Separation of Ribonuclease H and RNA Directed DNA Polymerase (Reverse Transcriptase) of Murine Type-C RNA Tumor Viruses

(anion exchange chromatography/core structure/antibody to reverse transcriptase)

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ABSTRACT Ribonuclease H (RNA·DNA-hybrid ribonucleotidohydrolase, EC 3.1.4.34) has been reported to copurify with reverse transcriptase (RNA directed DNA polymerase) of RNA tumor viruses. In addition, viral specific ribonuclease H and reverse transcriptase of avian type-C viruses are thought to be part of the same polypeptide. In this report we show that a fraction of the ribonuclease H activity from Rauscher murine leukemia and Kirsten murine sarcoma viruses was separated from reverse transcriptase by anion exchange chromatography while the remaining portion co-purified with the viral polymerase. The amount of this co-purified nuclease activity was about 4- to 8-fold lower than the activity found in avian myeloblastosis virus (with respect to the ratio of ribonuclease H to reverse transcriptase) and this nuclease activity can only be detected by using labeled substrate of high specific radioactivity. However, a complete separation of ribonuclease H activity from reverse transcriptase was obtained by purifying core structures of the virus by sucrose density gradient centrifugation. While reverse transcriptase was present in the cores, there was no detectable ribonuclease H. Furthermore, a specific antibody against Rauscher leukemia virus reverse transcriptase did not inhibit any virion associated ribonuclease H activity. Our results suggest that in these virions these two enzyme activities reside in two separate molecules and probably in two different compartments of the virus. These findings emphasize a basic difference between the avian and murine type-C virus DNA polymerases.

Ribonuclease H (RNase H) (RNA·DNA-hybrid ribonucleotidohydrolase, EC 3.1.4.34) has been found in association with reverse transcriptase in type-C RNA tumor virus particles (1-6), prompting some speculations on the mechanisms of integration of proviral DNA into host DNA (1, 3, 5). In avian myeloblastosis virus (AvMV), the virus-associated RNase H activity co-purified with reverse transcriptase through cation and anion exchange chromatography and sucrose or glycerol velocity gradient centrifugation (1, 3, 4, 6). These two activities co-migrated during nondissociating gel electrophoresis (6). It was concluded that two enzymatic activities reside on a single polypeptide (6). There is much less information on RNase H of mammalian viruses. Grandgenett *et al.* (2) studied RNase H activity associated with various types of RNA tumor viruses. Among these, reverse transcriptase from Moloney murine sarcoma virus (M-MuSV)

was partially purified. Similar to the avian system, the RNase H activity co-purified with reverse transcriptase activity, but this could be accounted for by similar properties of two distinct enzymes. In this respect, it is important to note that the estimated molecular size of both mammalian cellular RNase H (7-9) and reverse transcriptase of type-C mammalian viruses (10, 11) are similar.

In this paper we report our studies on the relationship between RNase H and reverse transcriptase activities associated with mouse type-C viruses. Our results indicate that at least in murine type-C viruses, RNase H and reverse transcriptase not only reside on two separate polypeptides but are probably located in two different "compartments" of the virus.

MATERIALS AND METHODS

Viruses. Rauscher murine leukemia virus (R-MuLV) (10^{11} particles per ml) grown in JLSV9 cells, was obtained through the Virus Cancer Program, National Cancer Institute. Kirsten murine sarcoma virus (Ki-MuSV) (10^{11} particles per ml) grown in normal rat-kidney cells was purchased from Electro-Nucleonics.

