Biochemical characteristics of the RNase H activity associated with immunoaffinity purified human immunodeficiency virus (HIV) reverse transcriptase (RT) were examined. Glycerol gradient centrifugation of HIV RT was performed in a single peak of RNase H, associated with RT activity, with an apparent molecular weight of 110,000. HIV RNase H exhibited a marked substrate preference for poly(dC)*[3H]poly(rG) compared to poly(dT)*[3H]poly(rA). It did not hydrolyze single-stranded RNA or the DNA component of DNA–RNA hybrids. Products of the HIV RT-associated RNase H reaction consisted primarily of monomers, dimers, and trimers with 3′ OH groups. This reaction was Mg2+ dependent, with greater than 90% of maximum activity at MgCl2 concentrations between 4 and 12 mM. The optimum KCl concentration for HIV RNase H was 50 mM, which was lower than that for HIV RT catalyzed polymerization with a poly(rA)–(dT)12 template. The optimum pH for HIV RNase H activity was between 8.0 and 8.5, in contrast to an optimum pH of 7.5 to 8.0 for HIV RT activity. The association of RNase H activity with the p66 component of HIV RT was demonstrated by activity gel analysis. These results indicate that HIV RT has an integral RNase H activity; however, some of its properties are different from those of RNase H associated with other retroviral RT's, and optimal assay conditions are different than those for HIV RT catalyzed DNA polymerization.

The human immunodeficiency virus (HIV) genome, like that of other retroviruses, contains three major genes, designated gag, pol, and env (1). The pol gene, thought to be expressed by fusion of gag and pol reading frames, codes for at least three distinct proteins. By analogy with similar viruses, these include a protease which is responsible for processing of precursor polypeptides, a reverse transcriptase (RT) which synthesizes a double-strand DNA copy of the viral ribonucleic acid genome, and an endonuclease/integrate (2). RT catalyzes several enzymatic reactions, including both enzyme sequences are present within the amino-terminal portion of p66, while the carboxyl terminus is homologous with RNase H from Escherichia coli (9). The association of RNase H activity with HIV RT produced by expression of a recombinant gene in E. coli (10), and with RT from lysed virions (6), was recently reported. RNase H and RT activity co-purify during ion exchange and DNA affinity chromatography, as well as glycerol gradient centrifugation. In addition, proteolytic processing of p66 results in production of p51 and a small polypeptide (p15) that retains RNase H activity but lacks polymerase activity (6).

There are several possible functions of RNase H in proviral DNA synthesis. These include removal of 5′ genome RNA in DNA–RNA after strong stop minus-strand DNA synthesis, removal of the tRNA primer at the 5′ end of minus-strand DNA, and a role in generation of the primer for plus-strand DNA synthesis (2). In view of the importance of RNase H in the generation of proviral DNA, this enzyme activity is a possible chemotherapeutic target for inhibition of HIV replication. This article examines some of the biochemical characteristics of the RNase H activity associated with immunoaffinity purified HIV RT.

**MATERIALS AND METHODS**

Poly(dC), poly(dT), oligo(dT)12-18, poly(rC)–(dG)12-18, poly(dC)–(dG)12-18, poly(rA)–(dT)12, E. coli RNA polymerase, phosphodiesterase I, Sephadex G-50, and unlabeled nucleotides were purchased from Pharmacia LKB Biotechnology, Inc. (Piscatway, NJ). Moloney murine leukemia virus (MMLV) RT, E. coli RNase H, T4 polynucleotide kinase, urea, and ultrapure reagents for polycrystalline gel electrophoresis (PAGE) were obtained from Bethesda Research Laboratories (Gaithersburg, MD). Gel filtration molecular weight standards, bovine serum albumin (BSA), guanylyl-(3′-5′)guanosine (GpG), and calf thymus DNA were obtained from Sigma. Avian myeloblastosis virus (AMV) RT and dithiothreitol (DTT) were purchased from Boehringer Mannheim. ACS scintillation mixture was obtained from Amersham Corp. Molecular weight markers for denaturing PAGE and a 4-chloro-1-naphthol containing horseradish peroxidase color development reagent were acquired from Bio-Rad. Biotinylated anti-human IgG and avidin D-horseradish peroxidase conjugate were
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purchased from Vector Laboratories, Inc. (Burlingame, CA). All 3H- and 32P-labeled compounds were purchased from ICN Radiochemicals (Irvine, CA).

