



The Impact of the M184V Substitution in HIV-1 Reverse Transcriptase on DNA 3'-end and RNA 5'-end-Directed Ribonuclease H (RNase H) Activity



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ABSTRACT

BACKGROUND: Mutations in the polymerase domain of HIV-1 reverse transcriptase (RT) that confer non-nucleoside RT inhibitor (NNRTI) resistance reduce RNase H activity. Little is known, however about the effects on RNase H activity of nucleoside RT inhibitor (NRTI) resistance mutations.

METHODS: Purified RT heterodimer was prepared by expressing cloned p66 and p51RT subunits of wild-type (WT; 18A) and AZT-resistant (18C) isolates. The 184V mutation for lamivudine (3TC) resistance was introduced into the 18A and 18C RTs by site-directed mutagenesis. In addition, thymidine analog resistance mutations (TAMs: 41L, 67N, 70R, 210W, 215Y, 219Q) and the 184V mutation were introduced into p51 and p66 subunits of HIV-1 RT from Hxb2. The DNA 3'-end-directed and RNA 5'-end-directed RNase H cleavage activity of purified recombinant RTs was characterized using RNA-DNA hybrid substrates.

RESULTS: Introduction of 184V into the 18A RT substantially reduced the overall rate of both DNA 3'-end-directed and RNA 5'-end-directed RNase H cleavage relative to the WT (18A) enzyme. However, 184V appeared selectively to enhance the secondary cleavage step. The 18C RT (41L/67N/70R/215Y/219Q) showed a moderate reduction in RNase H activity compared to 18A. RNase H activity of the 18C-184V RT was similar to 18C. Likewise, introduction of 184V into an Hxb2 RT substantially reduced 3'-end- and 5'-end-directed RNase H cleavage relative to WT Hxb2 RT. However, introduction of TAM-1 pattern mutations (41L, 210W, 215Y) did not substantially alter the 5'-end-directed cleavage rate.

CONCLUSIONS: The 184V mutation had a marked effect on RNase H function, reducing rates and modifying kinetics during both DNA 3'-end- and RNA 5'-end directed cleavages. The effect of TAMs on RNase H activity depended on the specific combination of mutations and the genetic backbone of the RT. These findings provide evidence that NRTI resistance mutations, like NNRTI resistance mutations, can alter RNase H function.

RESULTS

Effect of M184V on RNase H Cleavage

Figure 1. RNA 5'-end directed cleavage

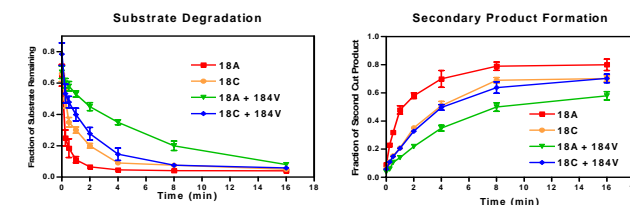
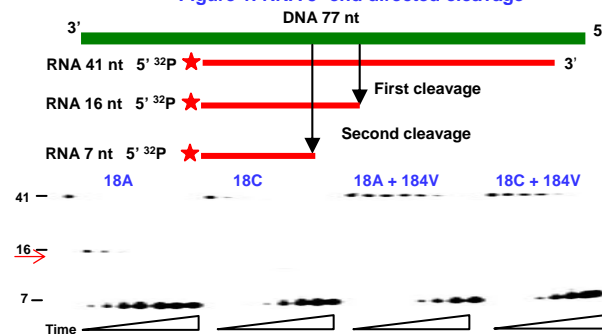
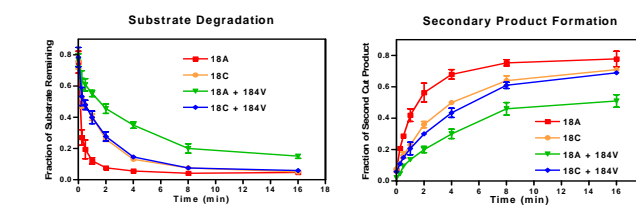
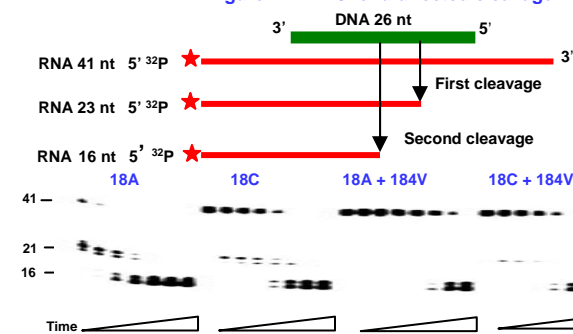


Figure 2. DNA 3'-end directed cleavage



INTRODUCTION

RNA degradation via the ribonuclease H (RNase H) activity of human immunodeficiency virus type 1 (HIV-1) reverse transcriptase (RT) is a critical component of the reverse transcription process. Cleavage of RNA-DNA hybrids by HIV-1 RNase H has been shown to be accomplished through two different modes, DNA 3'-end-directed and RNA 5'-end directed cleavage.