Preparation of Substrates. $[^3\text{H}](\text{A})_n \cdot (\text{dT})_m$ was prepared by incubating 0.24 μmoles of $[^3\text{H}]\text{ATP}$ (20.9 Ci/mmol) and 5 A_{260} units of poly(dT) with 330 μg of *Escherichia coli* RNA polymerase in a reaction mixture of 2.5 ml containing 50 mM Tris·HCl (pH 7.9), 5 mM MgCl_2 , 1 mM MnCl_2 , 5 mM dithiothreitol (DTT), and 5% glycerol at 37° for 30 min. The reaction mixture was deproteinized with phenol-m-cresol- H_2O (50:7:5, w/v/v containing 0.1% 8-quinolinol) and the polymeric product isolated by precipitation after adjusting to 0.3 M NaCl with 2 volumes of absolute ethanol followed by Sephadex G-50 column chromatography using a buffer containing 10 mM Tris·HCl (pH 7.4), 0.1 M NaCl, and 1 mM EDTA. $[^{32}\text{P}](\text{A})_n \cdot (\text{dT})_m$ was prepared as above except that $[\alpha\text{-}^{32}\text{P}]\text{ATP}$ was used in place of $[^3\text{H}]\text{ATP}$. $(\text{A})_n \cdot [^3\text{H}](\text{dT})_m$ was prepared by the polymerization of $[^3\text{H}]\text{deoxythymidine triphosphate (TTP)}$ (54 Ci/mmol) with R-MuLV reverse transcriptase employing the template-primer, $(\text{dT})_{12-18} \cdot (\text{A})_n$. The details of the reaction are as described under the assay system for reverse transcriptase (see below). The product was isolated and purified as described above. $[^3\text{H}](\text{A})_n$ was prepared by a modified procedure of Singer and Guss (3, 12). The incubation mixture (150 μl) contained 0.28 μmole of $[^3\text{H}]\text{ADP}$ (18 Ci/mmol), 0.1 M glycine buffer (pH 9.0), 10 mM MgCl_2 , 0.4 mM EDTA, 15 μg of bovine-serum albumin (BSA), and

Abbreviations: RNase H, ribonuclease H; R-MuLV, Rauscher murine leukemia virus; Ki-MuSV, Kirsten murine sarcoma virus; AvMV, avian myeloblastosis virus; M-MuSV, Moloney murine sarcoma virus; BSA, bovine serum albumin; PC, phosphocellulose; DTT, dithiothreitol.

FIG. 1. DEAE-cellulose column chromatography of RNase H-like activities and reverse transcriptase activity from the flow-through fractions obtained from the PC column step. The details of DEAE-cellulose column chromatography were described in *Materials and Methods*. The sample applied was the flow-through fractions from the PC column chromatography of Ki-MuSV as described in Table 1. Reverse transcriptase activities were assayed with $(dT)_{12-18} \cdot (A)_n$ as template-primer and RNase H activities with $[^3H](A)_n \cdot (dT)_m$ as substrate. The specific activity of $[^3H]TTP$ was 13,000 cpm/pmole and that of $[^3H](A)_n \cdot (dT)_m$ was 15,000 cpm/pmole. (The numbers on the outer left and inner left ordinates have been multiplied by 10^{-2} and 10^{-3} , respectively.)

0.65 units of polynucleotide phosphorylase (Miles Laboratory). At the end of 3-hr incubation at 37° , the reaction was terminated by adding sarkosyl to 0.3%. The product was purified by gel filtration as described above. ϕX -174 DNA- $[^3H]RNA$ hybrid was prepared using the procedure of Stavrianopoulos *et al.* (13), substituting ϕX -174 DNA in place of f1 DNA as the template, and using $[^3H]UTP$ (12.0 Ci/mmol) to label the RNA strand. The final product was a 1:1 DNA-RNA hybrid as determined by analyses in a cesium sulfate density gradient.

Enzyme Assays. The assays of purified and endogenous viral reverse transcriptase (14, 15) and RNase H (3, 7) were performed according to the procedures previously described. Triton X-100 (0.05%) was present when viruses or virus cores were used as the enzyme source. This concentration of detergent is sufficient to solubilize the enzymes from virus or from cores.

