



The L74V Mutation in HIV-1 RT Diminishes Synthesis of Viral DNA in Real-Time PCR and Impairs Rescue of ZDV-Terminated DNA Synthesis

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Abstract

Background

The M184V, K65R and L74V mutations in HIV-1 RT share several characteristics: discrimination against incorporation of relevant NRTIs, diminished RT processivity and error rates in biochemical assays and reduced viral replicative capacity. In addition, both M184V and K65R mutations cause a reduction in the efficiency of excision of ZDV-terminated DNA. We wished to assess the effect of L74V in synthesis of viral DNA in real-time PCR assays and whether L74V might also compromise rescue of ZDV-terminated DNA synthesis.

Methods

Viral replication capacity was determined by measuring copy numbers of (-)ssDNA and full length DNA by real-time PCR in a single round of infection. Recombinant wt, L74V, M184V and L74V/M184V-containing RTs were purified and rescue of chain-terminated DNA synthesis was studied at a single template position. A DNA/DNA duplex template/primer was incubated with either wt or mutant RT in a buffer containing 10 μ M dCTP and 10 μ M ZDV-TP. ATP or PPI-dependent excision of the ZDV-terminated primer and DNA synthesis were monitored in time-course experiments.

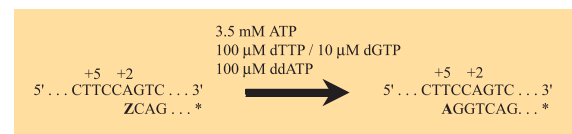
Results

Real-time PCR showed that L74V-containing viruses were compromised by more than 50% in synthesis of both (-) ssDNA and full-length DNA. The simultaneous presence of the M184V mutation further impaired reverse transcription with differences being especially significant in regard to full-length viral DNA. In the presence of ATP, L74V-containing RTs displayed a 50% reduction in the efficiency of excision of ZDV-MP from newly synthesized viral DNA. Wt enzyme was able to unblock 50% of the ZDV-terminated primer after 59 min whereas L74V RT required more than 90 min. M184V and L74V-M184V-containing RTs showed a dramatic impaired excision mechanism. In contrast, PPI-mediated excision was only impaired at very early time points of the rescue reaction.

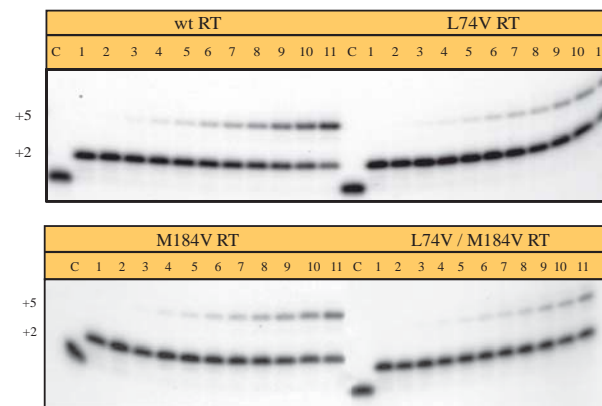
Conclusions

Diminished viral fitness of L74V may be due to reduced synthesis of (-)ssDNA as well as full-length DNA. Although ATP-mediated excision was compromised in RT containing L74V, PPI-mediated excision showed differences only at early time points, potentially highlighting the biological significance of ATP vs PPI in excision reactions.

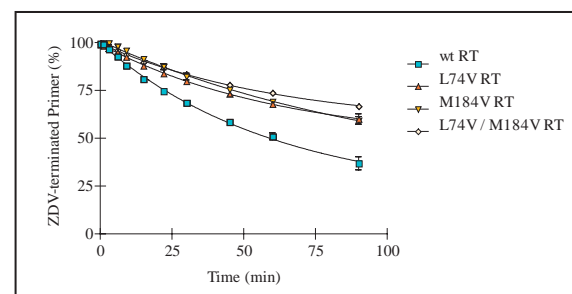
Efficiency of excision of incorporated ZDV-MP in the presence of 3.5 mM ATP



Graphic representation of the cell-free system (PPT-57/PPT-18) used to monitor the excision of ZDV-MP from newly synthesized HIV-1 DNA.



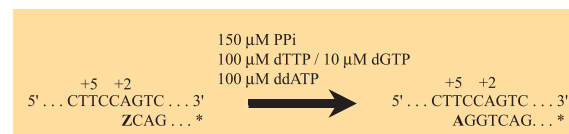
Comparison between wt RT, L74V RT, M184V RT and L74V/M184V RT. Lane C corresponds to control labeled primer. Lanes 1 to 11 show reaction products at 0, 1, 3, 6, 10, 15, 22, 30, 45, 60 and 90 minutes after addition of the excision mix.



