Mechanistic Independence of Avian Myeloblastosis Virus DNA Polymerase and Ribonuclease H

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Differential inhibition conditions were established for the DNA polymerase and RNase H activities of avian myeloblastosis virus (AMV) with ether-disrupted AMV and a purified enzyme preparation. The RNase H activity of ether-disrupted AMV with (rA)ₙ -(dT)ₙ and (rA)ₙ -(dT)₁₁, as substrates was inhibited 80 to 100% by preincubation with NaF at a final reaction concentration of 27 to 30 mM. Under these conditions, the DNA polymerase activity was inhibited only 0 to 20%. Similar inhibitions were found with exogenous Rous sarcoma virus 35S and 70S RNA-DNA hybrid and φX174 DNA-RNA hybrid as substrates. Studies were also performed with a purified enzyme preparation, in which the two activities essentially co-purified. The RNase H activity was inhibited >80% by 150 mM KCl with three different hybrid substrates, whereas the DNA polymerase activity was uninhibited. The DNA polymerase was completely inactivated by heat denaturation at 41 C or by omission of the deoxytriphosphates from the reaction mixture; the RNase H remained active. These differential inhibition conditions were used to compare the size of the DNA product synthesized with and without simultaneous RNase H action and to examine the effect of inhibition of the DNA polymerase on the size of the RNase H products. The size of the products of one activity was not affected by inhibition of the other activity. These results suggest that the AMV DNA polymerase and RNase H are not coupled mechanistically.

RNase H, which specifically degrades the RNA strand of a DNA-RNA hybrid, is one of the numerous nucleic acid metabolizing activities associated with RNA tumor viruses. Several recent studies (1, 2, 9, 10, 13, 14, 15, 22, 28) demonstrated the co-purification of the avian myeloblastosis virus (AMV) RNase H and DNA polymerase activities, and models were proposed which described a role for RNase H in the replication cycle of these viruses (5, 13, 14, 22). In addition, there was a precedent for exonucleases as integral parts of DNA polymerases: both the Escherichia coli (4) and Micrococcus luteus (18, 19) DNA polymerases are also 5'-specific exonucleases which are specific for double-stranded nucleic acids to give oligonucleotides as products. Hence, the notion of coupled enzyme activities was readily accepted.

In an effort to understand the process of DNA synthesis in RNA tumor virus systems, we wished to determine if the mechanisms of the AMV DNA polymerase and RNase H activities were obligately coupled. Since attempts at physical separation of the two activities in AMV had been unsuccessful to date, our experimental approach was to establish conditions under which one of the activities was preferentially inhibited, leaving the other activity essentially unaltered. Inhibition studies were performed with ether-disrupted AMV and with a purified enzyme preparation using four template-primer/substrate mixtures. The conditions which resulted in preferential inhibition of one activity were used to study the size of the products of the other activity. The results indicate that there is no apparent coupling between the mechanisms of the AMV DNA polymerase and RNase H.

MATERIALS AND METHODS

Nucleic acids. φX174 DNA-³²P]RNA was prepared by transcribing φX174 DNA with E. coli RNA polymerase as described previously (R. M. Wartell, J. E. Larson, and R. D. Wells, J. Biol. Chem., in press) and was purified further by hydroxyapatite chromatography. ³²P]Rous sarcoma virus (RSV) 70S and 35 S RNA (gift of A. M. Q. King) was isolated as described (6; unpublished work). This RNA was used instead of labeled AMV RNA due to the ease of purification of a high-specific-activity, relatively non-fragmented RNA from RSV. However, some studies were performed with ³H-labeled 70S AMV RNA, and
similar results were obtained with both RNAs. 

\[ ^{3}H] rA \], was purchased from Schwarz/Mann, Inc. or 
was prepared (26) with polynucleotide phosphorylase (Miles) to obtain a higher specific activity (90 mCi/
mmol). Nonradioactive \((rA)\), and \((rU)\), were gifts of J. E. Larson. All synthetic RNAs were purified as 
described previously (29). (dT)\(_{n}\) was prepared with 
the calf thymus terminal addition enzyme or with the 
\( M. \) luteus DNA polymerase (11, 18) followed by 
strand separation (3). The preparations had molecular 
weights (30) ranging from \(1 \times 10^{4}\) to \(13 \times 10^{4}\). 
(dT)\(_{10}\) and (dT)\(_{11}\) were prepared and characterized by 
\((rA)\) was a gift from U. RajBhandary. Unlabeled and 
labeled triphosphates were purchased and checked for 
purity as previously described in systems A–C (6, 29, 
31).