HIV positive human serum and immunoaffinity purified HIV RT were kindly provided by Biotech Research Laboratories (Rockville, MD). 7.5 ml of lysis virions from HTLV-III-b-infected H9 cell culture supernatant was added to a 10-ml volume consisting of monoclonal antibody specific for HIV p66 and p51 covalently linked to Sepharose (5). The column was washed with 275 ml of phosphate-buffered saline, then with 30 ml of phosphate-buffered saline with 0.5 M NaCl, followed by elution with 40 ml of 0.2 M NH4OH. Fractions of 3 ml were collected during elution, and were neutralized by addition of an equal volume of 0.5 M Tris-HCl, pH 6.8. Fractions which corresponded to the peak of RT activity were pooled, and BSA was added to a final concentration of 100 µg/ml. Pooled enzyme was dialyzed against 50 mM Tris-HCl, pH 7.5, 2 mM DTT, 20% glycerol, and was stored at -70 °C. General properties of this enzyme preparation have been described (11).

Substrate Preparation

Poly(dT)·-[3H]poly(A) and Poly(dC)·[3H]poly(G)—These complexes were synthesized with E. coli RNA polymerase and the respective DNA homopolymer. Reaction mixtures (250 µl) contained 50 mM Tris-HCl, pH 7.8, 100 mM KCl, 5 mM MgCl2, 5 mM DTT, 5% glycerol, and 1 A260 unit of poly(dC) with 500 µM [3H]ATP (2 Ci/mmol), or 1 A260 unit of poly(dC) with 500 µM [3H]GTP (2 Ci/mmol). These were prewarmed to 37 °C, and reactions were initiated with 20-70 units of E. coli RNA polymerase. After 10 min, reactions were terminated with 10 µl of 0.5 M EDTA, and products were analyzed by electrophoresis on a 15% polyacrylamide gel of poly(dC)/poly(dG) and Sephadex G-50 columns. Peak void volume fractions were pooled and extracted with an equal volume of phenol/chloroform/isooamyl alcohol (1:1:0.04). The aqueous phase was precipitated with 2 volumes of 100% ethanol, and the precipitate was resuspended in sterile H2O.

Polynucleotides—[3H]DNA and RNA—This complex was prepared as described above in a 50-µl reaction which contained 10 µg of heat-denatured calf thymus DNA and 500 µM [α-32P]GTP (10 Ci/mmol). DNA-[3H]RNA—The complex was prepared as described above in a 50-µl reaction which contained the same buffers and salts as for E. coli RNA polymerase reactions, and 1 A260 µg of poly(A)* RNA (prepared from KB cells as described in Ref. 12), 5 µg of oligo(dT)40·60, 500 µM (2 Ci/mmol) of each [3H]dNTP, and 20 units MMLV RT. Enzyme Assays

DNA- and RNA-dependent DNA Polymerase—Standard reaction mixtures (50 µl) contained: 50 mM Tris-HCl, pH 8.0, 2 mM DTT, 100 µg/ml BSA, 100 mM KCl, 8 mM MgCl2, 1 A260 units/ml of oligonucleotide (dT)40·60, poly(dC)·(dT)18·18, oligo(dC)·(dT)12·12, or poly(dC)·(dT)12·12, and 10 µM (20 Ci/ml) of [γ-32P]ATP or [γ-32P]GTP. Reactions were initiated with 2-5 µl of enzyme and incubated at 37 °C for 30 min. 40-µl aliquots were spotted on glass fiber filters (Whatman GF/A) and processed for determination of trichloroacetic acid-insoluble radioactivity. One unit is defined as the amount of enzyme which catalyzes incorporation of 1 nmol of deoxynucleotide into acid-insoluble material. Unless otherwise stated, units of RT were determined with a poly(A)* (dT)40·60 template using standard reaction conditions.

RNase H—Standard reaction mixtures (50 µl) contained: 50 mM Tris-HCl, pH 8.0, 2 mM DTT, 100 µg/ml BSA, 50 mM KCl, 8 mM MgCl2, and 2 x 105 cpm of [3H]-labeled substrate (poly(dC)·[3H]poly(rG)) unless otherwise indicated. Reactions were initiated with 2.5-5 µl of enzyme and incubated at 37 °C for 30 min. Reactions were terminated by transfer to ice followed by addition of 50 µl of ice-cold 10% trichloroacetic acid. After 30 min on ice, the precipitate was pelleted by centrifugation at 10,000 rpm for 15 min. 75 µl of the supernatant was added to 10 ml of scintillation fluid. One unit is defined as the amount of enzyme which produces 1 nmol of acid-soluble ribonucleotide/hr at 37 °C.

