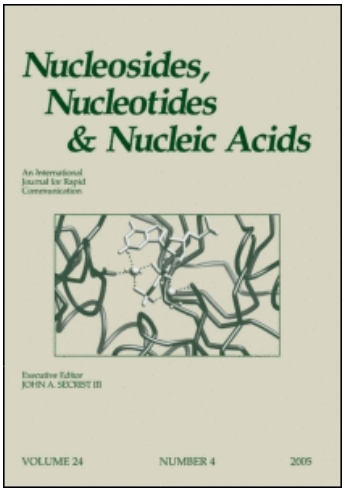

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INHIBITION OF THE REVERSE TRANSCRIPTASE OF HIV-1 BY 3'-AZIDOTHYMIDINE TRIPHOSPHATE AND 3'-AZIDO-OLIGOTHYMIDYLATE

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ABSTRACT

The inhibition of HIV-1 reverse transcriptase by 3'-azidothymidine triphosphate (N_3-dTTP) and 3'-azidooligothymidylate (N_3-$(dT)_{16}$) 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[1]. Most likely it inhibits the reverse transcriptase of HIV-1 in its 5'-triphosphate form[2]. 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[3,4]. In an attempt to clarify the inhibition mechanism we have undertaken the present study.

Using $(dT)_{15}$-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 μM[5]. dTMP is incorporated with an apparent K_m of 2.5 μM. 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 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[5]. 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_3-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_3-dTMP onto the 3´-end of an oligothymidylate by terminal transferase. Annealed to poly(rA) it competes with the primer-template for the free enzyme[5]. 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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