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# RNase H Activity Associated with Reverse Transcriptase from Feline Immunodeficiency Virus

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**Reverse transcription of retroviral genomes requires the action of an RNase H for template switching and primer generation. In this report, we compare enzymatic properties of the RNase H associated with the reverse transcriptase (RT) from feline immunodeficiency virus (FIV) and that from human immunodeficiency virus (HIV). Both enzymes displayed substrate preference for poly[$^3$H](rG) · poly(dC) hybrid over poly[$^3$H](rA) · poly(dT) and cation preference for $Mg^{2+}$ over $Mn^{2+}$. Activity of the FIV RNase H upon poly(rG) · poly(dC) produced hydrolysis products from 1 to 6 nucleotides in length, similar to that reported for HIV. Dextran sulfates were effective inhibitors of both the FIV and HIV RNase H and RT activities. Nearly identical inhibition constants (0.12 nM) were obtained for all enzyme activities with dextran sulfate 500,000, while different inhibition constants were observed with dextran sulfate 8,000. Our results suggest that FIV and HIV RTs contain a conserved region that is sensitive to the larger dextran sulfate and that dextran sulfate 8,000 may interact at a different site or by a different mechanism.**

Reverse transcriptase (RT) is an essential enzyme for replication of retroviruses, catalyzing the synthesis of proviral DNA by using genomic RNA as a template (41). This enzyme has proven to be an attractive target in retroviral chemotherapy, as the RT from human immunodeficiency virus type 1 (HIV) can be selectively inhibited by the active form of competitive (12, 16, 20, 22) and noncompetitive (38, 42) inhibitors. RT exists as a bifunctional enzyme, showing both DNA polymerase activity and an RNase H activity that hydrolyzes the RNA of RNA-DNA hybrids (2, 25). This activity resides in the C-terminal domain of RT, as demonstrated by homology with *Escherichia coli* RNase H (13), as well as analysis of deletion mutants (9, 14), point mutagenesis (24, 33, 34), and limited proteolysis (15, 43). RNase H is required for several steps in provirus formation, including degradation of RNA in transitional RNA-DNA hybrids (18, 19), generation of an RNA primer for synthesis of plus-strand DNA (32), and removal of tRNA and plus-strand primers (6, 30). The central role of RNase H in provirus formation makes this unique activity a potential target for AIDS chemotherapy (11, 17, 23, 26, 37).

Feline immunodeficiency virus (FIV) is a retrovirus that has been isolated from domestic cats showing an AIDS-like disorder (31, 44). This lentivirus shows considerable similarity to HIV with respect to morphology, cell tropism, and pathogenesis (4, 31). The RT from FIV shows a high degree of similarity with HIV RT, with greater than 45% homology at the genome sequence level (39). We purified FIV RT (28) and have shown it to be nearly identical to HIV RT in template preference, divalent metal requirement, and sensitivity to active forms of several important antiviral compounds (27, 29). These similarities make FIV a promising lentivirus model of RT-targeted AIDS chemotherapy. In this study, we have further characterized the enzymatic properties of FIV RT by directly comparing its RNase H activity with that of the RNase H associated with HIV RT. In addition, we have examined the sensitivity of these two enzymes to polyanions which have been shown to inhibit HIV RNase H activity in vitro (26) and HIV replication in cultured cells (1, 5, 21).

The FIV RT was purified as previously reported (28). HIV RT was kindly provided by Stephen Hughes (National Cancer Institute, Frederick, Md.). This recombinant HIV RT has been previously described (7, 10) and has been shown to be enzymatically indistinguishable from the virion-associated enzyme (35). FIV and HIV RTs each displayed single bands with molecular weights of 67,000 and 66,000, respectively, when subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis. RNA polymerase from *E. coli* was obtained from Boehringer Mannheim.