Glycerol Gradient Centrifugation

Continuous 20-30% glycerol gradients which contained 25 mM Tris-HCl, pH 7.5, 1 mM DTT, and 200 mM KCl in a total volume of 4.8 ml were prepared. 60 units of HIV RT in a final volume of 200 µl (10% glycerol) was layered onto the top of the gradient. Identical gradients were layered with solution containing molecular weight standards (20 µg each) in buffer containing 10% glycerol. Ultracentrifugation was performed at 44,000 rpm in a Beckman SW 60.1 rotor. Fractions of approximately 20 µl each were collected from the top of the gradient and assayed for the presence of various enzyme activities as described above. Positions of molecular weight standards were identified after PAGE in the presence of sodium dodecyl sulfate (SDS) as previously described (13), followed by staining with Coomassie Blue (14). Recovery of all enzyme activities was greater than 80%.

Product Analysis

0.05 or 0.5 units of HIV RNase H were incubated with poly(dC)·[3H]poly(rG) using standard reaction conditions for the time periods indicated in Fig. 2. Two 10-µl portions of each reaction were treated with 10 µl of 7% perchloric acid and processed for determination of acid-soluble radioactivity as described above. One 5-µl portion of each reaction was diluted with 20 µl of H2O and 12.5 µl of sequencing gel sample buffer (50 mM EDTA and 0.02% bromophenol blue in 88% formamide). 5 µl of an 80% acid-soluble reaction was further treated for 15 min with 0.1 unit of phosphodiesterase I at 37 °C, followed by addition of 44 µl of H2O and 25 µl of sequencing gel sample buffer. [5-32P]GpppA was prewarmed by 5° for phosphorylation of Gpp with T4 polynucleotide kinase in the presence of [γ-32P]ATP as recommended by the kinase supplier. Part of the [5-32P]GpppA was treated with phosphodiesterase I to produce [5-32P]GMP. Samples were boiled for 5 min and fractionated by electrophoresis on a 15% polyacrylamide-urea DNA sequencing gel as previously described (15), followed by autoradiography with Kodak X-Omat R film.

RNase H Activity Gel

A 10% polyacrylamide gel which contained SDS was prepared essentially as described by Laemmli (13), except for the addition of poly(dC)·[3H]poly(rG) (106 cpm) prior to polymerization. Enzyme samples for activity recovery were HIV RT (12 units), MMLV RT (200 units), and E. coli RNase H (10 units), each in a final volume of 0.4 µl which contained 5% glycerol, 2 mM EDTA, 1% SDS, 50 mM Tris-HCl, pH 7.5, 0.02% bromophenol blue, 1 mg/ml BSA, and 0.5 mM 2-mercaptoethanol. These were incubated for 5 min at 37 °C before application to the gel. One lane was reserved for protein standards and another for HIV RT template without nucleoside triphosphates. These were mixed with standard sample buffer (13) and boiled before application to the gel. After electrophoresis, the lane with molecular weight standards was removed and stained with Coomassie Blue (14). The lane with HIV RT in standard sample buffer was transferred to nitrocellulose (10), and transferred proteins were stained by incubation with Human HIV positive serum, followed by biotinylated anti-human IgG, and avidin D-horseradish peroxidase conjugate, in a blocking buffer which consisted of 5% Carnation non-fat dry milk and 4% IgG-free calf serum in phosphate-buffered saline. Development was performed with a 4-chloro-1-naphthol-based reagent in the presence of H2O2 as recommended by the horseradish peroxidase substrate supplier.

The remainder of the gel, which contained enzymes for activity recovery, was shaken gently at room temperature for 1 h in 2 changes (1 liter each) of 50 mM Tris-HCl, pH 8.0, 2 mM DTT, 20% glycerol, followed by 16 h in 2 changes (1 liter each) of the same buffer plus 50 mM KCl and 8 mM MgCl2. The gel was shaker for another 8 h in 50 mM Tris-HCl, pH 8.0, 50 mM KCl, 2 mM DTT, 8 mM MgCl2, in the absence of glycerol, followed by 16 h in the same buffer at 37 °C without shaking. This was followed by gentle shaking for 4 h in 1 liter changes of cold 5% trichloroacetic acid, 10 mM pyrophosphate. The gel was dried under vacuum and autoradiography was performed with Kodak X-Omat K film.

