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INHIBITION OF HUMAN TELOMERASE BY L-ENANTIOMERS OF NATURAL 2'-DEOXYRIBONUCLEOSIDE 5'-TRIPHOSPHATES

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INHIBITION OF HUMAN TELOMERASE BY L-ENANTIOMERS OF NATURAL 2'-DEOXYRIBONUCLEOSIDE 5'-TRIPHOSPHATES

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ABSTRACT

In order to clarify whether L-enantiomers of natural 2'-deoxyribonucleoside 5'-triphosphates (dNTPs) are recognized by human telomerase, a quantitative telomerase assay based on the 'stretch PCR' method was developed and used for kinetic analysis. Among the four L-dNTPs, L-dTTP and L-dGTP inhibited telomerase activity and the others showed slight or no inhibitory effect. Lineweaver-Burk plot analysis showed that the inhibition mode L-dGTP was competitive with dGTP.

Telomerase, which catalyses telomere DNA elongation in animal cells through addition of the repeat 5'-TTAGGG-3' using an RNA template in the enzyme molecule, is classified as one of the reverse transcriptases (RTs) (1). Interestingly, HIV-1 RT is able to bind to the L-enantiomer of dTTP when acting on poly(A)-oligo(dT) as the template-primer (2,3). To clarify the chiral discrimination ability of telomerase for L-enantiomers of 2'-deoxyribonucleoside 5'-triphosphates (dNTPs)

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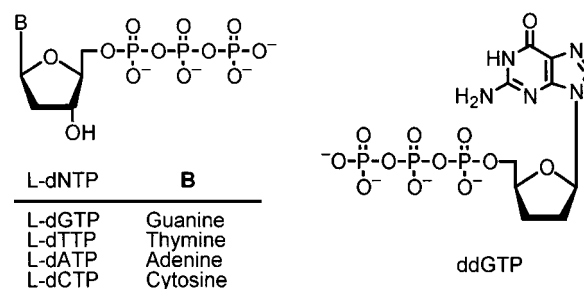


Figure 1. Nucleotide analogues examined in this study.

(4) (Fig. 1), a quantitative telomerase assay based on the ‘stretch PCR’ method was established and utilized for kinetic analysis.

MATERIALS AND METHODS

Quantitative Telomerase Assay Based on the ‘Stretch PCR’ Method (5)

Telomere DNA extension—A reaction mixture (20 μ l) for telomere DNA extension comprised 50 mM Tris-AcOH (pH 8.5), 50 mM KOAc, 1 mM DTT, 1 μ M TAG-U (5'-GTA AAA CGA CGG CCA GTT TGG GGT TGG GGT TGG GGT TG-3'), 10–500 μ M dGTP, dTTP and dATP, 1 mM MgCl₂ or 0.1 mM MnCl₂, and HeLa cell S100 extract (1–1.7 μ g of protein). Incubation was performed for 10 min at 30°C.

PCR amplification and detection of the PCR products—The DNA products were extracted with phenol/CHCl₃, and precipitated by addition of ethanol for removal of low-molecular-weight materials. Then, PCR amplification of the purified DNA was performed using CTA-R (3'-TCC CAA TCC CAA TCC CAA TCC CCA GTA TCG ACA AAG GAC-5') as the reverse primer. The PCR products were separated by 10% polyacrylamide gel electrophoresis and detected by staining with SYBR green I. The amounts of PCR products constituting a telomere DNA ladder were estimated with a fluorescent image analyzer FLA 3000 (Fuji Film).

RESULTS AND DISCUSSION

In the present study, we have optimized the stretch PCR method to perform a kinetic analysis of telomerase. In the procedure, the use of a fluorescent image analyzer was essential to estimate the amounts of DNA stained with SYBR green I. With this assay method, Lineweaver-Burk plot was used to analyze the inhibition by ddGTP, which is known to be a potent telomerase inhibitor (6) (Fig. 2). The mode of inhibition of human telomerase by ddGTP was competitive with dGTP. The K_i value of ddGTP was estimated to be 0.65 μ M. This K_i value is approximately 20 times

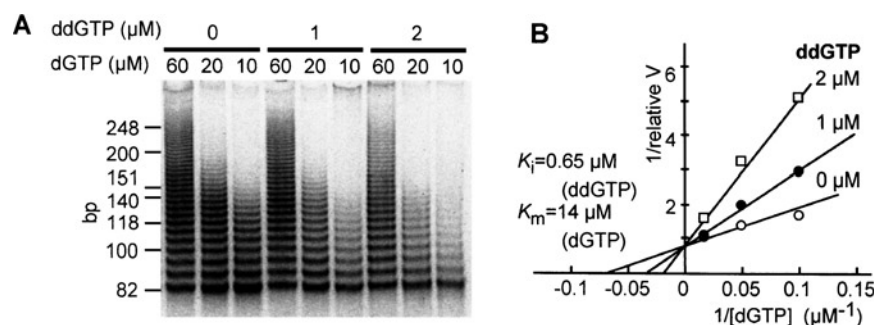


Figure 2. Lineweaver-Burk plot analysis of the inhibitory effect of ddGTP on telomerase. Telomerase activity in the presence of the indicated concentrations of ddGTP (A). Lineweaver-Burk plot analysis of the inhibition by ddGTP (B). Relative reaction velocity (relative V) was calculated relative to the activity without ddGTP in the presence of 60 μM dGTP, which was taken as 1.