**Enzyme preparations.** Avian myeloblastosis virus 
in (plasma) from J. E. Beard was stored at \(-20 \text{ C}\). 
The virus was purified (24) and disrupted with ether 
especially as described previously (6–8).

The purified AMV DNA polymerase-RNase H 
preparation was obtained using the following steps: 
ether-disruption of purified virus, sucrose gradient 
centrifugation, and DEAE-Sephadex column chromato-
graphy. The enzyme activity was eluted from the 
DEAE-Sephadex column with 0.01 M potassium 
phosphate buffer (pH 6.9), 25% ethylene glycol, 5 mM 
dithiothreitol, 1% Nonidet P-40, and 0.3 M NaCl. The 
column fractions were assayed for RNase H and 
DNA polymerase activities as described below using 
\((rA)\)–(dT)\(_{n}\) as substrate or template-primer. The ratio 
of the two activities varied as much as fivefold 
across the column peak; both activities were assayed in 
the linear range of enzyme concentration. In some 
presentations, tailing was observed with the RNase H 
activity, whereas the DNA polymerase consistently 
elicited as a sharp peak. The purified enzyme prepara-
tion rendered 590 nmol of \(^{3}H] rA \), acid soluble per 
mg of protein in 90 min in a standard \[^{3}H] rA \), (dT)\(_{n}\) 
assay and incorporated 615 nmol of dTMP per mg 
of protein in 60 min in the standard \( rA \), (dT)\(_{n}\) assay.

Throughout the purification, the ratio of the two 
activities (total units of DNA polymerase to total 
units of RNase H) at the various stages varied no more 
than twofold; however, a greater variance in this ratio 
was found across the DEAE-Sephadex column.

The enzyme preparation was not homogeneous as 
d judged by SDS-polyacrylamide gel electrophoresis. It 
contained no detectable phosphatase or DNase activity, 
but it did contain nuclease diaphosphokinase activity as 
reported previously (17). The purification procedure 
was originally designed by P. F. Schendel (this laboratory); details of the procedure are available 
on request to the authors.

**DNA polymerase assay.** The DNA polymerase 
activity was assayed with the synthetic template-
primers, \((rA)\)–(dT)\(_{n}\) and \((rA)\)–(dT)\(_{11}\), and also with 
the natural templates, \( \Phi X 174 \) DNA-RNA hybrid, 
AMV 70S RNA, and RSV 70S and 35S RNA. Unless 
otherwise stated, the standard 0.1 ml reaction con-
tained: Tris-hydrochloride buffer (pH 8.1), 50 mM; 
\( MgCl_{2}, 10 \text{ mM}\); \( NaCl \) or \( KCl, 30 \text{ to } 60 \text{ mM}\); 
dithiothreitol, 20 mM; rATP, 0.5 mM; \((rA)\), and (dT)\(_{n}\) or 
(dT)\(_{11}\), 60 \text{ M } \mu \text{ M each}; dTTP, 108 \text{ M } \mu \text{ M}; \[^{3}P] \text{dTTP or } 
[^{3}H] \text{dTTP, } \geq 1 \times 10^{4} \text{ counts per min per reaction; } 
and enzyme as indicated. When necessary, the 
enzyme was diluted with dilution buffer (0.05 M Tris-
hydrochloride (pH 8.1), 5.0 mM \( MgCl_{2}, 2.0 \text{ mM } \beta-
d-mannoselactose, 1 mg/ml bovine serum albumin). 
The polynucleotide template–primer components were 
mixed and annealed at 65 \text{ C} for 10 min and then 
slowly cooled. When natural templates were utilized 
instead of the synthetic polymers, the same 
conditions were used except that all four dNTPs were 
added, three unlabeled at concentrations of 90 \text{ M } \mu \text{ M} 
each and the labeled dNTP at a concentration of 10 
\text{ M}. The concentrations of the AMV 70S RNA and 
RSV 70S and 35S RNA were not known; \( \Phi X 174 \) hybrid 
was used at a final reaction concentration of 40 \text{ M}. 
Control reactions (no enzyme) had backgrounds of 10 
to 20 pmol/ml. The reactions were incubated at 41 \text{ C} 
for the length of time indicated. The incorporation of 
radioactive dNMP into acid-insoluble material was 
monitored essentially as described previously (11, 23).