Purification of RNase H. For phosphocellulose column chromatography, phosphocellulose (PC) (P 11, Whatman), precycled by immersing in 4 M NaCl solution for 4–6 hr, was equilibrated with 50 mM Tris-HCl buffer (pH 7.9). The PC column (2.9×25 cm) was equilibrated with buffer 1 [10 mM Tris-HCl (pH 7.9), 0.1 mM EDTA, 1 mM DTT, and 20% glycerol]. The virus suspension (50 ml) was disrupted at 4° with buffer 1 containing 1% Triton X-100 and 0.8 M KCl. After centrifugation ($150,000 \times g$, 1 hr), the supernatant was dialyzed against 100 volumes of buffer 1 and then applied to the PC column. The column was developed with a 360-ml linear gradient between 0.2 and 0.7 M KCl. Fractions of 2.5 ml were collected and aliquots assayed for reverse transcriptase activity using $(dT)_{12-18} \cdot (A)_n$ as template-primer and for RNase H activity using $[^3H](A)_n \cdot (dT)_m$ as substrate. Enzyme

TABLE 1. Reverse transcriptase and RNase H-like activities from Ki-MuSV and R-MuLV in the flow-through and 0.4 M KCl eluate from phosphocellulose column chromatography step*

Virus	Fractions	Reverse transcriptase activity† (pmoles/ml)	RNase H-like activity‡ (pmoles/ml)
MuSV (Kirsten)	Flow-through	131	2675
	0.4 M KCl fraction	9513	17
MuLV (Rauscher)	Flow-through	27	5234
	0.4 M KCl fraction	5119	20

* For this study, fifty milliliters each of Ki-MuSV or R-MuLV were used. See *text* for the details of enzyme purifications. The flow-through fractions from both Ki-MuSV and R-MuLV were 75 ml each and the 0.4 M KCl fractions 60 ml each. Peak fractions were pooled and dialyzed before assay.

† Reverse transcriptase was assayed as follows. Reaction mixtures of 50 μ l contained 50 mM Tris-HCl (pH 7.9), 1 mM Mn acetate, 60 mM KCl, 1 mM DTT, 0.2 mM EDTA, 10 μ g/ml $(dT)_{12-18} \cdot (A)_n$, 5.6 μ M $[^3H]TTP$ (13,000 cpm/pmole), and 20 μ l of enzyme fraction. The reactions were carried out for 45 min at 30° and then the acid-insoluble radioactivity was determined.

‡ RNase H was assayed as follows. Reaction mixtures of 50 μ l contained 50 mM Tris-HCl (pH 7.9), 1 mM Mn acetate, 1 mM DTT, and 10,000 cpm $[^3H](A)_n \cdot (dT)_m$. The reactions were carried out at 37° for 45 min and terminated by adding 250 μ g of yeast tRNA and 0.2 ml of 20% CCl_3COOH containing 20 mM Na pyrophosphate. After standing at 0° for 10 min, the precipitate was removed by centrifugation at $3500 \times g$ for 5 min. Aliquots of 0.2 ml supernatant were added to 10 ml of Aquasol to determine the acid soluble radioactivity. The enzyme activities were converted to picomoles of labeled nucleotide incorporated or released per 45 min/ml of enzyme. The data show the activities of RNase H and reverse transcriptase for the same aliquot of the pooled fractions.

activities were obtained in the flow-through and the 0.4 M KCl eluate. These were separately pooled, dialyzed against 100 volumes of buffer 1, and the enzyme activities further purified as described below.

For DEAE-cellulose column chromatography, precycled DEAE-cellulose (DE 52, Whatman) was equilibrated with 0.05 M Tris-HCl (pH 7.9). The two dialyzed fractions obtained from the PC column described above were separately applied to the DEAE-cellulose column (1.9×15 cm) equilibrated with buffer 1 and eluted with a 200 ml linear gradient between 0.01 and 0.5 M KCl. Fractions of 2.5 ml were collected and aliquots assayed for reverse transcriptase and RNase H activities.

