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Inhibition of reverse transcription in rat liver intracisternal A-particles by thymidine derivatives

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The thymidine derivatives araAzT, dTTP(3'N_3), TTP(3'NH_2), and araTTP(3'N_3), were studied as inhibitors of the reverse transcription taking place within endogenous retroviral A-type particles, where retroviral RNAs served as templates and primers. dTTP(3'N_3) was shown to be the most efficient inhibitor of retroviral particle reverse transcription. Termination of DNA chain elongation is the basic mechanism of the inhibitory action of dTTP(3'N_3). The compound has a very low inhibitory effect on mammalian DNA-dependent DNA polymerases α, β and γ.

Retroviral particle, A-type; DNA synthesis; Selective inhibitor

1. INTRODUCTION

Previously it has been shown that the postmitochondrial supernatant of liver homogenates of adult Wistar rats possesses RT activity [1]. The source of the activity was found to be the virus-like particles identified by hybridization with the appropriate DNA probe as IAP, an endogenous A-type retrovirus [2]. The DNA sequences related to the mouse IAP genes have been detected in the rat genome [3]. In this genome the proretroviral IAP *pol* gene was revealed and its expression in adult rat tissues demonstrated [4]. The rat liver IAP RT was found in the particle-bound and free forms and the properties of the purified enzyme studied [1,5].

Data are available suggesting that the RT of endogenous retroviruses and particularly IAP RT may have in animal cells some physiological functions [6]. The application of RT inhibitors can help in elucidation of the putative role of this enzyme in normal animal cells. Efficient RT inhibitors are employed as drugs for treatment of diseases caused by retroviruses. Some types of dideoxynucleoside triphosphates were shown to act as selective inhibitors of RT of the C-type retroviruses, avian myeloblastosis virus [7,8], mouse Rausher leukemia virus [9] and human acquired immunodeficiency virus [10,11]. It was found that a highly efficient inhibitor of these enzymes in cell-free systems is dTTP(3'N_3) [7–11]. The inhibition was evoked by incorporation of this dideoxynucleotide residue into the growing DNA chain and termination of chain elongation, AzT, which is phosphorylated in animal cells and forms dTTP(3'N_3), was found to be an efficient inhibitor of reverse transcription in animal cells transformed by the retroviruses of the C-type [12–14].

It was shown previously that reverse transcription takes place in the dNTP-permeable Triton X-100-treated IAP where it is catalyzed by the IAP RT and templated by the retroviral RNA [1].

We studied the effect of thymidine derivatives

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Abbreviations: dTTP(3'N_3) and dTTP(3'NH_2), 5'-triphosphates of 3'-azido-2',3'-dideoxythymidine and 3'-amino-2',3'-dideoxythymidine; araTTP(3'N_3), 3'-azido-3'-deoxyarabinothymidine 5'-triphosphate; araAzT, 3'-azido-3'-deoxyarabinothymidine; IAP, intracisternal A-type particles; RT, reverse transcriptase

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on the reverse transcription which takes place in such Triton X-100-treated retroviral particles. dTTP(3'N_3) was shown to be the most efficient and selective inhibitor of reverse transcription. The inhibitory action of dTTP(3'N_3) on IAP reverse transcription is concerned with termination of DNA chain elongation.

2. MATERIALS AND METHODS

Particles of the endogenous retrovirus, IAP, were obtained from the liver microsomal fraction of adult Wistar rats (120–150 g body wt) as described [1]. According to DEAE-cellulose and phosphocellulose chromatography, the preparations of retroviral particles contained no detectable DNA-dependent DNA polymerase activity [5]. The standard reaction mixture for determination of IAP reverse transcription contained (in 50 μl): 50 mM Tris-HCl (pH 7.8) at 37°C, 1 mM DTT, 0.02% Triton X-100, 10 mM $MgCl_2$, 80 μg/ml actinomycin D, 10 μM [^{3}H]dCTP (450 cpm/pmol) and 100 μM other dNTPs, 50–100 μg IAP-rich microsomal fraction (calculated on a protein basis). The mixture was incubated for 30 min at 37°C; aliquots (45 μl) on DEAE-cellulose DE-81 filters were washed with 0.5 M K-phosphate buffer (pH 7), water and then dried as in [5].

dTTP(3'N_3) and araTTP(3'N_3) were synthesized by triphosphorylation of 3'-azido-2',3'-dideoxythymidine [15] and araAzT [16] using established methods [17,18]. dTTP(3'N_3) was reduced to dTTP(3'NH_2) with triphenylphosphine as in [15,17]. These compounds were added to reaction mixtures in the amounts indicated in the figure legends. DNA polymerase α (500 U/mg) was isolated from calf thymus according to [19], DNA polymerase β (220 U/mg) and γ (250 U/mg) being prepared from rat brain nuclei and synaptosomes, respectively, according to [20]; activated calf thymus DNA was used as a template. One unit of DNA polymerase activity was defined as 1 pmol labelled deoxynucleotide incorporated into DNA at 37°C per 30 min.

3. RESULTS AND DISCUSSION

The effect of thymidine derivatives on reverse transcription in IAP is presented in fig.1. dTTP(3'N_3) was shown to be the most efficient inhibitor of reverse transcription templated by the IAP RNA catalyzed by IAP RT. It was demonstrated earlier that this compound also acts as a potent inhibitor of the RT of avian myeloblastosis virus [11]. Other TTP analogues studied were less effective as inhibitors while araAzT did not inhibit IAP RT activity at all.