The in vitro assay used to quantitate RNase H activity was identical to that reported by Starnes and Cheng (37). The substrate utilized in this assay was $^3$H-labeled RNA transcribed from poly(dC) or poly(dT) templates with *E. coli* RNA polymerase. RNA-DNA hybrids were purified by Sephadex G-50 chromatography and phenol-chloroform extraction. Final hybrid preparations yielded approximately 460,000 cpm/nmol of GMP or AMP. Reaction mixtures (50 μl) for determination of Michaelis and inhibition ($K_i$) constants were incubated for 20 min at 37°C; for all other reactions, incubations were 30 min. Each reaction was initiated by adding 0.5 U of RNase H and terminated by adding an equal volume of ice-cold 7% perchloric acid. Aliquots (75 μl) were counted by liquid scintillation spectrometry in 10 ml of scintillation cocktail. One unit of RNase H activity is defined as the amount of enzyme that produces 1 nmol of acid-soluble ribonucleotide per hour at 37°C. RT activity was assayed as previously described (28) by using poly(rA) · oligo(dT)$_{10}$ as a template and 0.05 U of RT per 50-μl reaction mixture. One unit of RT is the amount of enzyme required to incorporate 1 nmol of dTMP per h into acid-insoluble product at 37°C.

Under our assay conditions, FIV RNase H showed optimal activity at pH 8.5 and 60 mM KCl. These values are similar to those obtained in direct comparisons with HIV RNase H, which showed a pH optimum of 8.0 with 50 mM KCl. The FIV and HIV RNase H's were absolutely dependent upon the presence of divalent cation and showed a preference of $Mg^{2+}$ over $Mn^{2+}$ (Fig. 1). The FIV activity

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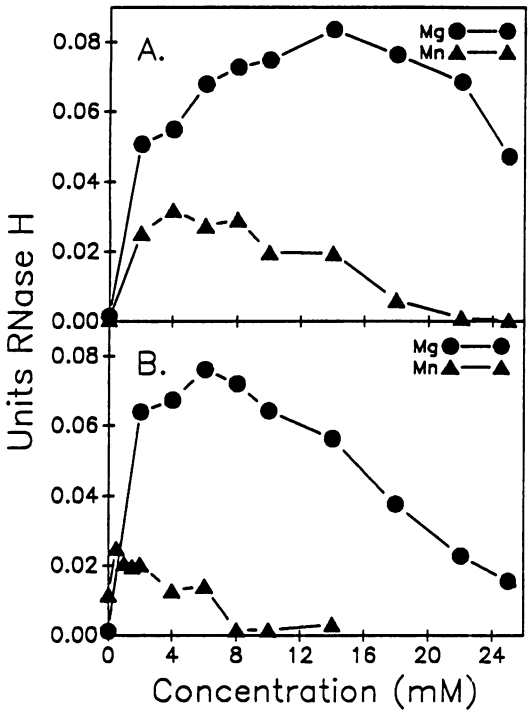


FIG. 1. Divalent metal ion dependence of (A) FIV and (B) HIV RNase H's. Activities were measured by using standard reaction conditions, except that $MgCl_2$ or $MnCl_2$ was added to the indicated final concentrations. Each point represents the average of two determinations.

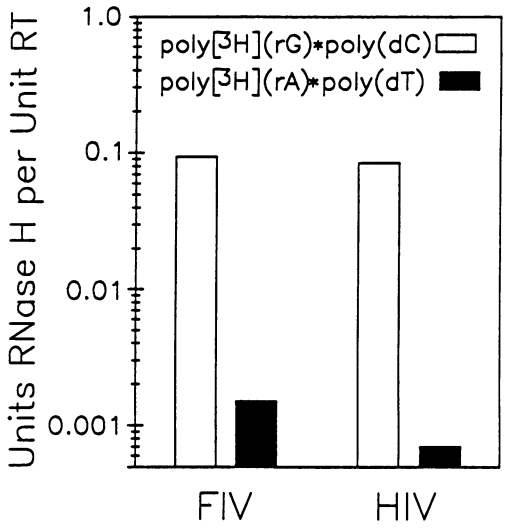


FIG. 2. Substrate specificity of FIV and HIV RNase H's. Activities were measured by using standard reaction conditions with 50,000 cpm of either [$^3$H]poly(rG) · poly(dC) or [$^3$H]poly(rA) · poly(dT) substrate per 50-μl mixture. Values were normalized for RT activity using standard RT assay conditions. Each bar represents the average of two determinations.