Preparation of Core Structures from R-MuLV. To prepare R-MuLV cores, 1-ml aliquots of the virus particles were treated with various concentrations of Triton X-100 at 30° for 5–10 min. These partially disrupted particles were then placed on a 20–60% (w/w) sucrose density gradient prepared in buffer 1. They were centrifuged at $200,000 \times g$ in an SW 41 rotor (Beckman, Spinco) for 16 hr at 4° . Fractions of 0.5 ml were collected, the density of each fraction was determined, and the location of the particles and cores in the gradient was determined by assaying reverse transcriptase. Normally, the density of intact virus particles was around 1.16 g/ml and the cores around 1.21–1.26 g/ml, which is in agreement with previous studies (16, 17). Very often there are particle struc-

TABLE 2. Substrate specificity for various RNase H-like activities

Source of enzyme†	Activity (pmoles/ml)*			
	[³ H](A) _n ·(dT) _m	[³ H](A) _n	[³ H](dT) _m ·(A) _n	φX-174 DNA·[³ H]RNA
PC flow-through				
Before DEAE-Cellulose	5.1	2.1	1.6	10.2
DEAE-Cellulose eluates				
0.1 M KCl	2.5	0	0	0.36
0.25 M KCl	2.5	1.5	2.2	5.6
0.35 M KCl	1.7	0	0	2.0
PC 0.4 M KCl eluate				
Before DEAE-Cellulose	4.2	0	0	8.8
DEAE-Cellulose eluate				
0.22 M KCl	3.1	0	0	9.2

* The details of RNase H assay are described in Table 1. Mn⁺⁺ was used as divalent cations for assaying all of the enzyme peaks except the 0.35 M KCl DEAE-cellulose eluates in which Mg⁺⁺ was used.

† See Fig. 1 and text for the identification of each enzyme peak.

tures with intermediate density, especially around 1.19 g/ml or with low density around 1.13 g/ml. The formation of these particles with various densities is determined by the concentration of Triton X-100.

RESULTS

RNase H-Like Activities Associated with Murine Type-C Viruses. When Ki-MuSV or R-MuLV was disrupted with Triton and processed on a PC column, nuclease activities which release soluble radioactivity from [³H](A)_n·(dT)_m were found in the flow-through fractions and in the 0.4 M KCl eluate. We call these RNase H-like activities since at this stage of purification it is difficult to distinguish between true RNase H and "pseudo" RNase H. The latter can occur from a combination of deoxyribonucleases and ribonucleases, from exonuclease III-like activity (18), and from phosphodiesterase. The RNase H-like activity in the 0.4 M KCl eluate copurified with the peak activity of reverse transcriptase while the flow-through fractions contained little reverse transcriptase activity, but the majority of RNase H-like activity. Table 1 shows the relative amounts of RNase H-like and reverse transcriptase activities in these fractions.

The RNase H-like activities were further purified by DEAE-cellulose column chromatography (see *Materials and Methods*). When the flow-through fractions from the PC step were then applied to the DEAE-cellulose column, three peaks of nuclease activities were obtained, eluting at 0.1 M, 0.25 M, and 0.35 M KCl, respectively. The reverse transcriptase from the flow-through fraction of the PC column eluted from the DEAE-cellulose column at 0.25 M KCl (Fig. 1).

When the 0.4 M KCl eluate from the PC column was applied to the DEAE-cellulose column, the reverse transcriptase activity eluted in one major peak at 0.22 M KCl with two to three shoulder activities at lower ionic strength. The RNase H-like activity was present in all of these activity regions. The reverse transcriptase activity at the 0.22 M KCl region was then pooled for further study.

Substrate Specificity of RNase H. RNase H is defined as a nuclease which releases acid soluble nucleotides only from the RNA strand of matched DNA·RNA hybrid structures. To determine which of the above mentioned RNase H-like activities are true RNase H, each of these activities was

assayed with [³H](A)_n·(dT)_m, [³H](A)_n, [³H](dT)_m·(A)_n, and φX-174 DNA·[³H]RNA hybrid as substrates. RNase H should only release soluble radioactivity from the RNA strands of [³H](A)_n·(dT)_m and φX-174 DNA·[³H]RNA hybrid and not from [³H](A)_n or [³H](dT)_m·(A)_n. Table 2 shows that the PC flow-through fractions are able to hydrolyze all four substrates. Upon further purification of this fraction on DEAE-cellulose, both the single-strand specific nuclease and exonuclease III-like activities (which would attack both strands of DNA·DNA or DNA·RNA duplex structures) are removed from the 0.1 M and 0.35 M KCl eluates, but the 0.25 M KCl eluate still contains activities which degraded the four substrates tested. However, both the PC 0.4 M KCl eluate and the 0.22 M KCl eluate from the subsequent DEAE-cellulose chromatography of this fraction contain true RNase H activity. Since these fractions contained reverse transcriptase, we conclude that the true RNase H activity copurified with reverse transcriptase in these two fractions. However, the ratio between the RNase H and reverse transcriptase in murine type-C viruses was 4–8-fold lower than the ratio found with these enzyme activities from AvMV. It is important to emphasize that a substrate of high specific radioactivity was used to detect this amount of RNase H. We do not detect this activity with low specific activity substrate. Similarly, Leis *et al.* (19) and Wang and Duesberg (20) did not detect RNase H in purified reverse transcriptase from murine type-C viruses using substrates of low specific activity.