**RNase H assay.** The RNase H activity was as-
sayed with the following hybrid substrates: 
\[^{3}H] rA\)–(dT)\(_{n}\), \[^{3}H] rA\)–(dT)\(_{11}\), \( \Phi X 174 \) DNA-RNA, 
\[^{3}P] \text{SSS} \) or 70S RSV RNA, and \[^{3}H] \text{70S AMV RNA. The RSV 
and AMV RNAs were used with the } \text{DNA transcribed from them by AMV DNA polymerase to }

provide the hybrid structure for RNase H. 
(\( rA \)–(dT)\(_{n}\) hybrid components were mixed in an 
equimolar ratio, heated to 65 \text{ C} for 10 min, and then 
slowly cooled.

The components and conditions of the standard 
RNase H reactions were the same as for the DNA 
polymerase reactions, except that the labeled dNTPs 
were omitted. At specific time intervals, portions 
were withdrawn and acid-soluble radioactivity was 
determined as previously described (16, 18). The control 
assays for single-stranded and double-stranded 
RNAs were the same as the standard assay, except 
that \[^{3}H] rA\) or \[^{3}H] rA\)–(rU)\(_{n}\), were used as substrates.

**Size analysis of the DNA polymerase products.** The 
DNA polymerase reactions were prepared as 
described above with conditions varied to inhibit the 
RNase H activity as indicated. The reactions were 
stopped by adding EDTA (pH 7.5) to a final concen-
tration of 50 mM on ice. Potassium hydroxide was 
added (0.3 M final concentration) to hydrolyze the 
RNA template. After hydrolysis at 37 \text{ C} for 16 h, the 
alkali was neutralized by titrating with HCl or by 
adding Dowex-50 in the acid form. The product then 
was dialyzed essentially as described (31) to remove 
the dNTPs and salt, or the product was desalted by 
column chromatography on Sephadex G-50 columns 
(2.4 cm\(^{3}\) column volume, 0.75 cm diameter). The 
method of neutralization and salt removal had no 
influence on the size of the product although the 
recoveries varied from 50 to 90%. The purified prod-
ucts were layered onto 5 to 20% sucrose (in 0.01 M 
Tris-hydrochloride, pH 8.1; 0.1 M NaCl; \(^{10-5} \) M 
EDTA) gradients and were centrifuged at 40,000 rpm 
for 4 h in an SW50.1 rotor. The gradients were dripped 
into 200-\mu l fractions. The acid-insoluble radioac-
tivity in 20- to 100-\mu l volumes and the density
across the gradients were determined. The recovery from the sucrose gradients was 80 to 100%.

Other methods. Descending paper chromatography on Whatman #1 paper was performed in the following systems: (A) isobutyric acid-concentrated ammonium hydroxide-H2O (66:1:33); (B) ethanol:1.0 M ammonium acetate buffer (pH 7.5) (7:3); (C) 0.1 M sodium phosphate (pH 6.8)-ammonium sulfate-n-propanol (100:60:2, vol/vol/vol). Radioactivity was detected by liquid scintillation counting on 1-cm strips of the chromatograms. Other details were described previously (7, 8, 29).

