Telomerase-inhibitory effects of sugar-modified nucleotide analogs

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ABSTRACT
Telomerase is an endogenous reverse transcriptase that uses its internal RNA moiety as a template for the synthesis of telomere repeats, thus maintaining telomere length. To study the susceptibility of telomerase to sugar-modified nucleotide analogs, inhibition by arabinofuranosylguanine 5'-triphosphate (araGTP), 3'-azido-2',3'-dideoxyguanosine 5'-triphosphate (AZdGTP), 2',3'-dideoxy-2'-fluoroarabinofuranosylguanine 5'-triphosphate (FaraGTP), and their thymine counterparts was investigated. Among these compounds, all dGTP analogs showed potent inhibitory activity against human telomerase. Conversely, dTTP analogs showed moderate or weak inhibition. Partially purified telomerase from cherry salmon testis utilized dDGTP and AZdGTP as substrates into the 3'-terminus of DNA.

INTRODUCTION
Telomerase, which catalyzes telomere DNA elongation in animal cells through addition of TTAGGG using an RNA template in the enzyme molecule, thus contributing to the maintenance of telomeres, is classified as one of the reverse transcriptase (1,2). In mammals, telomerase activity is present in renewing tissues, such as germ-line tissues, stem cells and tumor cells, but not in most somatic cells. Since the telomerase activity is low even in these telomerase-positive cells, it is generally difficult to detect without using a method for amplifying the DNA products that are synthesized by telomerase. It is also difficult to prepare a large amount of telomerase from these mammalian cells. Recently, Klapper et al. reported that high telomerase activity was detectable in several normal organs of the rainbow trout, being 10–100-fold higher than the activity in the human tumor cell line L-428 (3). Therefore, we have partially purified telomerase from cherry salmon testis, a source that is readily available in Japan. To investigate the inhibition mechanism of dGTP analogs (Figure 1), which are potent telomerase inhibitors, we have analyzed whether these inhibitors are used as substrates by the enzyme.

Fig. 1. Nucleotide analogs examined in this study.

MATERIALS AND METHODS
Nucleotide analogs. AZdGTP and FaraGTP were obtained from the corresponding nucleosides (4,5) by chemical phosphorylation.

Telomerase activity measurement. To study the properties of telomerase and for inhibition studies with some compounds, the stretch PCR assay was performed as described for human HeLa cell extracts (6,7).

Partial purification of telomerase from immature cherry salmon testis. S-100 extracts from cherry salmon testis (5 g) were prepared as described by Tatematsu et al (7). Telomerase was partially purified from the extracts by DEAE cellulose column chromatography and (NH4)2SO4 fractionation.

Primer extension assay. The reaction mixture (40 μl) comprised 50 mM Tris-HOAc, pH 8.5, 50 mM KOAc, 1 mM MgCl2, 1 mM dithiothreitol, 2 μM (TTAGGG)3, 5 μM [α-32P]dTTP, 5 μM dATP, 5 or 0 μM dGTP, several
concentrations of dGTP analogs and 50% by volume of partially purified enzyme preparation. Incubation was performed for 1 h at 25°C. DNA product purification and imaging were performed as described by Tendian and Parker with slight modification (8).

RESULTS AND DISCUSSION

Cherry salmon testis telomerase. The enzymatic properties of the partially purified telomerase from cherry salmon testis were similar to those of human telomerase (data not shown). As expected for a ribonucleoprotein, salmon telomerase activity was abolished in the enzyme preparation pretreated by heating or with RNase A. Salmon telomerase activity was also abolished after omitting dTTP, dATP, or dGTP, but unaffected by the absence of dCTP. Cherry salmon telomerase showed maximum activity at around 25°C, which is lower than the optimal temperature for human telomerase.

Fig. 1. Inhibition by dGTP analogs and incorporation of dGTP analogs into DNA. The inhibitory effects of the sugar-modified analogs shown in Figure 1 on human telomerase activity were investigated using the stretch PCR assay (data not shown). The guanine analogs ddGTP, araGTP, AZdGTP, and FaraGTP showed potent inhibitory effects. On the other hand, the thymine counterparts showed moderate or weak inhibition. Next, we analyzed the primer extension products that were synthesized by cherry salmon telomerase in the presence of ddGTP, araGTP or AZdGTP instead of dGTP. Among them, ddGTP and AZdGTP were effectively incorporated into the 3'-terminus of the primer strand (Figure 2). Telomerase incorporates dGMP as much as three residues during an extension reaction of six residues. Therefore, dGTP analogs may be promising telomerase inhibitors. Further study of some dGTP analogs containing a fluorine atom in the sugar moiety is now underway in our laboratory.

ACKNOWLEDGMENTS

We thank Dr. Teruo Azuma, Nikko Branch, National Research Institute of Aquaculture, Fisheries Research Agency, for kindly providing immature male cherry salmon. This work was supported in part by a Grant-in-Aid for Scientific research on Bioscience/Biotechnology Areas from the Ministry of Education, Culture, Sports, Science and Technology, and a grant from the Promotion and Mutual Corporation for Private Schools in Japan.

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