RESULTS

Glycerol Gradient Centrifugation—Immunofluorescence purified HIV RT was subjected to ultracentrifugation in a 10-30% glycerol gradient in the presence of 200 mM KCl, and fractions were analyzed for the presence of polymerase and RNase H activities (Fig. 1). A single peak of RNase H activity coincident with DNA polymerase activity measured with a poly(dC)-oligo(dG) template, and with RT activity measured with poly(rC)-oligo(dG) and poly(rA)-oligo(dT) templates.
HIV Reverse Transcriptase-associated RNase H

FIG. 1. Glycerol gradient centrifugation of HIV RT. Ultracentrifugation of immunoaffinity purified HIV RT was performed as described under "Materials and Methods." Fractions of approximately 200 µl were collected from the top of the gradient and assayed for the presence of RNase H activity (●), or for polymerase activity with a poly(rA). (dT)₁₀ (●), poly(rC). (dG)₁₂₋₁₈ (△), or poly(dC). (dG)₁₂₋₁₈ (◆) template. Positions of standard proteins are indicated with arrows: carbonic anhydrase (29,000), BSA (66,000), alcohol dehydrogenase (150,000), and β-amylase (200,000).

TABLE I
Substrate preference of RNase H activity associated with HIV and AMV reverse transcriptase

<table>
<thead>
<tr>
<th>Substrate</th>
<th>HIV RT</th>
<th>AMV RT</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA-[³H]RNA</td>
<td>0.026</td>
<td>0.032</td>
</tr>
<tr>
<td>DNA-[³H]RNA, heat-denatured</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>RNA [³H]DNA</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Poly(dT). [³H]poly(rA)</td>
<td>0.004</td>
<td>0.056</td>
</tr>
<tr>
<td>Poly(dC). [³H]poly(rG)</td>
<td>0.150</td>
<td>0.033</td>
</tr>
</tbody>
</table>

* Labeled substrate rendered acid-soluble (nanomole/h) per unit of RT. Nuclease activity with the indicated substrate was plotted as a function of RT activity, and the slope was determined by linear regression analysis. Numbers represent the average slope from at least two such experiments, each with at least four different enzyme concentrations. The lowest average correlation coefficient was 0.954.

The complex had an apparent molecular weight of approximately 110,000. When a similar experiment was conducted in the absence of KCl, a broad peak of polymerase and RNase H activity appeared in fractions 11–21, which corresponded to apparent molecular weights between 110,000 and 250,000 (data not shown).

Template Preference of HIV RNase H—HIV RT-associated RNase exhibited a marked substrate preference for poly(dC). [³H]poly(rG) compared to poly(dT). [³H]poly(rA), in contrast with AMV RT/RNase H which preferred the latter substrate (Table I). Intermediate activity was obtained with HIV RNase H and a mixed DNA-[³H]RNA hybrid. There was an absence of nonspecific nuclease activity in our preparation of HIV RT as demonstrated by the lack of degradation of single-stranded RNA, and an inability to hydrolyze [³H]DNA in a [³H]DNA-RNA hybrid.

Products of the HIV RNase H Reaction—HIV RT/RNase H was incubated with poly(dC). [³²P]poly(rG) under standard conditions, and the reaction products were identified after

FIG. 2. Products of the HIV RNase H reaction. Products of the HIV RNase H reaction with a poly(dC). [³²P]poly(rG) substrate were analyzed as described under "Materials and Methods." Lanes a and b contain 5'-³²P-labeled pGpG and GMP, respectively. Lane c is a control reaction, incubated for 40 min at 37 °C in the absence of enzyme. Lanes d, e, and f are reactions with 0.05 units of HIV RNase H incubated for 20, 40, and 120 min, respectively. Lane g contains reaction products from a 120-min incubation with 0.5 units of HIV RNase H. The percentages of acid-soluble material in lanes d–f were 30, 54, 80, and 100%. Lane h contains one-half the amount of reaction products in lane f, treated with venom phosphodiesterase as described under "Materials and Methods."