RESULTS

Characteristics of the enzyme preparations. Studies were performed with both ether-disrupted AMV and a purified DNA polymerase-RNase H preparation using several template-primer substrates. Before differential inhibition attempts were made, studies were performed to establish the linear range of enzyme concentration for the DNA polymerase and RNase H activities in order to determine their relative amounts.

Ether-disrupted AMV. The RNase H activity, measured by the degradation of [3H](rA)n hybridized with (dT)n, was essentially linear over the range of 0 to 5.0 µl of disrupted AMV in a 100-µl reaction; 8 nmol of [3H](rA)n per ml was made acid soluble in 60 min by 5 µl of disrupted AMV. The reaction kinetics were linear for at least 90 min of incubation at 41 C with an enzyme concentration of 20 µl or less in a 100-µl reaction (data not shown). The reaction using [3H](rA)n-(dT)11 was also linear for at least 90 min of incubation at 41 C with 20 µl of AMV per 100-µl reaction; however, the extent of degradation in 1 h was approximately 1.3-fold greater than observed with [3H](rA)n-(dT)n.

The DNA polymerase activity, measured by the incorporation of dTMP into acid-insoluble material with (rA)n-(dT)n as template-primer, was linear over the concentration range of 0 to 5.0 µl of enzyme in a 100-µl reaction; 18 nmol of dTMP per ml was incorporated in 60 min by 5 µl of disrupted AMV. The DNA polymerase reaction with (rA)n-(dT)n remained linear for only the first 30 min of incubation at 41 C with 5.0 µl or more of disrupted AMV in a 100-µl reaction. With less than 5.0 µl of enzyme in the reaction, synthesis proceeded linearly for at least 90 min. Similar results were found with (rA)n-(dT)11 (data not shown). The amount of DNA synthesis in 1 h with (rA)n-(dT)11 was approximately threefold greater than with (rA)n-(dT)n. When (rA)n-(dT)n was considered, the ratio of the amount of dTMP incorporated to the amount of [3H](rA)n made acid-soluble with 2 µl of enzyme after 60 min was approximately 3:1. Studies in this and other laboratories (1, 2, 5–7, 9, 13, 22) have established optimum reaction conditions for the disrupted AMV DNA polymerase and RNase H activities.

The presence of ribonucleases (12, 25) other than RNase H was studied by providing (rA)n or (rA)n-(rU)n as substrate. The amount of activity observed with these substrates was only 3.0 and 1.4%, respectively, of that found using the hybrid substrate. Thus, RNase H was the predominant RNase under these assay conditions.

Purified DNA polymerase-RNase H preparation. The purified enzyme preparation, in which the DNA polymerase and RNase H activities essentially co-purified, was studied to determine maximum reaction conditions and to quantitate the relative amounts of the two activities. The influence of the following variables on the enzyme activities was determined: (dT)n concentration from 0 to 160 µM or the (dT)11 concentration from 10 to 200 µM with the (rA)n concentration constant at 40 µM; (rA)n-(dT)n concentration from 0 to 70 µM; deoxytriphosphate concentration from 0 to 0.18 mM; the divalent metal ion (MgCl2, MnCl2, or MgCl2 + MnCl2); and MgCl2 concentration from 0 to 30 mM. The optimum reaction conditions are described in Materials and Methods. Detailed results of these studies are not described herein but are available on request from the authors.

Experiments were then performed to determine the linear range of enzyme concentration for the purified activities. The ratio of the amount of dTMP incorporated to the amount of [3H](rA)n made acid soluble in 2 h with (rA)n-(dT)n was approximately 1:1. Since the RNase H activity was more stable during storage (unpublished data; 22), this ratio changed after storage of the enzyme preparation at 5 C.

Inhibition studies. To study the association and possible coupling of the two activities, it seemed appropriate to find methods that would preferentially inhibit one of the activities, leaving the other activity essentially uninhibited. Several reaction requirements and inhibitors were studied.

