of Rous sarcoma virus. Nature (London) 228:424–427.
18. Mizutani, S., H. M. Temin, M. Kodama, and R. O. Wells. 1971. DNA ligase and exonuclease activities in virions of Rous sarcoma virus. Nature N. Biol. 230:232–235.
19. Mölling, K., D. P. Bolognesi, H. Bauer, W. Büsen, H. W. Plassmann, and P. Hausen. 1971. Association of viral reverse transcriptase with an enzyme degrading the RNA moiety of RNA-DNA hybrids. Nature N. Biol. 234:240–243.
20. Quintrell, N., L. Fanshier, B. Evans, W. Levinson, and J. M. Bishop. 1971. DNA polymerase(s) of Rous sarcoma virus: effects of virion-associated endonuclease on the enzymatic product. J. Virol. 8:17–27.
21. Roy, P., and D. H. L. Bishop. 1971. Nucleoside triphosphate phosphotransferase. A new enzyme activity of oncogenic and non-oncogenic "budding" viruses. Biochim. Biophys. Acta 235:191–206.
22. Schlom, J., and S. Spiegelman, 1971. Simultaneous detection of reverse transcriptase and high molecular weight RNA unique to oncogenic RNA viruses. Science 174:840–843.
23. Stein, H., and P. Hausen. 1970. Factors influencing the activity of mammalian RNA polymerase. Cold Spring Harbor Symp. Quant. Biol. 35:709–717.
24. Strand, M., and J. T. August. 1971. Protein kinase and phosphate acceptor proteins in Rauscher murine leukemia virus. Nature N. Biol. 233:137–140.
25. Temin, H. M., and S. Mizutani. 1970. RNA-dependent DNA polymerase in virions of Rous sarcoma virus. Nature (London) 226:1211–1213.