Defective RNA-Directed Strand Displacement due to HIV-1 Ribonuclease H Inactivation

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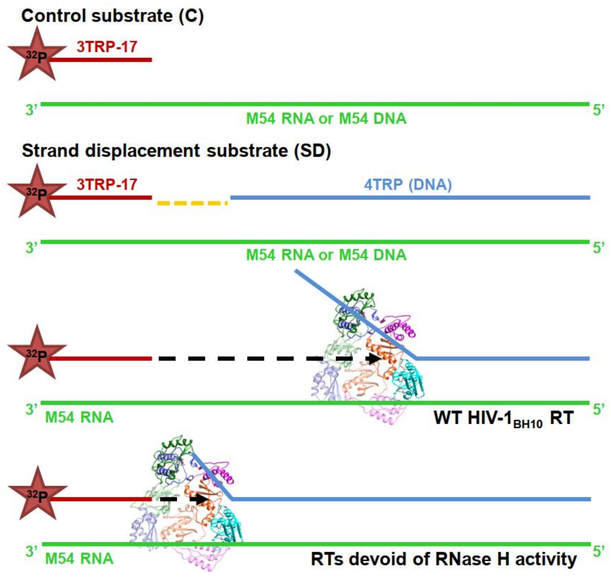
BACKGROUND

In retroviruses, strand displacement DNA-dependent DNA synthesis catalyzed by the viral reverse transcriptase (RT) is required to obtain double-stranded proviral DNA. Strand displacement facilitates the elimination of uncleaved genomic RNA fragments during plus-strand DNA synthesis. In addition, strand displacement during RNA-dependent DNA synthesis is critical to generate high-quality cDNA for use in a variety of biotechnological applications.

METHODS

Wild-type (WT) HIV-1_{BH10} RT and mutant RTs deficient in ribonuclease (RNase H) activity (i.e. carrying the substitutions D443N or E478Q) were tested in strand displacement DNA synthesis assays, in the absence or presence of RNase H active site inhibitors.

RTs were expressed as heterodimers and purified by cationic-exchange chromatography and metal-affinity chromatography. Control DNA synthesis reactions were carried out with RNA/DNA and DNA/DNA template-primers (54/17-mers), while strand displacement complexes included an additional displaced-oligonucleotide of 32 bases complementary to the 5' sequence of the template.



RESULTS

After screening a panel of HIV-1 RTs, we identified several mutants with reduced strand displacement activity while copying RNA and DNA templates. The loss of RNase H activity due to inactivating mutations in the HIV-1 RT (e.g. D443N or E478Q) had a minimal effect on strand displacement when copying DNA templates. However, we observed a remarkable reduction in DNA polymerization if reactions were carried out with RNA templates. Similar results were obtained when using β-thujaplicinol, an RNase H active site inhibitor.

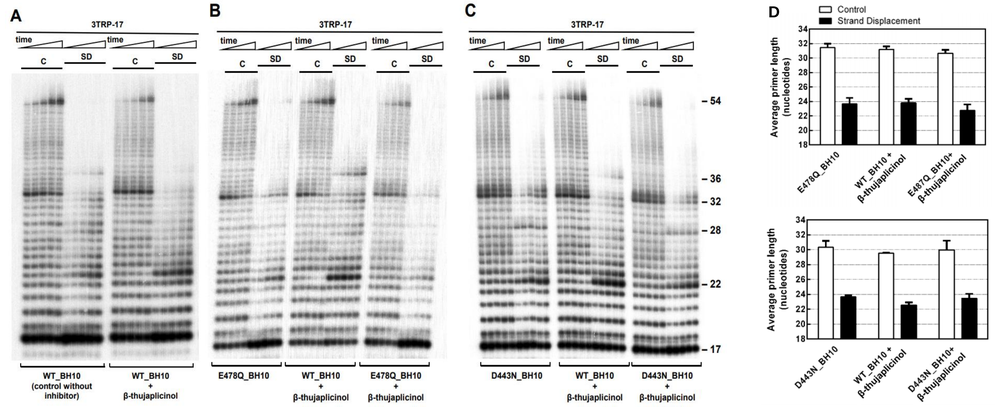


Figure 1. Strand displacement in RNA-dependent DNA polymerization. Tested enzymes in the gel were WT HIV-1_{BH10} RT (A), RNase H-deficient mutants E478Q (B) and D443N (C) in the absence or presence of β-thujaplicinol. Primer extension reactions were carried out in the presence of the template-primer M54RNA/3TRP-17 (C, control) or the hybrid M54RNA/3TRP-17/4TRP (SD, strand displacement conditions) at a 30 nM concentration, 20 nM active site RT and 100 μM of each dNTP. Aliquots were taken at 0.5, 1, 2, 5 and 10 min. Quantitative analyses of primer extensions obtained at 2 min incubation are shown in panel (D).

