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Regions of HIV Reverse Transcriptase Involved in Resistance to Several Nonnucleoside Inhibitors. V.V. Sardana, E.A. Emini, L. Gotlib, D.J. Graham, D.W. Lineberger, W.J. Long, A.J. Schlabach, J.A. Wolfgang, and J.H. Condra. Department of Virus & Cell Biology, Merck Sharp & Dohme Research Laboratories, West Point, PA 19486.

Several structurally distinct nonnucleoside compounds, L-697,639, TIBO, and BI-RG-587 have been shown to be potent inhibitors of HIV-1 reverse transcriptase but not of the related enzyme from HIV-2 (RT2). In order to address the structural basis for this differential drug sensitivity, we have constructed a series of molecular chimeras between RT1 and RT2, and have correlated drug sensitivity of the purified enzyme *in vitro* with the presence of specific amino acid residues from the parental enzymes. RT2 was completely sensitized to these compounds by substituting amino acids 101-106 and 155-217 of RT1. Within these regions, residues 176-190, and particularly 181 and 188 are responsible for RT2's resistance to this class of compounds. Mutations K103N/Y181C have been shown to confer >1000-fold drug resistance on HIV-1 virus grown in cell culture (Nunberg *et al.*, **J. Virol. 65:**4887 (1991)). We examined the effects of these and other amino acid substitutions on drug sensitivity of the purified enzyme *in vitro*. Mutations that conferred resistance to this class of compounds included Y181C, Y181S, Y181H, Y181W, Y181I, Y188H, Y188L, and Y188C. Mutants Y181F and Y188F remained fully sensitive. These data suggest that clinical resistance to any of these nonnucleoside inhibitors will probably extend to other members of this same pharmacologic class.

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Characterization of the dNTP Binding Sites on HIV-1 Reverse Transcriptase Using ^{1}H NMR. G. R. Painter, A. E. Aulabaugh and P.A. Furman. Burroughs Wellcome Co., 3030 Cornwallis Rd. Research Triangle Park, NC U.S.A.

Binding studies carried out on heterodimeric HIV-1 reverse transcriptase (RT) using intrinsic Trp fluorescence have shown that each of the four natural 2'-deoxynucleoside 5'-triphosphate (dNTP) substrates and the 5'-triphosphate of zidovudine (AZTTP) bind to single sites on the p66 subunit of the enzyme (as evidenced by Hill coefficients of one; Painter *et al.*, J.Biol. Chem. **29**, 19357 (1991)). Furthermore, substrate binding is competitive due to either (1) direct steric hinderance or (2) allosteric interactions. Here we report 500 MHz ^{1}H NMR studies carried out on dNTP-RT and AZTTP-RT complexes using transferred nOes (TRNOEs) to identify amino acid residues in the ligand binding site. TRNOEs provide a means of determining if there are amino acid residues common to the binding sites of the different ligands. Protein resonances between 0.5 and 2.8 ppm (aliphatic) and between 6.6 and 7.4 ppm (aromatic) were irradiated at 20 Hz intervals for 0.4 sec and TRNOEs to dTTP, dATP and AZTTP measured. A complex pattern of TRNOEs was observed between the aliphatic region of the protein spectrum and the three ligands. TRNOEs common to dTTP, AZTTP and dATP occurred upon irradiation at 0.6 and 0.8 ppm, while TRNOEs common only to the two pyrimidines were produced by irradiation at 0.7 and 1.64 ppm. There were no common TRNOEs between the aromatic region of the protein spectrum and the ligands. Irradiation at 6.75 and 7.00 ppm produced TRNOEs to the H8 and H1', H2' and H2" resonances of dATP whereas irradiation at 6.71 and 7.20 ppm produced TRNOEs to H6 and H1', H2' and H2" resonances of dTTP. No TRNOEs were observed to AZTTP upon irradiation of this region of the protein spectrum. The results show that while there are common contact points on the protein surface for dTTP, AZTTP and dATP and therefore the binding sites are proximal, each ligand is oriented somewhat differently. The topography of the binding sites and the types of amino acids that make up these sites will be discussed.