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Recombinant Human Immunodeficiency Virus Type-1 Reverse Transcriptase is Conformationally Heterogeneous. JE. Wilson, LL Wright, JL Martin, GR Painter, and PAFurman, Division of Virology, Burroughs Wellcome Co, Research Triangle Park, NC, USA

Human Immunodeficiency Virus Type-1 (HIV-1) encodes a p66 reverse transcriptase (RT), which is processed by the HIV-1 protease to a p66/p51 heterodimer. A major effort in our laboratory has been to establish a link between site-mutations associated with resistance to dideoxynucleoside analogues in the RT coding region and changes in inhibitor sensitivity at the enzyme level. Wild type (wt) HXB2D HIV-1 RT and variant T215Y, which is associated with resistance to AZT, were subcloned from M13mp18HXBRT into the expression vector pKK233 and transfected into *E.Coli* strain TG1. Following induction, the protein was purified using anion-exchange, cation-exchange, and immunoaffinity chromatography. Three species of RT, designated A, B, or C, were isolated depending upon the purification regimen used, for both wt RT and T215Y. The three species of wt RT differed in apparent charge (as indicated by affinity to cation and anion exchange resin), apparent dimer association, and C_m values as determined from equilibrium urea denaturation profiles, but had similar secondary structure as indicated by circular dichroism spectra. Additionally, T215Y had an identical purification profile and had physical properties identical to wt, suggesting that the point mutation did not alter the conspeciesational heterogeneity of the variant RT. The three species of the wt RT were kinetically indistinguishable with respect to dTTP turnover on poly(rA)p(dT)$_{10}$. However, while the kinetic constants for dTTP and AZTTP were similar to wt protein for both T215Y(B) and T215Y(C), T215Y(A) exhibited a two-fold elevated K_m for dTTP and a thirteen-fold elevated K_i for AZTTP with respect to wt protein purified in the same manner. These results indicate that the ability to identify resistance associated with a mutation at T215 is dependent upon the isolation procedure used.

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Biochemical Analysis of Human Immunodeficiency Virus Type-1 Reverse Transcriptase with Mutations at Methione 184 in the Highly Conserved YMDD Region: Mechanism for 1-β-L Nucleoside Drug Resistance. JE Wilson, JL Martin, A Aulabaugh, LL Wright, S McPherson[2], JK Wakefield[2], S Jablonski[2], CD Morrow[2], and PA Furman. Division of Virology, Burroughs Wellcome Co., Research Triangle Park, NC, USA, and [2]Department of Microbiology, University of Alabama at Birmingham, Birmingham, Al, USA.

A mutation at position 184 from methionine to valine in the highly conserved YMDD region of the human immunodeficiency virus type-1 (HIV-1) reverse transcriptase (RT) has been identified in nucleoside analogue drug resistance. This mutation was generated during *in vitro* selection to generate variant HIV-1 resistant to ddI and cross-resistant to ddC, creating a five-fold elevated IC_{50} to ddI and ddC. A more significant effect is seen in resistance to FTC ((-) Cis-5-fluoro-1-[2-(hudroxymethyl)-1,3-oxathiolane-5-yl] cytosine), with greater than a 5000-fold elevated IC_{50} to the 1-β-L nucleoside analogue in virus containing a mutation at this site in the RT region. Mutations at this position to a serine or alanine has also been shown to result in virus less infectious than wt virus. Mutations at M184 to valine, alanine, and serine were introduced in recombinant BH10 HIV-1 RT. The proteins were expressed in *E.coli* BL21 and purified using phosphocellulose and DEAE cellulose chromatography. The relative purity of the enzyme preparations was judged to be 95% by Coomassie Blue staining of SDS-polyacrylamide gels. Steady-state enzyme assays were done with the purified wt and variant HIV-1 RTs for both natural dNTPs as well as chain-terminating 1-β-L and 1-β-D nucleotide analogues. M184V RT catalyzed product with similar K_m and k_{cat} values to wt RT. Two- to five-fold elevated K_i values were seen during RNA-directed DNA synthesis for 1-β-D nucleotide analogues using M184V RT but not during DNA-directed DNA synthesis. 100- to 1000-fold elevated K_i values were seen during both RNA-directed and DNA-directed DNA synthesis with this enzyme for 1-β-L nucleoside analogues. M184A and M184S had 10-fold decreased K_m/k_{cat} values with respect to wt RT, indicating that this region is important in catalysis.