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KINETIC MECHANISM OF HIV-1 REVERSE TRANSCRIPTASE CATALYSED AZT EXCISION

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BACKGROUND: The phenotypic mechanism of HIV- 1 resistance to 3'-azido-2',3'-dideoxythymidine (AZT) involves reverse transcriptase (RT)-catalysed phosphorolytic excision of chain-terminating AZT. To further define the kinetic events involved in the AZT phosphorolytic reaction we have compared the ability of wild-type (wt) and AZT-resistant RT to incorporate and excise phosphorothioate analogues of AZTtriphosphate (AZTTP). The stereo selectivity of an enzyme toward the Rp and Sp isomers of phosphorothioate analogues of dNTP has long been established as a useful tool in probing detailed interactions between the metal ions and nucleotides at the enzyme active site.

METHODS: Pre-steady-state kinetic parameters for the incorporation and excision of AZT triphosphate (AZTTP) and the Rp and Sp diastereomers of AZTTPaS were determined for wt and D67N/K70R/ T215F/K219Q and M41L/L210W/T215Y mutant enzymes.

RESULTS: The wt and AZT-resistant enzymes incorporated AZTTP and Sp-AZTTPaS with equivalent catalytic efficiencies, but were significantly less efficient (~12-fold) in incorporating Rp-AZTTPaS. The decreased catalytic efficiency of incorporation of the Rp-analog by wt and AZT-resistant RT was due to decreased nucleotide affinity (Kd) and decreased rate of incorporation (kpol). However, none of the enzymes exhibited a significant phosphorothioate elemental effect [defined as kpol(AZTTP)/ kpol(AZTTPaS)] for the incorporation of these analogues. In contrast to nucleotide incorporation, the mutant RTs were significantly more efficient than wt RT in the phosphorolytic excision of the different analogues of AZT. However, RT was much less efficient in excising both the Rp and Sp isomers of AZTMPaS than in excising AZTMP. Indeed, large

phosphorothioate elemental effects (>10) were determined for the excision of Rp and Sp AZTMPaS by wt and AZT-resistant RT.

CONCLUSIONS: The absence of any observed phosphorothiate elemental effects in RT-catalysed incorporation of AZTTP and AZTTPaS is consistent with the hypothesis that the chemistry step is not rate-limiting during nucleotide incorporation. However, the large phosphorothioate elemental effects observed for the excision of both Rp and Sp isomers of AZTTPaS by wt and mutant RT indicates that the rate-limiting step of the phosphorolytic reaction is the chemistry step. The difference in rate-limiting steps for RT catalysed DNA synthesis and phosphorolytic excision suggests that inhibitors selective for the excision process may be possible.

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