FIG. 3. MgCl₂ dependence of HIV RT and RNase H activities. HIV RT (●) and RNase H (○) activities were measured using standard reaction conditions, except that MgCl₂ was added at the indicated final concentrations. Data points represent the average values from two experiments, each performed in duplicate.
three determinations incorporated poly(dC). poly(rG) was prepared and processed as described under "Materials and Methods," except that KC1 was added at the indicated final concentrations. Each data point represents the average of at least three determinations ± standard deviation.

**FIG. 4. Effect of KCl on HIV RT and RNase H activities.** HIV RNase H (●) and polymerase activities with poly(dC).-poly(dG)12-18 (A) and poly(rA).-poly(dT)10 (■) templates were measured using standard assay conditions, except that KCl was added at the indicated final concentrations. Each data point represents the average of at least three determinations ± standard deviation.

**FIG. 5. pH profile of HIV RT and RNase H activities.** HIV RT (■) and RNase H (●) activities were measured using standard reaction conditions except that Tris (---) or Hepes (—) buffers, adjusted at 37 °C to the indicated pH, were substituted for the standard buffer.

**FIG. 6. RNase H activity gel.** A polyacrylamide gel with incorporated poly(dC).-poly(rG) was prepared and processed as described under "Materials and Methods." A shows the immunostain pattern of HIV RT after transfer to nitrocellulose. The RNase H activity gel in B contains (a) HIV RT, (b) MML RT, and (c) E. coli RNase H.

electrophoresis in a polyacrylamide/urea DNA sequencing gel (Fig. 2). Incubation with 0.05 units of RT for 20, 40, or 120 min resulted in the increasing appearance of a series of 32P-labeled oligomers, primarily monomers, dimers, and trimers. 80% of the substrate was acid-soluble at 120 min. When a 10-fold higher concentration of HIV RT was employed, 100% of the substrate was rendered acid-soluble, and the products consisted almost exclusively of monomers, dimers, and trimers. Treatment of HIV RNase H reaction products with venom phosphodiesterase resulted in complete conversion to [5'-32P]GMP.

**Divalent Cation Requirement**—The HIV RNase H reaction with poly(dC).-poly(rG) as the substrate was absolutely dependent on the presence of divalent cation. MgCl2 was preferred to MnCl2 (not shown). Greater than 90% of the maximum RNase H activity was obtained at MgCl2 concentrations between 4 and 12 mM (Fig. 3). This is in contrast to the MgCl2 dependence of HIV RT activity measured with a poly(rA).-poly(dT)10 template, which exhibited a comparatively sharp MgCl2 optimum of 6–8 mM, and retained only 54% of maximum activity at a concentration of 12 mM.

**Effect of Salt**—The effect of KCl on RNase H activity and polymerase activity with several different templates was examined (Fig. 4). KCl was added to various assay mixtures at 25 mM increments to 0.25 M. RT activity with a poly(rA).-poly(dT)10 template exhibited a maximum activation of about 2.3-fold at 75 mM KCl, and KCl concentrations greater than 175 mM resulted in less activity than observed in the absence of KCl. This was similar to the salt activation profile observed with a poly(rC).-poly(dG)12-18 template (not shown). In contrast, DNA polymerase activity with poly(dC).-poly(dG)12-18 retained 164% of control activity in the presence of 250 mM KCl. RNase H activity exhibited a salt profile different from that of polymerase activity with any of these templates. Maximum activation of 2.1-fold was obtained at 50 mM KCl, and the presence of KCl at concentrations greater than 100 mM was strongly inhibitory.

**pH Optimum**—HIV RT-associated RNase H had an alkaline pH optimum of 8.0–8.5, with either Tris or Hepes buffers (Fig. 5). In contrast, polymerase activity with a poly(rA).-poly(dT)10 template had a sharp pH optimum between 7.5 and 8.0, and retained about 60% of maximum activity at pH 8.5.

**RNase H Activity Gel**—The RNase H substrate poly(dC).-poly(rG) was incorporated into a polyacrylamide gel prior to polymerization. Purified HIV RT was denatured and subunits were separated during electrophoresis through this gel. One lane which contained HIV RT was transferred to nitrocellulose and immunostained with a monoclonal antibody against this enzyme (Fig. 6a). The remainder of the gel was washed extensively to allow protein renaturation, followed by incubation at 37 °C under conditions optimal for detection of HIV RNase H activity. The gel was then fried, and autoradiography performed (Fig. 6b). RNase H activity was associated with the p66 subunit of HIV RT as demonstrated by the absence of radioactive substrate in the portion of the gel occupied by p66. A smaller band of nuclease activity which corresponded to a molecular weight of approximately 200,000 was also noted.

