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Comparison of the Conformations of the 5'-Triphosphates of Zidovudine® (AZT) and Thymidine Bound to HIV-1 Reverse Transcriptase. G.R. Painter*, A.E. Aulabaugh*, C.W. Andrews†, Divisions of Virology* and, Organic Chemistry† , Burroughs Wellcome Co., Research Triangle Park, NC 27709

X-ray analysis of AZT has revealed that the 3'-azido-2',3'-dideoxyribose ring can assume an unusual C4'endo/C3'exo pucker (P-215°). It has been suggested that the ability to assume this conformation, which has been observed in the solid state for other 2',3'-dideoxynucleoside analogs active against HIV, may contribute significantly to the anti-HIV activity of the compound by increasing the affinity for nucleoside kinases involved in phosphorylation and/or by increasing the affinity of the 5'-triphosphate for HIV RT. In this report, we describe nOe studies carried out at 500 MHz to determine the conformations of the furanose rings of the 5'-triphosphates of AZT (AZTTP) and 2'-deoxythymidine (dTTP) when bound to HIV-1 RT. The results show the conformations of AZTTP and dTTP are virtually identical in the bound state. Both glycosidic bond torsional angles are anti with the χ of AZTTP being -120° ± 12 and the χ of dTTP being -110 ° ± 8. The C4'-C5' rotor appears to be +sc. The pseudorotational phase angles of the 3'-azido,2',3'-dideoxyribose ring of AZTTP and the 2'-dideoxyribose ring of dTTP are similar, having values of 60° ± 10 and 55° ± 8, respectively. Whether the rings are locked in the C4'-exo conformation (P=54°) or are in rapid equilibrium between the C2'-and C3'-endo conformations with the position of the equilibrium favoring C3'-endo cannot be determined from this data. In conclusion, the unusual conformation observed for the 3'-azido-,2',3'-dideoxyribose ring of AZT in the solid state (P=215°) is not observed for AZTTP when bound to HIV-1 RT. Since a chain terminating inhibitor must be recognized and utilized as a substrate by the enzyme, perhaps it is the similarity rather than the dissimilarity of the conformations of AZTTP and dTTP in the bound state that contributes to the effectiveness of AZTTP as an antiviral agent. Preliminary results on analogues with a heteroatom in the 3'-position will also be discussed.

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Local Perturbation of Recombinant HIV-1 Reverse Transcriptase by Urea Occurs Prior to Global Denaturation. LL Wright, JE Wilson, JL Martin, SE Haire, PH Ray, GR Painter, Division of Virology, Burroughts Wellcome Co, Research Triangle Park, NC USA

The equilibrium unfolding transition induced by urea of recombinant, heterodimeric HIV-1 reverse transcriptase was monitored by spectroscopic methods (fluorescence emission intensity and circular dicroism) and changes in enzymatic activity. This study was undertaken to characterize the structural stability of wild-type enzyme for comparison to future studies on AZT-resistant mutants. The equilibrium denaturation profiles from both spectroscopic methods were coincident. The transition is well described by a two-state mechanism in which only native and fully denatured protein are present in the transition region. The loss of activity induced by increasing concentrations of urea is also well described by a two-state mechanism, but the transition, involving active and fully inactivated forms of the protein, is not coincident with the spectroscopic profile. This result suggests that local perturbation of the catalytically active site occurs prior to global denaturation, as evidenced by the midpoint of the transitions occuring at 2.0 M and 4.1 M urea respectively. A slight disturbance to the spatial geometry of the functional groups at the active site must destroy the enzyme's activity before any gross global conformational change can be detected. The active site is probably located in a more flexible or mobile region of the enzyme. Additionally the spectroscopic transition is 100% reversible; the renaturation profile of the enzyme coincides with the denaturation profile. However, the return of catalytic activity by dilution from 8 M urea is not coincident with the loss of activity of native enzyme, only 9% of the native enzyme activity is recovered after dilution. The role of enzyme solubility and the kinetic competition of refolding vs. aggregation in the renaturation process monitored by changes in catalytic activity will be discussed.