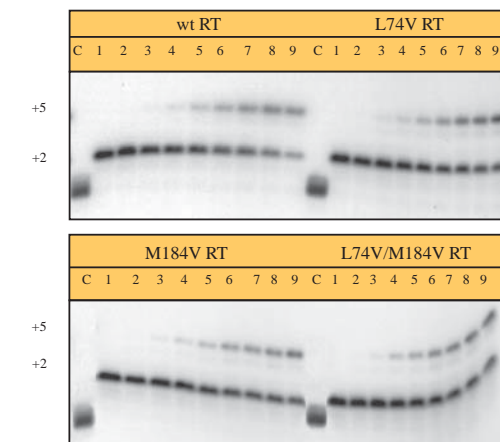
Graphic representation of gel-based assays shown above. Values are means of at least three independent experiments \pm standard errors.

The L74V mutation in RT causes an approximate 50% reduction in the efficiency of excision of ZDV-MP.

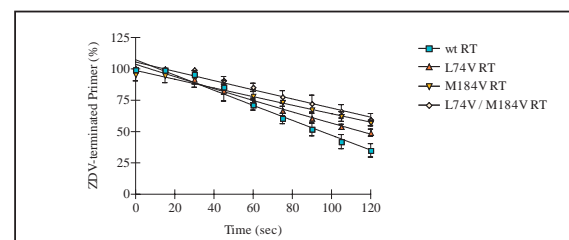
Efficiency of excision of incorporated ZDV-MP in the presence of 150 μ M PPI



Graphic representation of the cell-free system (PPT-57/PPT-18) used to monitor the excision of ZDV-MP from newly synthesized HIV-1 DNA.



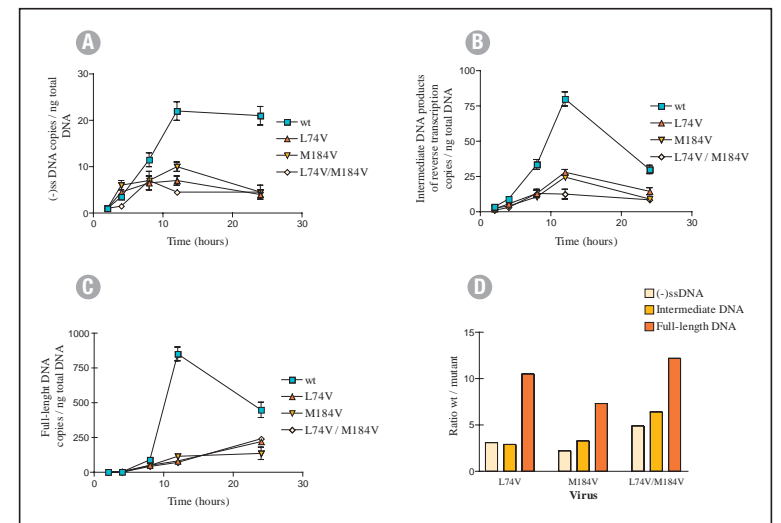
Comparison between wt RT, L74V RT, M184V RT and L74V/M184V RT. Lane C corresponds to control labeled primer. Lanes 1 to 9 show reaction products at 0, 15, 30, 45, 60, 75, 90, 105, and 120 seconds after addition of the excision mix.



Graphic representation of gel-based assays shown above. Values are means of at least three independent experiments \pm standard errors.

Differences were observed between wt and L74V RTs although they were not as pronounced as seen with ATP

Viral replication kinetics of different reverse transcribed HIV-1 DNAs.



1x10⁶ H9 cells were infected with wt and mutant HIV-1 at a MOI of 0.0001. After 2, 4, 8, 12 and 24 hours infection, reverse transcribed DNAs were quantified by real time PCR. (A) (-)ssDNA, (B) Intermediate products of reverse transcription, (C) Full-length DNA, (D) Ratio between newly synthesized wt HIV-1 DNA and mutant HIV-1 DNA for the different reverse transcribed products at 12 hours infection. Values are means of at least three independent experiments \pm standard errors.

Viruses harboring L74V display decreased synthesis of (-)ssDNA, DNA produced after the first strand transfer and full-length DNA in reverse transcription.

These findings support previous evidence that K65R, L74V and M184V should be considered as a group with regard to common mechanisms of resistance to NRTIs and their effects on RT biochemistry.

Acknowledgments

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