NaF inhibition. Results obtained previously in this lab (5) indicated that an appropriate concentration of NaF would inhibit the RNase H and not the DNA polymerase activity in ether-disrupted AMV. The effect of 0 to 75 mM NaF (final reaction concentration) on the DNA polymerase and RNase H reactions with (rA)n-(dT)n is shown in Fig. 1. At 24 to 30 mM
NaF, the RNase H was inhibited greater than 70%, whereas the DNA polymerase was inhibited less than 25%. Titration experiments also were performed using (rA)$_n$-(dT)$_n$ and [H]$I(rA)_n$-(dT)$_n$, respectively. A 20-μl volume of disrupted AMV was used in each 100-μl reaction after preincubation with an amount of NaF necessary to provide the indicated final concentrations after addition to the reaction mixtures. The preincubations were for 30 min at 0 °C. The KCl concentration of the reactions was decreased as the NaF concentration was increased to maintain a constant salt concentration. The arrow at 27 mM indicates the inhibitor concentration used for further studies.

NaF, the RNase H was inhibited greater than 70%, whereas the DNA polymerase was inhibited less than 25%. Titration experiments also were performed using (rA)$_n$-(dT)$_n$, and the inhibition patterns were similar to those observed with (rA)$_n$-(dT)$_n$ (data not shown). The RNase H reaction kinetics using (rA)$_n$-(dT)$_n$, with and without 27 mM NaF, were linear; the reaction was inhibited 87% in 90 min by 27 mM NaF. Alternatively, kinetic analysis showed that the DNA polymerase reaction was inhibited only 15% in 30 min and 27% in 90 min with 27 mM NaF (results not shown).

The NaF inhibition effect on the RNase H of ether-disrupted AMV was confirmed with [P]$T$0S and 35S RSV RNA and ϕX174 DNA:[H]RNA as substrates. In all cases, at least 70% inhibition (average of 81% for four test cases) was observed after preincubation of the disrupted AMV with 30 mM NaF (final reaction concentration) for 30 min at 0 °C. Sucrose gradi-
enzyme, the RNase H activity of the disrupted AMV using (rA)ₙ-(dT)ₙ was less sensitive to salt inhibition; only 25% inhibition was found at 150 mM KCl. As seen for the purified enzyme, the DNA polymerase was not inhibited; in fact, the DNA polymerase was slightly stimulated (up to 128%) by the higher KCl concentrations. In an effort to understand the difference between the inhibitions found in the two systems, Nonidet P-40 (2.0% final concentration) was added to the ether-disrupted virus reactions. No change was found for the DNA polymerase reactions; however, the RNase H was more sensitive to KCl inhibition (≥40% inhibition at 150 mM KCl). Thus, it is apparent that subtle differences in reaction conditions influence the observed inhibition patterns.

Heat inactivation studies. Figure 3 shows that the purified DNA polymerase using (rA)ₙ-(dT)ₙ was inactivated greater than 80% by preincubation at 41 C for 15 min, whereas the RNase H remained greater than 99% active. After preincubation for 15 min at 65 C, the RNase H still was inactivated only 42%. When heat inactivation studies were performed with (rA)ₙ-(dT)₁₁, the DNA polymerase was inactivated 50% after 5 min at 41 C, 80% after 15 min at 41 C, and 100% after 5 min at 50 C.

Therefore, heat denaturation of the purified enzyme provided a method for studying the RNase H degradation in the absence of the DNA polymerase activity. Differential heat denaturation of the DNA polymerase and RNase H in a purified AMV enzyme preparation had been suggested previously (14, 15).

Attempts were made to preferentially inactivate the DNA polymerase activity in disrupted AMV by heat treatment. The RNase H was substantially more sensitive to the treatment in disrupted AMV (82% inactivation by preincubation for 15 min at 41 C) than in the purified system; the DNA polymerase inactivation was not markedly different. Therefore, this technique was not applicable to the disrupted virus system as a tool for preferential inhibition of the DNA polymerase activity.