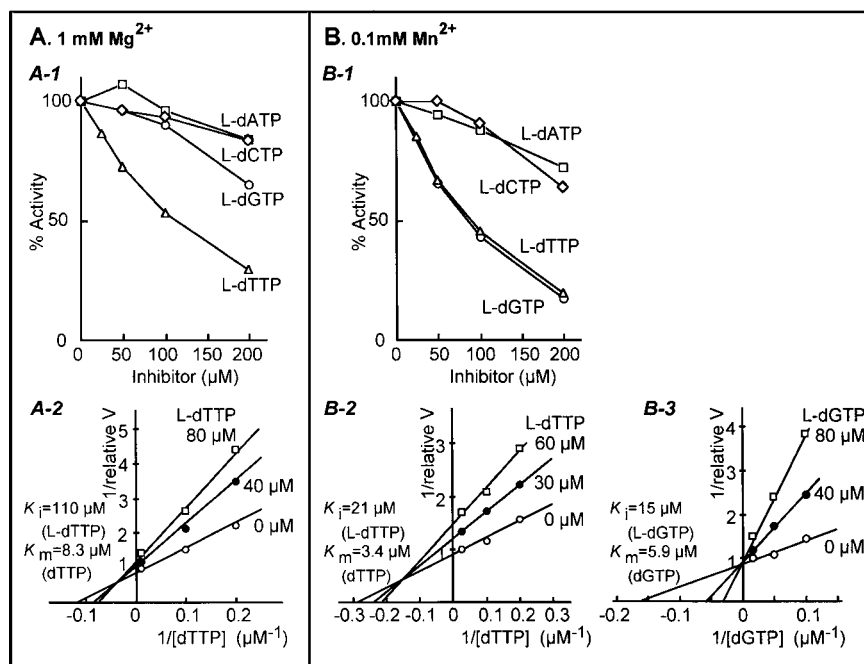


Figure 3. Inhibition of telomerase activity by L-enantiomers of natural dNTPs in the presence of 1 mM Mg^{2+} (A) or Mn^{2+} (B) as a divalent cation. **A-1 & B-1.** Telomerase activity in the presence of the indicated concentrations of L-enantiomers. Telomerase activity was measured as described in the text in the presence of various concentrations of inhibitor, the D-dNTP (10 μM) corresponding to the inhibitor, and the other two dNTPs (200 μM each). When L-dCTP was examined, the concentrations of dGTP, dATP and dTTP were 200, 200 and 10 μM , respectively. Activity without inhibitor was taken as 100%, and the activities remaining are shown. **A-2, B-2 and B-3.** Lineweaver-Burk plot analyses of the inhibition by L-dTTP (A-2 and B-2) and L-dGTP (B-3). Relative reaction velocity (relative V) was calculated relative to the activities without inhibitor in the presence of 100 μM dTTP (A-2), 40 μM dTTP (B-2) and 60 μM dGTP (B-3), which were taken as 1.



smaller than the K_m of dGTP ($14 \mu\text{M}$). The K_m value ($14 \mu\text{M}$) of dGTP was similar to that (approximately $8 \mu\text{M}$) of telomerase from human leukemic cells (7).

Among the four L-enantiomers of the natural substrate dNTPs, L-dTTP and L-dGTP inhibited telomerase activity and the others showed slight or no inhibitory effect (Fig. 3). Human telomerase was inhibited partially competitively (mixed type inhibition) by L-dTTP in the presence of Mg^{2+} or Mn^{2+} (Fig. 3A-2 and B-2). On the other hand, telomerase was significantly inhibited by L-dGTP in the presence of Mn^{2+} (Fig. 3B-1). The mode of inhibition was competitive with dGTP (Fig. 3B-3). The competition between L-dGTP and dGTP indicates that both nucleotides bind to the same active site of the enzyme. The K_i value of L-dGTP ($15 \mu\text{M}$) was approximately three times larger than the K_m of dGTP ($5.9 \mu\text{M}$). In the case of HIV-1 RT, the K_i value of L-dTTP ($1.2 \mu\text{M}$) is smaller than the K_m of dTTP ($17 \mu\text{M}$) when poly(A)-oligo(dT) is used as the template-primer in the presence of Mn^{2+} (2). Thus, the active site of telomerase also exhibits low chiral discriminatory ability, although not as low as that of HIV-1 RT.

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