

Differential Effects of L74V and M184V Mutations on ATP-mediated Primer Unblocking in HIV-1 Reverse Transcriptase Carrying Thymidine Analog Resistance Mutations

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ABSTRACT

Background: Thymidine analog resistance mutations (TAMs) in HIV-1 reverse transcriptase (RT) confer resistance to zidovudine (ZDV/AZT) by increasing the rate of ATP-dependent phosphorylation of the terminal nucleotide monophosphate (primer unblocking). These mutations occur in one of 2 clusters: M41L210W/215Y, or D67N/67R/K219Q(E); presence of all six mutations confers high-level AZT resistance. The didanosine resistance mutation (L74V) partially restores AZT susceptibility in HIV-1 carrying TAMs. To determine the extent to which the 74V mutation reverses the effect of TAM on primer unblocking, we studied purified recombinant RT carrying various combinations of TAMs, and compared the effect of L74V to that of the M184V mutation for lamivudine (3TC) resistance.

Methods: TAMs (41L, 67N, 70R, 210W, 215Y, and 219Q), 74V, and 184V were introduced into p51 and p66 subunits of wild type HIV-1 RT (HXB2) by site-directed mutagenesis. Recombinant RT was purified using a double-tag strategy. ATP-dependent primer unblocking was assayed using an AZT-terminated 32P-labeled primer (PPT-18) hybridized to a DNA template (PPT-57). Rescue of AZT-terminated DNA was performed in the presence of 3.5 mM ATP and 100 µM dTTP, dGTP, and ddATP. Reaction products were resolved on 8% polyacrylamide-7M urea gels, analyzed on a phosphorimager, and bands quantified using ImageQuant software. All experiments were performed in triplicate.

Results: Presence of L74V in an otherwise wild type RT reduced ATP-mediated primer unblocking to a degree similar to that observed with the M184V mutation. RT carrying different TAMs (41L/210W/215Y, 67N/70R/219Q, or 41L/67N/70R/210W/215Y) showed increased primer unblocking as expected. Introduction of 74V into RTs carrying TAMs partially counteracted the effect of these mutations on the rate of primer unblocking, but did so to a lesser extent than the 184V mutation. The 184V mutation reduced the rate of ATP-mediated primer unblocking to wild-type levels when introduced into the 41L/210W/215Y enzyme; the effect of 184V on reducing primer unblocking in RT carrying all 6 TAM was comparable to that of 74V.

Conclusions: Both 74V and 184V mutations reverse the effect of TAM on ATP-mediated primer unblocking in HIV-1 RT. The effect of 184V was greatest in viruses carrying the 41L/210W/215Y combination of TAM.

BACKGROUND

Resistance to zidovudine (ZDV/AZT) results from sequential accumulation of thymidine analog mutations (TAMs) at HIV-1 reverse transcriptase (RT) codons 41, 67, 70, 210, 215, and 219. These mutations emerge as two distinct patterns involving the 41L/210W/215Y and 67N/70R/219Q pathways. Recent evidence has shown that AZT monophosphate can be efficiently removed from AZT-terminated primers by ATP-mediated phosphorylation (primer unblocking). This mechanism could account for high levels of resistance to AZT. In fact, RTs carrying TAMs possess intrinsic rates of unblocking higher than the wild type enzyme. The extent of unblocking is proportional to the number of TAMs present in RT. Interestingly, L74V, a mutation that confers resistance to didanosine (ddI), restores AZT susceptibility to viruses carrying TAMs, which suggest that this mutation could decrease the rate of unblocking conferred by TAMs. To test this hypothesis, we performed well-established primer unblocking assays of RTs bearing both TAMs and 74V. The effect of 74V on unblocking was compared to that of the M184V substitution which confers resistance to lamivudine (3TC) and also decreases susceptibility to AZT.

MATERIALS AND METHODS

TAMs (41L, 67N, 70R, 210W, 215Y, and 219Q), 74V, and 184V were introduced into p51 and p66 subunits of wild type HIV-1 RT (HXB2) by site-directed mutagenesis. Recombinant RT was purified using a double-tag strategy. ATP-dependent primer unblocking was assayed using an AZT-terminated 32P-labeled primer (PPT-18) hybridized to a DNA template (PPT-57). Rescue of AZT-terminated DNA was performed in the presence of 3.5 mM ATP and 100 µM dTTP, dGTP, and ddATP (Figure 1). Reaction products were resolved on 8% polyacrylamide-7M urea gels, analyzed on a phosphorimager, and bands quantified using ImageQuant software. All experiments were performed in triplicate.