Effect of dNTPs on RNase H activity. Previous reports indicated that dNTPs were not required for the AMV RNase H reaction when using [³H](rA)ₙ-(dT)ₙ or natural hybrids (1, 2, 9, 10, 14, 15, 22, 28). Results of studies with [³H]poly(rA)-oligo(dT) as substrate indicated that the omission of dTTP was inhibitory (1, 2, 14, 15). The effect of dNTPs on the RNase H activity of the purified enzyme was examined using [³H](rA)ₙ-(dT)ₙ, [³H](rA)ₙ-(dT)₁₁, and...
φX174 DNA-[3H]RNA as substrates. Three (rA)-to-(dT) ratios (5:1, 2:1, and 1:1) were studied with both (rA)n-(dT) and (rA)- (dT)11. If the dNTPs were omitted, or a triphosphate was included which could not be incorporated [i.e., dCTP in an (rA)- (dT) reaction], less than 20% inhibition was observed in all cases. Therefore, the omission of dNTPs provided a second method for inactivating the DNA polymerase and retaining RNase H activity with the purified enzyme.

Although numerous techniques were studied, no completely satisfactory method was found for preferential inhibition of the DNA polymerase in the disrupted virus preparation. Omission of dNTPs should, in principle, inhibit the DNA polymerase activity. However, prior studies showed the presence of nucleotides and nucleotide kinases in disrupted virus preparations (20, 21). Thus it was not possible to be certain of the absence of substrates for the DNA polymerase. A reaction was performed using only the labeled deoxyribosephosphate (0.25 μM [3H]dTTP; no unlabeled dNTP was added). If the assumption was made that the only substrate used by the enzyme was that added as labeled dTTP, the amount of DNA synthesized was only 10% that synthesized under normal reaction conditions.

To test the effect of the apparent absence of a DNA polymerase reaction on the RNase H activity, studies were performed in the absence of added dNTPs. When either [3H](rA)n-(dT)n or φX174 DNA-[3H]RNA was used as the substrate, less than 5% inhibition was observed when the dNTPs were omitted. However, when the dTTP was omitted with [3H](rA)n-(dT)11 as substrate, inhibition of the RNase H was observed. At a 1:1 (rA)-to-(dT) ratio, 50% inhibition resulted; 80% inhibition resulted at a 2:1 ratio; and 90% inhibition was observed at a 5:1 ratio. Therefore, when [3H](rA)n-(dT)n and φX174 DNA-[3H]RNA hybrid substrates were used, there was no dNTP requirement for the RNase H activity. However, when [3H](rA)n-(dT)11 was used as the substrate, omission of dTTP from the reaction resulted in inhibition of the disrupted AMV RNase H.

Other inhibition studies. Other inhibitors were tested including 1,10-phenanthroline, iodoacetate, HgCl2, and N-ethylmaleimide. In no case were the differential inhibitions as pronounced as found above; however, some preferential inhibition of the purified DNA polymerase was found with 1,10-phenanthroline, iodoacetate, and N-ethylmaleimide.

Effect of RNase H inhibition on the size of the DNA polymerase products. The NaF and KCl inhibition results discussed above provided tools for studying the possible role of RNase H in DNA synthesis by the AMV DNA polymerase. Size analysis was performed on the DNA polymerase products to determine if the size of the products was different when the RNase H activity was inhibited.

The DNA polymerase reactions were performed under standard reaction conditions using (rA)n-(dT)11 and ether-disrupted AMV, with and without NaF preincubation. The products from a 90-min reaction were alkali treated to hydrolyze the RNA template and centrifuged on 5 to 20% sucrose gradients as described in Materials and Methods. Figure 4 shows that the DNA polymerase synthesized a 6S product in both reactions. Studies using ether-disrupted AMV and (rA)n-(dT)n, with and without NaF preincubation, gave similar results.

The size of the DNA polymerase product synthesized by the partially purified enzyme with (rA)n-(dT)n also was examined. The reactions were performed at both 30 and 150 mM KCl as described in Fig. 2. The products synthesized in 90 min were alkali treated and analyzed on sucrose gradients as described in Materials and Methods. The product synthesized in the presence of 150 mM KCl was 6S, whereas that formed in 30 mM KCl was 9S (results not shown). However, this size difference was probably a result of the high salt concentration and not a direct result of the inhibition of RNase H. When ether-disrupted virus was used with (rA)n-(dT)n, the product was 12 to 13S (with and without NaF preincubation). High salt (150 mM KCl) had very little effect on the RNase H activity of ether-disrupted AMV. However, the DNA product synthesized by the disrupted virus system with 150 mM KCl was 3S units smaller than that synthesized with 30 mM KCl.