Mode of Action. Keller and Crouch (8) reported that the RNase H associated with AvMV has a higher proportion of dinucleotides compared to tetranucleotides in the digested products. In contrast, the RNase H preparations purified from chick embryo and KB cells gave a higher proportion of tetramers than dimers. We found that the products of RNase H activity in enzyme fractions (post-DEAE-cellulose) of R-MuLV (Fig. 2) and Ki-MuSV (not shown) were mainly tetranucleotides. However, the RNase H activity in the enzyme preparation was extremely low (see Table 2) and it was difficult to drive the reaction to completion. For comparison, products of AvMV RNase H and leukemic cellular RNase H (7) are included in Fig. 2. The results with these two activities confirm that dinucleotides are the predominant

FIG. 2. Analysis of the reaction products of RNase H from viral and cellular origin. The reaction mixtures (25 μ l) contained 50 mM Tris-HCl (pH 8.3), 10 mM MgCl₂ (for AvMV and human leukemic cell enzymes) or 1 mM MnCl₂ (for R-MuLV enzyme), 10 mM β -mercaptoethanol, 500,000 cpm of [³²P](A)_n·(T)_m, and 40 ng each of AvMV (21) or R-MuLV reverse transcriptase (0.2 M KCl eluate from DEAE-cellulose column) or 80 ng of human leukemic cell RNase H (7), as indicated. After incubation at 37° for 60 min, the reaction mixtures were spotted on a DEAE-cellulose sheet (Whatman, DE-81) and subjected to electrophoresis at 33 V/cm using 50 mM KPO₄ buffer (pH 7.5) containing 7 M urea. The electrophoretogram was cut into 1-cm slices along the length of the run and the radioactivity determined in a liquid scintillation counter. (The numbers on the ordinate have been multiplied by 10⁻³.)

digestion products of AvMV RNase H, while the tetramers predominate with the cellular enzyme (8). Apparently, then, the property of RNase H associated with murine viral reverse transcriptase is different from that of AvMV and is not distinguished from cellular RNase H. However, we should emphasize that the isolation of oligonucleotide products does not distinguish between an endonucleolytic or exonucleolytic mode of cleavage (8, 22), nor does a particular distribution of these products indicate that the enzyme is of viral or cellular origin.

Absence of RNase H Activity in R-MuLV Core Structures. Viral reverse transcriptase exists in the core of virus particle (16, 17). To see if RNase H activity was also located in the cores, core structures from R-MuLV were prepared using nonionic detergent. As shown in Fig. 3a, R-MuLV virions banded at a density of 1.16–1.18 g/ml and both RNase H activity and reverse transcriptase activity were located in this density region. When the particles were treated with 0.2% Triton X-100 some cores were liberated. The density of these structures ranged from 1.21 to 1.26 g/ml, similar to previous reports (16, 17). Although these cores contained abundant reverse transcriptase, there was no detectable RNase H activity (Fig. 3c). All RNase H activity banded at a buoyant density of 1.18 g/ml associated with intact virions or migrated to the top of the gradient as a second peak, presum-