showed a broad $Mg^{2+}$ optimum and retained 90% of its maximum activity between 10 and 18 mM $MgCl_2$. RNase H activity was reduced by substituting $Mn^{2+}$ in the reaction mixture, showing a narrow optimum for $MnCl_2$ between 0.5 and 1.0 mM $MnCl_2$. The HIV RNase H showed a similar metal ion dependence, with a broad optimum at 6 mM for $MgCl_2$ and a narrow optimum at 0.5 mM for $MnCl_2$.

The FIV RNase H demonstrated a substrate preference for [$^3$H]poly(rG) · poly(dC) relative to [$^3$H]poly(rA) · poly (dT), hydrolyzing the (rG) hybrid at a rate at least 50 times greater than that of the (rA) hybrid (Fig. 2). Direct comparison showed that the HIV enzyme displayed a similar preference, confirming earlier results reported by Starnes and Cheng (37). Both enzymes showed nearly identical ratios of RNase H activity to RT activity (ca. 0.1 U of RNase H per U of RT) when hydrolyzing the preferred substrate.

To characterize products from the action of FIV RNase H, approximately 100,000 cpm of [$^{32}$P]poly(rG) · poly(dC) substrate was incubated with 0.25 U of FIV RNase H in a 25-μl volume under optimal assay conditions. Reactions were terminated by adding 25 μl of 20 mM citrate buffer (pH 5.0) containing 7.0 M urea and 1.0 mM EDTA, and the mixtures were immediately applied to a 17.5% polyacrylamide–urea DNA sequencing gel (40). GMP standards and RNA ladders were generated by complete or limited (3) base hydrolysis of a [$^{32}$P]RNA · DNA hybrid. Incubation with FIV RNase H resulted in the appearance of several bands, ranging from 1 to 6 nucleotides in length (Fig. 3). Analysis of excised bands by liquid scintillation spectrometry showed that approximately 40% of the digested [$^{32}$P]poly(rG) was hydrolyzed to fragments smaller than 3 nucleotides.

Polyanions such as dextran sulfates have been shown to inhibit the RNase H associated with HIV RT (1, 26). While these compounds do not exert their antiviral activity by directly targeting RNase H in vivo (1, 21), they can provide a useful reference for comparing the polyanion sensitivity

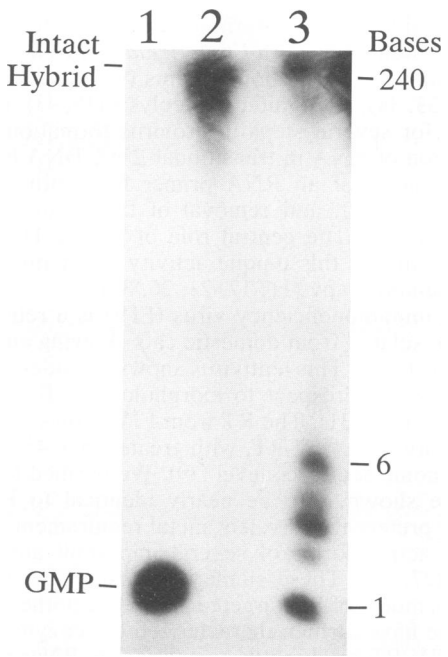


FIG. 3. Products of the FIV RNase H hydrolysis. Products of the FIV RNase H reaction with [$^{32}$P]poly(rG) · poly(dC) substrate were separated by using a 17.5% DNA sequencing gel. Lane 1, complete base hydrolysis of hybrid; lane 2, control reaction containing no FIV RNase H; lane 3, hydrolysis from a 60-min reaction with 0.5 U of FIV RNase H. Approximately 45% of the hybrid was hydrolyzed to acid-soluble material.