Therefore, when either (rA)n-(dT)n or (rA)n-(dT)11 was used as template-primer, inhibition of the RNase H did not affect the size of the product synthesized by the DNA polymerase.

The size of the DNA polymerase product synthesized with ether-disrupted AMV in 5 h using 70S RSV RNA as the template also was examined. The reactions were performed with and without preincubation with NaF. The products were alkali treated and centrifuged on 5 to 20% sucrose gradients as described in Materials and Methods. Figure 5 shows that the DNA polymerase synthesized a 6 to 7S product in both reactions. The same result was observed when the endogenous disrupted AMV DNA polymerase reaction was studied. Therefore, as observed with the (rA)-(dT) reactions, inhibi-
under three different conditions: (i) standard reaction conditions, (ii) standard conditions except that dTTP was omitted, and (iii) standard conditions except that the DNA polymerase was heat-inactivated before addition of the enzyme to the reaction mixture. In all three cases, approximately 1% of the total radioactivity was rendered acid-soluble (Fig. 6), and greater than 70% of the acid-soluble products migrated in a relatively sharp peak just ahead of the (rA)_n marker.

The RNase H reactions using disrupted AMV were performed under standard reaction conditions, except that in one case the dTTP was omitted to inhibit the DNA polymerase. Approximately 20% of the total radioactivity was rendered acid-soluble; 50 to 60% of the acid-soluble radioactivity profile was not precipitable with NaF or NaF inhibited by NaF.

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soluble product migrated in a relatively sharp peak just ahead of the \((rA)_8\) marker (results not shown). The remainder of the acid-soluble product was predominantly smaller oligomers. When \(^{32}P\)\(70\)S RSV RNA was degraded by the disrupted AMV RNase H, the products were the same size as those described above for the \(^{3}H\)\(70\)S AMV RNA reaction.

Therefore, the products of the RNase H reaction were oligonucleotides (two to eight nucleotides in length), and inactivation of the DNA polymerase did not affect the size of the RNase H products.

**DISCUSSION**

Different reaction requirements and different sensitivities to several inhibitors of DNA polymerase and RNase H were observed with both ether-disrupted AMV and the purified enzyme preparation. When size analyses were performed on the DNA polymerase reaction products, no differences were observed when the RNase H activity was inhibited. In addition, inhibition of the DNA polymerase activity did not affect the size of the RNase H products. These results, in conjunction with the slight separation of the two activities during purification, demonstrate a lack of obligate coupling of the two reactions and indicate that the two activities have different active sites. The two active sites may be on the same or on different protein molecules.

Other recent studies are consistent with the notion of the non-necessity of RNase H for in vitro reverse transcriptase activity. Wang and Duesberg (27) reported that Kirsten murine sarcoma-leukemia virus DNA polymerase lacked detectable RNase H activity. In addition, R. C. Gallo et al. (personal communication) recently purified and characterized a reverse transcriptase from human leukemic cells and found little or no RNase H associated with the DNA polymerase; these authors also found a similar result for murine RNA tumor virus reverse transcriptases (32).

Although our results show that the two activities are not closely coordinated, as found for other DNA polymerase-exonucleases (4, 18, 19), it is still possible that RNase H has a vital role in provirus synthesis. Inhibition of RNase H had no influence on the size of the products formed by the DNA polymerase under the reaction conditions employed. However, it is conceivable that the nuclease could have a role in other aspects of DNA synthesis. For example, the RNase H could influence the secondary structure of the DNA product, the rate of release of the DNA product from the RNA template (primer?), or the extent of transcription of the template. The differential inhibition conditions described in this paper provide a tool for further study of the possible roles of AMV RNase H in DNA synthesis by the AMV DNA polymerase.

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LITERATURE CITED