Interestingly, the inhibitory effects observed with β-thujaplicinol in RNA-dependent DNA polymerization under strand displacement conditions were further confirmed using a panel of RNase H active site inhibitors obtained and previously characterized in our laboratories.

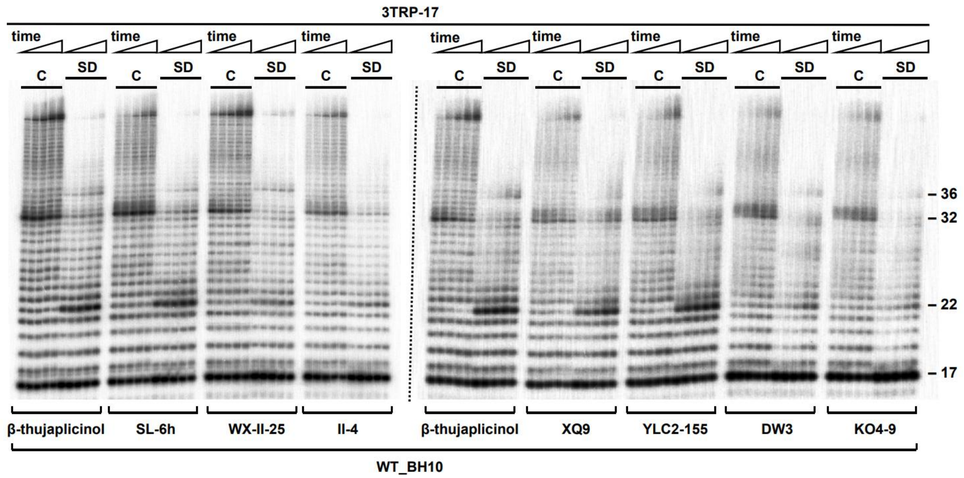


Figure 2. Effect of active site RNase H inhibitors on strand displacement RNA-dependent DNA synthesis. Assays were carried out using the template-primer M54RNA/3TRP-17 (C) or the hybrid M54RNA/3TRP-17/4TRP (SD) at a 30 nM concentration, 20 nM active site WT HIV-1_{BH10} RT and 100 μM of each dNTP in the presence of different compounds at a final concentration of 25 μM. Aliquots were taken at 0.5, 1, 2, 5 and 10 min.

Highlights:

- In HIV-1 RT, **RNase H inactivation** (either by mutational inactivation or using active site inhibitors) has a **major impact on RNA-dependent DNA polymerization under strand displacement conditions** but has no effect when using DNA templates.
- We demonstrate the **inhibition of RNA-dependent strand displacement DNA synthesis** when using **β-thujaplicinol** and other active site **RNase H inhibitors.**

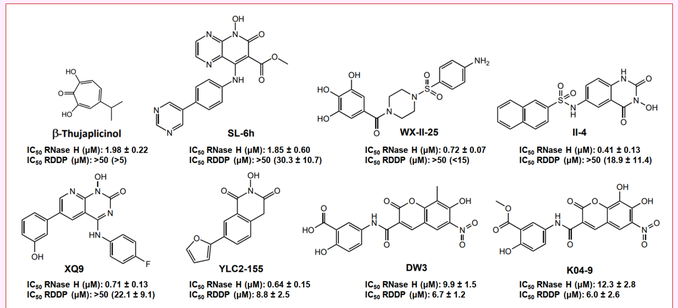


Figure 3. Chemical structures of active site HIV-1 RNase H inhibitors. Concentration required to inhibit by 50 % (IC_{50}) the *in vitro* RNase H and RNA-dependent DNA polymerase (RDDP) activities. Numbers between parentheses indicate the maximum percent inhibition observed with a 50 μM concentration of the compound.

CONCLUSIONS

Our results demonstrate that the loss of RNase H activity reduces the efficiency of RNA-directed strand displacement DNA synthesis by HIV-1 RTs. These findings are expected to be helpful to design novel strategies to inhibit HIV reverse transcription while improving transcriptomics technologies aimed to obtain more uniform read coverages when copying long RNAs.

ACKNOWLEDGEMENTS

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