The data suggest that low substrate specificity is characteristic of the IAP RT as for RT from other sources [7]. 50% inhibition of IAP reverse transcription by dTTP(3'N_3) occurs at a

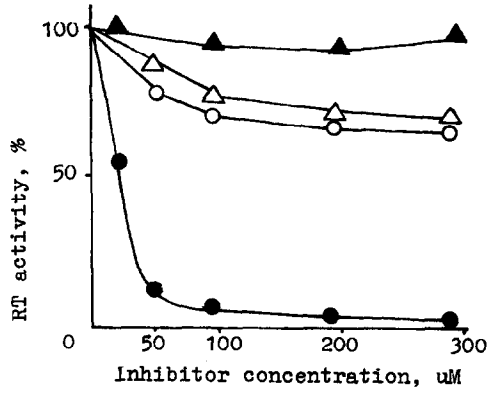

Fig.1. Effect of different thymidine derivatives on RT activity in rat liver retroviral A-particles: (○—○) dTTP(3'NH_2), (●—●) dTTP(3'N_3), (▲—▲) araTTP(3'N_3), (△—△) araAzT; 100% activity corresponds to incorporation of 14 pmol [^{3}H]dGMP into DNA over 30 min.

dTTP(3'N_3)/dTTP molar ratio of 1:4 (fig.1). If competitive inhibition of reverse transcription by dTTP(3'N_3) takes place, one must assume that $K_i < K_m$ for dTTP and that the affinity of IAP RT for the inhibitor is higher than for substrate. However, termination of the growing DNA chain as the mechanism of inhibition seems to be more plausible.

Direct study of the molecular mechanism of dTTP(3'N_3) action by gel electrophoresis as in [7,11] is impossible because of the presence of nucleases in preparations of IAP RT. Therefore, we sought an indirect approach. DNA synthesis was studied after preincubation of IAPs with dTTP(3'N_3) in the presence or absence of dNTPs. Since reverse transcription is templated by the natural retroviral RNA, dTTP(3'N_3) could be efficiently incorporated into DNA only in the presence of other dNTPs.

In the case of termination of DNA synthesis, the effective concentration of DNA chains being elongated should decrease during preincubation with dTTP(3'N_3). The velocity of subsequent DNA synthesis depends on the number of DNA chains elongated under conditions when DNA polymerase is not template-primer saturated.

The appropriate experiments were performed, the results being presented in fig.2. The IAP fraction was preincubated with 10 μM dTTP(3'N_3), 50 μM dCTP, 100 μM dATP and dGTP, while dTTP was omitted (fig.2, curve 3). In controls, the

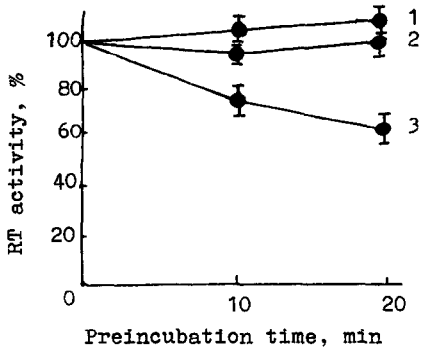

Fig.2. Effect of conditions of IAP preincubation on rate of subsequent reverse transcription: (1) preincubation with all components of the reaction mixture except dNTPs (control); (2) control + dTTP(3'N_3); (3) control + dTTP(3'N_3) + dATP, dGTP, dCTP. Averages from 3–5 experiments are given; 100% activity corresponds to incorporation of 55 pmol [^{3}H]dGMP into DNA over 30 min.

reaction mixture for preincubation contained dTTP(3'N_3), but all dNTPs were omitted (fig.2, curve 2) or both dTTP(3'N_3) and dNTPs were omitted (fig.2, curve 1). For the next 30 min of incubation [^{3}H]dCTP, dTTP at 100 μM and other components were added to the reaction mixture so that their final compositions were similar in all variants.

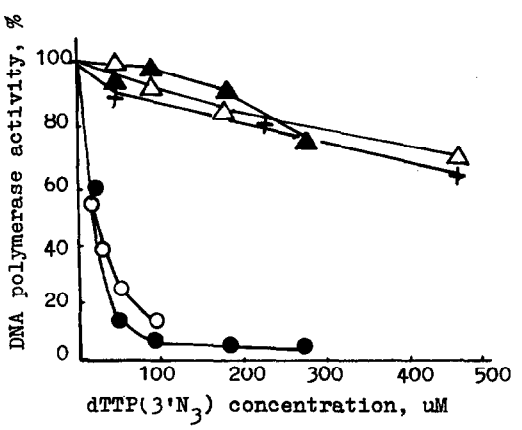

Fig.3. Effect of dTTP(3'N_3) on activity of various DNA polymerases: (▲) DNA polymerase α; (△) DNA polymerase β; (+) DNA polymerase γ; (○) AMV RT; (●) IAP RT. 100% polymerization activity of the various enzymes ranged from 3 to 100 pmol [^{3}H]dNMP incorporated into DNA. The following templates were employed for studying DNA polymerase activities: DNA polymerase α, β and γ, activated DNA; AMV RT, total rat liver RNA with random hexanucleotide mixture as primer; IAPs, own retroviral RNA; concentration of dTTP was 20 μM for DNA polymerases α, β and γ, and 100 μM for RT.

If dTTP(3'N_3) inhibits DNA synthesis by competition with dTTP for the RT in all three variants, similar RT activity should be found independently of the preincubation conditions. However, clear-cut differences between the experiment and controls suggest that dNTP(3'N_3) inhibits reverse transcription in IAP by termination of DNA chain elongation.

Specificity of the inhibitory effect of dTTP(3'N_3) on RT activity was also investigated. At 100–150 μM, dTTP(3'N_3) almost completely inhibits activities of IAP and AMV RT and has a low inhibitory effect on mammalian DNA polymerases α, β and γ (fig.3).

Therefore, dTTP(3'N_3) was shown to be an efficient and highly specific inhibitor of RT in retroviral particles when the natural templates and primers were employed.

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