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**INHIBITION OF THE REVERSE TRANSCRIPTASE OF HIV-1 BY 3'-AZIDOTHYMININE TRIPHOSPHATE AND 3'-AZIDO-OLIGOTHYMYDYLATE**

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**ABSTRACT**

The inhibition of HIV-1 reverse transcriptase by 3'-azidothymidine triphosphate ( $N_3$ -dTTP) and 3'-azidooligothymidylate ( $N_3$ -(dT)<sub>16</sub>) was investigated by kinetic analysis. The inhibition patterns suggest for both compounds a dead-end inhibition mode.

3'-Azidothymidine was the first nucleoside analogue which was used in the chemotherapy of AIDS<sup>1</sup>. Most likely it inhibits the reverse transcriptase of HIV-1 in its 5'-triphosphate form<sup>2</sup>. Based on the results of several research groups it could not be decided whether  $N_3$ -dTTP inhibits the reverse transcriptase by simple competition with dTTP or by incorporation of  $N_3$ -dTMP into the growing DNA chain followed by chain termination<sup>3,4</sup>. In an attempt to clarify the inhibition mechanism we have undertaken the present study.

Using (dT)<sub>15</sub>-poly(rA) as primer-template HIV-1 reverse transcriptase incorporates  $N_3$ -dTMP into a growing DNA chain with an apparent  $K_m$  of 3.0  $\mu$ M<sup>5</sup>. dTMP is incorporated with an apparent  $K_m$  of 2.5  $\mu$ M. In comparison to the very close  $K_m$  values the apparent  $v_{max}$  for  $N_3$ -dTMP incorporation (2.3 nmol dNMP/min/mg) is about 50 times lower than that for dTMP incorporation (120 nmol dNMP/min/mg). These results show that  $N_3$ -dTTP is a substrate for HIV-1 reverse transcriptase.

Analysis of the inhibition pattern of  $N_3$ -dTTP revealed a competition with dTTP for the enzyme-primer-template complex with an apparent  $K_i$

of 40 nM<sup>5</sup>. By varying the primer-template concentration an uncompetitive inhibition pattern with an apparent  $K_i$  of 140 nM was obtained. These findings suggest an incorporation of N<sub>3</sub>-dTTP into the primer and inhibition of the enzyme by formation of a dead-end complex.

3'-Azido-oligothymidylate was synthesized by addition of one N<sub>3</sub>-dTTP onto the 3'-end of an oligothymidylate by terminal transferase. Annealed to poly(rA) it competes with the primer-template for the free enzyme<sup>5</sup>. When the dTTP concentration is varied this primer analogue inhibits the RT in a mixed type fashion. The low apparent inhibition constant of this chain-terminated primer suggests further investigations on this new class of HIV inhibitors.

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