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Inhibition of the Human Immunodeficiency Virus Type 1 Reverse Transcriptase by 2'-Deoxynucleoside-5'-Triphosphate Substrates
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Substrate inhibition was observed with the heterodimeric (66/51), and the homodimeric (66/66,51/51) forms of the HIV-1 reverse transcriptase. An apparent Ki value of 195 ± 37uM was determined for dTTP using the cloned and expressed heterodimer Similar values were obtained with the homodimeric and the virus-encoded enzymes. Substrate inhibition was not confined to pyrimidine dNTPs. When poly(rC)·pd(G)$_{10}$ was used as template-primer, dGTP exhibited substrate inhibition with an apparent Ki value of 189 ± 32uM. Substrate inhibition was not observed with dTTP when the DNA-DNA homopolymer poly(dA).pd(T)10 was used as template-primer. Inhibition of enzyme activity also occurred with noncomplementary dNTPs, but at concentrations (>5mM) where the ionic strength of the reaction medium was responsible for the inhibition. Substrate inhibition of the heterodimer does not appear to be due to an allosteric mechanism involving the catalytically inactive p51 subunit, since Hill coefficients for substrate binding determined in the presence of saturating concentrations of template-primer are one. Furthermore, UV crosslinking experiments with [32P] dTTP shows that crosslinking only occurred to the p66 subunit, thereby eliminating low affinity binding to p51 as a source of substrate inhibition.

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Interaction of the 5'-triphosphate of the carbocyclic analog of 2'-deoxyguanosine with human immunodeficiency virus type-1 reverse transcriptase. William B. Parker, E. Lucile White, Sue C. Shaddix, Larry J. Ross, John A. Secrist, III and William M. Shannon. Kettering-Meyer Laboratories, Southern Research Institute, Birmingham, Alabama 35205

In an effort to better understand structural features in nucleoside analogs that result in the inhibition of HIV-1 reverse transcriptase, we have evaluated a number of nucleoside 5'-triphosphates as inhibitors of HIV-1 reverse transcriptase, regardless of the activity of their nucleosides against HIV-1 in cultured cells. We found that the 5'-triphosphate of the carbocyclic analog (substitution of the ring oxygen by a methylene group) of 2'-deoxyguanosine (CdG-TP) was a reasonably potent inhibitor of the RNA- and DNA-directed activity of HIV-1 reverse transcriptase. Inhibition was competitive with dGTP, and the $K_i$ was 1 $\mu$M. To determine whether or not CdG-TP could replace dGTP in this reaction, HIV-1 reverse transcriptase was incubated in the absence of dGTP and in the presence of CdG-TP, dATP, dCTP, TTP and a [$^{32}$P]labeled primer annealed to a DNA template. The reaction products were separated by electrophoresis and visualized by autoradiography. HIV-1 reverse transcriptase incorporated CdG-MP into the DNA chain, but was not able to extend the chain after the incorporation of CdG-MP. This result was unexpected, because CdG-MP retains the 3'-hydroxyl and could theoretically be extended by any DNA polymerase (Herpes Simplex Virus type 1 DNA polymerase is able to extend the DNA chain after the incorporation of CdG-MP). The $K_m$ and $V_{max}$ for incorporation of CdG-MP by HIV-1 reverse transcriptase were similar to the values for dGTP. CdG presumably does not inhibit HIV replication in cell culture assays due to its inadequate phosphorylation to the triphosphate. Similar experiments are planned using an RNA template containing all four bases. To our knowledge this is the only non dideoxy-nucleotide analog that has been shown to inhibit HIV-1 reverse transcriptase activity. Research supported by NIAID grant number U01 AI26054.