FIG. 3. Absence of RNase H in core structures of R-MuLV. The treatment of virus particles with various concentrations of Triton X-100 is described in *Materials and Methods*. The different preparations were centrifuged in a sucrose density gradient as described in *Materials and Methods*. RNase H assays were as described in Table 1. Reverse transcriptase was assayed (on 20- μ l aliquots) by following the endogenous DNA synthesis using the assay system described in Table 1, but omitting (dT)₁₂₋₁₈·(A)_n and adding 0.1 mM each of dATP, dCTP, and dGTP, and incubating at 37°. All activities are expressed as pmoles of [³H]TMP incorporated (for reverse transcriptase) or pmoles of [³H](A)_n solubilized (for RNase H) per 45 min/ml of enzyme. The specific activities of the substrates were the same as in Fig. 1. Fig. 3a, untreated virus; Fig. 3b, virus treated with 0.1% Triton X-100, and Fig. 3c, virus treated with 0.2% Triton X-100. Note the complete absence of detectable RNase H in the cores (density of about 1.21–1.24 g/ml) which do contain reverse transcriptase activity.

ably liberated from the virus by detergent. Similar results were obtained with core preparations from Ki-MuSV. Treatment of virions with lower Triton concentration yielded subviral particles with densities lower than that of cores. These structures still contained both reverse transcriptase and some RNase H activities (Fig. 3b).

The specific activity of the substrate was high (20.9 mCi/ μ mole). Therefore, even minute amounts of RNase H in the cores should have been detected. The relative sensitivity of detection and the ratio of reverse transcriptase activity to RNase H activity in virus particles is illustrated by the data shown in the noncore fractions of these gradients. We observed that when reverse transcriptase activity was measured using (dT)₁₂₋₁₈·(A)_n as template-primer and RNase H activity was measured using [³H](A)_n·(dT)_m as substrate, the ratio of reverse transcriptase to RNase H activities in a copurified enzyme fraction (DEAE-cellulose eluate) was about 600 to 1. Therefore, a minimal detectable RNase H is

TABLE 3. Effect of antibody to R-MuLV reverse transcriptase on the RNase H and reverse transcriptase activities associated with R-MuLV

Experiment	Enzyme fraction†	Percent inhibition of enzyme activity*	
		RNase H	Reverse transcriptase
A (10 µg of IgG)	Purified enzyme	0	80
	Core Fraction (no. 9, Fig. 3c)	N.A.‡	82
	Solubilized Fraction (no. 30, Fig. 3c)	0	70
B (30 µg of IgG)	Purified enzyme	0	89
	DEAE-cellulose column eluates (Fig. 1)		
	0.1 M KCl	0	N.A.
	0.35 M KCl	0	N.A.
	No enzyme (only IgG)	N.A.	N.A.

* The assay systems for reverse transcriptase and RNase H are the same as in Table 1. The enzymes were preincubated with anti R-MuLV reverse transcriptase IgG (10 µg per reaction in Exp. A, and 30 µg per reaction in Exp. B) for 10 min in ice before initiating the reaction by the addition of the remaining assay components. IgG from sera of preimmunized animals was used in control assays and did not affect these enzyme activities.

† For the source of enzymes, see Table 2, and Fig. 3c. The purified enzyme used in this experiment was that obtained from the 0.4 M KCl eluate of the phosphocellulose column chromatography step (Table 1).

‡ No enzyme activity detectable.

with that amount of reverse transcriptase which catalyzes an incorporation of about 2 pmoles of [³H]thymidine monophosphate into acid insoluble form. It should be noted that a 20-µl aliquot of a core fraction (no. 9, Fig. 3c) catalyzed the incorporation of 10.5 pmoles of [³H]thymidine monophosphate when (dT)₁₂₋₁₈·(A)_n was used as template-primer (data not shown) but did not contain any detectable RNase H activity.

One possibility which might account for the absence of RNase H activity in core fractions is the presence of a specific inhibitor of RNase H. With the assumption that dialysis does not inactivate the hypothetical inhibitor, this possibility was ruled out, since the addition of dialyzed core fractions into samples containing RNase H did not inhibit RNase H activity. Although we found that a high concentration of sucrose partially inhibited RNase H, reverse transcriptase was inhibited to the same degree. RNase H activity was still not detected after the core fractions were dialyzed until the sucrose was removed, while the activity of reverse transcriptase was retained and, in fact, increased 2-fold.