Resistance to zidovudine (AZT) results from sequential accumulation of thymidine analog resistance mutations (TAMs) at HIV-1 RT codons 41, 67, 70, 210, 215, and 219. Previous studies have shown that mutations associated with non-nucleoside RT inhibitor resistance alter RNase H function (Archer et al Biochem 2001;40:4087). However, the effect of nucleoside RT inhibitor resistance mutations on RNase H has not been characterized. To address this issue, we evaluate the impact of AZT resistance mutations and the lamivudine (3TC) resistance mutation M184V on RNase H activities in wild-type (WT) RT and in RTs carrying different combinations of TAMs.

MATERIALS AND METHODS

Generation of mutant RTs. Purified RT heterodimer was prepared by expressing cloned p66 and p51 RT subunits of WT (18A) and AZT-resistant (18C) clinical isolates. The 184V mutation was introduced into the 18A and 18C RTs by site-directed mutagenesis. In addition, site-directed TAMs and the 184V mutation were also made in HIV-1 RTHxb2.

Analysis of RT activity. The relative activity for DNA polymerization of each RT preparation was determined by measuring the rate of incorporation of 3H-dTTP into a poly(rA)-oligo(dT) template-primer.

RHase H assay. Two modes of RNase H cleavage were assayed: DNA 3'-end directed (polymerase dependent) and RNA 5'-end-directed (polymerase independent) cleavage. The relative input of each RT was normalized based on specific activity of RNA dependent DNA polymerization. RT was pre-bound to substrate and reactions were initiated by the addition of magnesium. Reaction were stopped with EDTA at time range from 15 sec to 16 min. Cleavage products were resolved by denaturing gel electrophoresis and quantified by phosphorimaging. Experiments were performed in triplicates.

Figure 3. RNA 5'-end Directed Cleavage

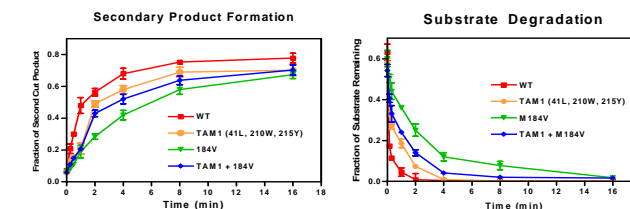
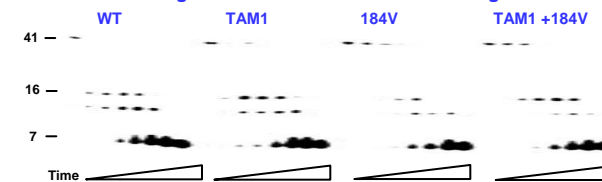
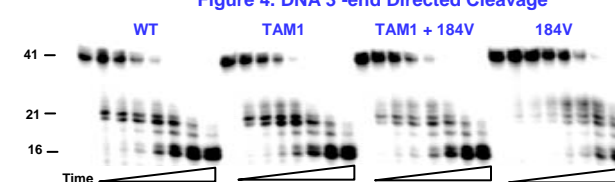


Figure 4. DNA 3'-end Directed Cleavage



CONCLUSIONS

- The M184V mutation had a marked effect on RNase H function.
 - reducing rates, slowed in both DNA 3'-end and 5'-end directed cleavages, and in both genetic backbone.
 - modifying kinetics, selectively enhanced the secondary cleavage step during both DNA 3'-end and RNA 5'-end directed cleavage.
 - the effect of M184V on the RNase H function was diminished when present together with both three and six TAMs.
- TAMs had a moderate effect on RNase H function. The reductions in rates of cleavage in both DNA 3'-end and RNA 5'-end directed are less marked than M184V.
- The specificity of RNA 5'-end directed RNase H cleavage can be affected by different genetic backbone.
- Our findings provide the evidence that NRTI resistance mutation, like NNRTI resistance mutations can alter RNase H function.

ACKNOWLEDGMENT

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