**DISCUSSION**

Immunofluorescence purified HIV RT possessed RNase H activity as well as DNA- and RNA-dependent DNA polymerase activity. All three activities co-migrated in a glycerol gradient during ultracentrifugation. We were unable to observe any small molecular weight RNase H in glycerol gradient fractions or by activity gel analysis. RNase H activity was an integral part of HIV RT, since it was associated with the p66 subunit. A smaller amount of RNase H activity with an apparent molecular weight of 200,000 was also observed, which raises the interesting possibility that the gag-pol precursor could have RNase H activity; however, we have not ruled out the
possibility that the presence of high molecular weight RNase H could be due to enzyme aggregation. Others have observed concurrent production of p51 and a 15,000 RNase H during proteolytic cleavage of a carboxyl-terminal fragment from p66 (6). Several factors may contribute to the absence of this activity in our preparation. The monoclonal antibody used for purification of p66 and p51, and would not be expected to recognize the carboxyl-terminal portion of p66. Therefore, p15 should not be present unless it forms a complex with RT, or is produced after purification of RT by the action of a contaminating protease.

HIV RT-associated RNase H demonstrated characteristics of a true RNase H, since it did not degrade single-stranded RNA or the DNA portion of a DNA-RNA hybrid. Unlike AMV RT, it demonstrated a marked substrate preference for poly(dC)-[3H]poly(rG). In addition, we have confirmed the substrate potential of mixed DNA-3H]RNA hybrids observed by others (6, 10). However, poly(dC)-[3H]poly(rG) is the preferred substrate for many applications due to higher activity, ease of preparation, and homogeneity of the substrate.

Like AMV RT/RNase H, HIV RT-associated RNase H produced a series of oligomers; however, these products are primarily monomers, dimers, and trimers, while AMV RNase H products are primarily larger oligonucleotides (2, 17). Venom phosphodiesterase was able to convert HIV RNase H products to 5'-3'PG. Since this enzyme requires a substrate with a free 5'-hydroxyl group, HIV RNase H products, like those produced by other retroviral RNase Hs (2), are probably terminated with 5'-phosphate and 3'-hydroxyl groups.

Several factors indicate that HIV RT contains distinct catalytic sites for polymerase and RNase H activity. Amino acid sequence analysis demonstrates homology with viral and bacterial polymerases within the amino-terminal portion of p66, and homology with other RNase Hs near the carboxyl terminus (9). Release of an active 15,000 RNase H which lacks polymerase activity during processing of p66 also supports this idea (6). Proteolytic fragments of AMV RT which possess RNase H activity but lack polymerase activity have also been reported (18). In spite of apparently distinct active sites for RNase H and polymerase, removal of the 15,000 fragment results in a 51,000 polypeptide that has greatly reduced RT activity. We previously reported that we were unable to detect polymerase activity associated with p51 by activity gel analysis (3); however, with highly concentrated enzyme, we are now able to detect a small amount of p51-associated polymerase activity. This is consistent with a recent report that p51 has 20-80-fold less RT activity than p66/mg of protein (19).

RNase H activity catalyzed by p15 H exhibits a random mode of action, in contrast with RT-associated RNase H, which is processive (6). The change to a random mode of RNase H action after loss of the polymerase active site could be due to the loss of a nucleic acid binding domain. That this is the case for HIV p15 is supported by a site-directed mutagenesis study which indicates that the template binding site is present within the amino-terminal half of p66 (20).

Our results obtained with immunoadfinity purified HIV RT indicate several differences in optimum assay conditions for polymerase and RNase H activities. RNase H exhibits a broader MgCl2 optimum, lower KCl optimum, and prefers a more alkaline pH, than polymerase. For these reasons and those discussed above, it may be possible to discover selective inhibitors of HIV RNase H.

RNase H plays an important role in proviral DNA synthesis catalyzed by RT. Studies in progress may indicate whether or not this enzyme activity will be a useful target for inhibition of HIV replication. In addition, degradation of viral genomic or messenger RNA by RNase H could be an important mechanism of action of antisense oligonucleotides, a chemotherapeutic approach which is under active investigation in several laboratories. This article provides important basic information for investigation of these possibilities.

REFERENCES