The viral origin of the reverse transcriptase activity in the core fractions which is free from RNase H activity was proved by its inhibition by a specific antibody to R-MuLV reverse transcriptase (Table 3, Exp. A).

Lack of Inhibition of RNase H by Antibody to R-MuLV Reverse Transcriptase. As described above, some RNase H copurified with reverse transcriptase during ion exchange chromatography while other RNase H activities did not, but a

clear separation of the two activities was obtained by the isolation of cores. These findings suggested that the copurification of RNase H and reverse transcriptase from intact murine virions reported earlier [(2) and data above] occurs from coincidentally similar physical properties of two distinct polypeptides. For example, the molecular weights of RNase H (7-9) and reverse transcriptase (10, 11) are both about 70,000. If their charge is similar, these two enzyme activities should copurify throughout most of the conventional enzyme purification schemes, and probably coassociate after detergent disruption of the virus.

Additional information bearing on this question was obtained from antibody studies. Specific antibody (IgG) prepared against purified reverse transcriptase from R-MuLV strongly inhibits this polymerase (23). If RNase H activity is separate from reverse transcriptase and if the purified reverse transcriptase used as antigen did not contain an immunogenic quantity of this nuclease, then the immune sera should not inhibit RNase H. The results in Table 3 (Exp. B) shows that 30 µg of this antibody inhibited 90% of reverse transcriptase activity of the enzyme fraction containing both reverse transcriptase and RNase H activities; the same amount of antibody did not inhibit RNase H. Similarly, the antibody inhibited the reverse transcriptase activity from the top fractions of the sucrose gradient (Fig. 3c) but not the RNase H activity in the same peak. All the remaining RNase H-like activities (see Fig. 1) were also not inhibited by the R-MuLV polymerase antibody. The IgG preparation was not contaminated with RNase H-like activity (see last row of Table 3).

DISCUSSION

The cores of R-MuLV and Ki-MuSV do not contain detectable RNase H. This observation combined with the lack of inhibition of RNase H activity by antibody prepared against purified mouse viral reverse transcriptase indicates that the two enzyme activities do not reside on the same polypeptide, and they do not appear to be in the same virion component. The observation that RNase H activity copurified with reverse transcriptase when whole virus was completely disrupted probably resulted from the association of these two physically distinct molecules when they were mixed together during the disruption procedure. This would lead to difficulty in subsequent separation by conventional enzyme purification techniques.

In view of these results with murine viruses, it was of interest to examine core fractions from other species of viruses for RNase H. In fact, Mölling *et al.* state that core fractions from AvMV do contain RNase H (1), and we have recently confirmed this. These observations indicate that there is a basic difference between the murine and avian type-C virus DNA polymerases. Furthermore, we confirmed that the antibody (IgG) to AvMV reverse transcriptase did inhibit both reverse transcriptase and the associated RNase H activity of this virus (4, 6, 24). However, the AvMV DNA polymerase used to prepare antibody probably contained RNase H. The positive antibody inhibition of RNase H then, although in keeping with the argument that the polymerase and RNase H reside on the same polypeptide of this virus, does not substantially add to the evidence. There are, as yet, no available data which conflict with the suggestion that AvMV RNase H does copurify with the polymerase (1, 3, 4, 6) and resides in the same polypeptide (6).

The degradation products of RNase H activity associated with avian viral reverse transcriptase have a high proportion of ribonucleotide dimers [(8) and confirmed here]. In contrast, the predominant products of the RNase H associated with murine virus transcriptase are tetramers. This is another difference between RNase H associated with murine reverse transcriptase and that with AvMV. This suggests that either there is a substantial qualitative difference in the properties of the avian and murine viral RNase H or that, in fact, there is no *viral* RNase H in some type-C viruses. It is possible that all the detected activity arises from cellular contaminants. One possible criterion for distinguishing these two alternatives is to determine whether the RNase H associated with murine viral reverse transcriptase is an exonuclease or an endonuclease, since so far all of the known cellular RNase H activities are endonucleolytic (5, 7, 8). A firmer distinction may be obtained when monospecific antibodies against cellular and virus associated RNase H are available.

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