TABLE 1. Inhibition constants for FIV and HIV enzymatic activities (RNase H and RT) using dextran sulfate 500,000 and dextran sulfate 8,000

| Enzyme activity | $K_i$ value (nM)[a] | |
|---|---|---|
| | Dextran sulfate 500,000 | Dextran sulfate 8,000 |
| FIV RNase H | 0.12 | 19 |
| HIV RNase H | 0.13 | 75 |
| FIV RT | 0.12 | 9,000 |
| HIV RT | 0.11 | 3,400 |

[a] The mode of inhibition for all activities and both inhibitors was pure noncompetitive. $K_i$ values were calculated by replots of slopes from double-reciprocal plots versus inhibitor concentrations (36).

and functional similarities of various retroviral RNase H activities. To see whether the functional similarities observed between FIV and HIV RTs (27, 29) extend to RNase H activity, we directly compared the sensitivity of these RNase H's to dextran sulfates 500,000 and 8,000. Both FIV and HIV RNase H were noncompetitively inhibited by dextran sulfate 500,000 with nearly identical $K_i$ values of 0.12 and 0.13 nM, respectively (Table 1). Interestingly, the RT activities from FIV and HIV were also noncompetitively inhibited by this dextran sulfate with nearly identical values of 0.12 and 0.11 nM, respectively. These results provide strong evidence that FIV and HIV RTs may contain a structurally conserved domain that interacts with dextran sulfate 500,000. This domain is not located at either the RNase H or RT substrate binding site but, rather, at a region that reduces both enzyme activities with nearly identical $K_i$ values. Dextran sulfate 8,000 also inhibited the RNase H and RT activities of the FIV and HIV enzymes noncompetitively, but the $K_i$ values were not so similar (Table 1). In general, the RNase H activities of FIV and HIV were considerably more sensitive than the RT activities, showing $K_i$ values of 19 and 75 nM for RNase H and 9,000 and 3,400 nM for RT activity, respectively. The variable $K_i$ values observed with dextran sulfate 8,000 suggest that this polysulfate either inhibits these RTs at a less homologous site or that the inhibitor has a different mode of action than dextran sulfate 500,000.

Previous studies on the RT-associated RNase H activities from avian myeloblastosis virus, Moloney murine leukemia virus, and HIV indicate there may be considerable variation between retroviral RNase H activities. The HIV RNase H is $Mg^{2+}$ dependent, displays a preference for poly(rG) · poly (dC), and hydrolyzes RNA-DNA hybrids into mononucleotides (37). The RNase H from avian myeloblastosis virus, while $Mg^{2+}$ dependent, does differ greatly from HIV RNase H with respect to substrate utilization and product formation, utilizing poly(rA) · poly(dT) at a rate much greater than poly(rG) · poly(dC) (2, 43) and hydrolyzing substrate into products between 2 to 10 nucleotides in size (2, 8). The Moloney murine leukemia virus RNase H shows a substrate specificity similar to that of HIV (43), but the activity is $Mn^{2+}$ dependent (8) and it hydrolyzes homopolymeric hybrids into products greater than 10 to 20 nucleotides in length (8). Our results demonstrate that the FIV RT-associated RNase H is very similar to the RNase H from HIV with respect to ion requirements, substrate preference, catalytic activity, and sensitivity to polyanionic inhibitors. As RNase H-targeted inhibitors become available for chemotherapeutic studies, animal models will greatly facilitate design and development. On the basis of our results, the FIV RNase H appears to be sufficiently similar to the same activity from HIV to warrant further development of FIV as a retrovirus model for chemotherapy of AIDS.

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