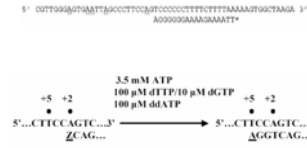


Figure 1. Top: Primer/template used in the experiments. Bottom: Primer unblocking strategy used to monitor ATP-mediated unblocking of an AZT-blocked primer. Z = incorporated AZTMP at position +2. Upon addition of the indicated reagents, DNA synthesis resumes and stops at position +5.

RESULTS

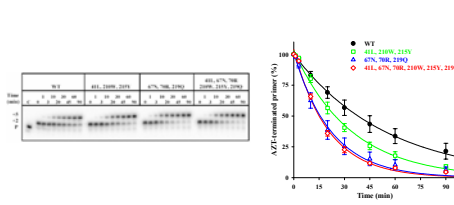


Figure 2. Effect of TAMs on ATP-mediated rescue of an AZT-blocked primer. The reactions were monitored at the indicated times.

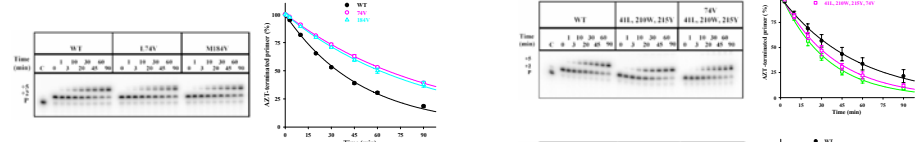


Figure 3. Effect of 74V and 184V on ATP-mediated rescue of an AZT-blocked primer in a WT background. The reactions were monitored at the indicated times.

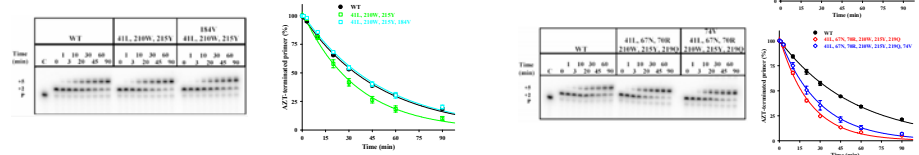


Figure 4. Effect of 184V on ATP-mediated primer rescue in the context of TAMs. The reactions were monitored at the indicated times.

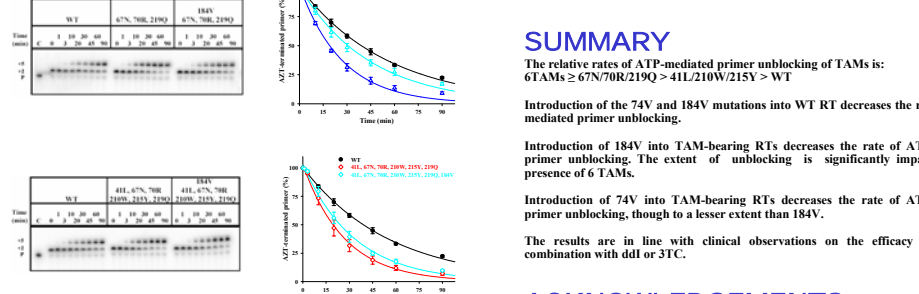


Figure 5. Effect of 74V on ATP-mediated primer rescue in the context of TAMs. The reactions were monitored at the indicated times.

SUMMARY

The relative rates of ATP-mediated primer unblocking of TAMs is: 6TAMs ≥ 67N/70R/219Q > 41L/210W/215Y > WT

Introduction of the 74V and 184V mutations into WT RT decreases the rate of ATP-mediated primer unblocking.

Introduction of 184V into TAM-bearing RTs decreases the rate of ATP-mediated primer unblocking. The extent of unblocking is significantly impaired in the presence of 6 TAMs.

Introduction of 74V into TAM-bearing RTs decreases the rate of ATP-mediated primer unblocking, though to a lesser extent than 184V.

The results are in line with clinical observations on the efficacy of AZT in combination with ddI or 3TC.

ACKNOWLEDGEMENTS

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