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Human Telomerase Inhibition by 7-Deaza-2′-deoxypurine Nucleoside Triphosphates†

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ABSTRACT: Telomeres play an important role in chromosome organization and stability. Human telomerase is a terminal transferase that adds TTAGGG units onto the telomere end. In general, telomerase activity is not detected in normal somatic cells but is present in immortalized cells. Consequently, telomerase might be a selective target for cancer chemotherapy. Using cell-free biochemical telomerase assay, we have found that 7-deaza-2′-deoxyguanosine-5′-triphosphate (7-deaza-dGTP) and 7-deaza-2′-deoxyadenosine-5′-triphosphate (7-deaza-dATP) were potent telomerase inhibitors. The concentrations of inhibitors in which 50% of the telomerase activity was inhibited (IC_{50} values) were 11 and 8 μM for 7-deaza-dGTP and 7-deaza-dATP, respectively. Additional studies show that both 7-deaza-dGTP and 7-deaza-dATP were also incorporated into telomeric DNA by telomerase. However, incorporation of 7-deaza-dATP or 7-deaza-dGTP results in a telomeric ladder that is prematurely shortened. No difference in the number or position of pause sites were observed when 7-deaza-dATP was compared to dATP as substrates. On the other hand, both a shift and an increase in pause sites was observed when dGTP was replaced by 7-deaza-dGTP. Incorporation of 7-deaza nucleotides by telomerase may be used as a tool for the study of telomerase mechanism and function. In addition, this may be a novel approach in the design of new telomerase inhibitors.

Telomeres, the ends of eukaryotic chromosomes, are composed of tandemly repeated guanine-rich sequences which have an important role in chromosome organization and stability. However, due to the nature of DNA synthesis, the 5′ ends of telomeres shorten with each round of replication leaving a 3′ overhang that is subject to degradation. This has been described as the "end-replication" problem of linear chromosomes (Watson, 1972; Olovnikov, 1973). The end-replication problem could be overcome by addition of nucleotides to the 3′ end of the telomere. A telomere terminal transferase (telomerase) activity was initially discovered in *Tetrahymena* (Greider & Backburn, 1985). Telomerase activity has since been found in other ciliates (Zahler & Prescott, 1988; Shippen-Lentz & Blackburn, 1989), *Xenopus* (Mantell & Greider, 1994), yeast (Cohn & Blackburn, 1995), mouse (Prowse et al., 1993), and human cells (Morin, 1989). Telomerase is a ribonucleoprotein in which the internal RNA component serves as a template for directing the appropriate telomeric sequences onto the 3′ end of a telomeric primer. The cloning (Greider & Blackburn, 1989) and secondary structure determinations of the *Tetrahymena* telomerase RNA have determined the template portion of the RNA which has suggested a model for the

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mechanism of telomerase activity. This mechanism includes (1) telomerase binding to the 3′ single-stranded overhang of the telomere (TTAGGG in humans) which base pairs with the complementary bases of the RNA component of telomerase; (2) nucleotide addition onto the 3′ end of the telomere by telomerase using its RNA component as a template; and (3) dissociation of the newly synthesized telomeric DNA from the RNA template and repositioning to allow for the next round of polymerization. This last step is called the translocation step.

The variety of secondary structures formed by the guanine-rich telomeric sequences involving G-quartets or hairpins (Guschlbauer, 1990; Williamson, 1994) can have an affect on telomerase activity. For example, there is evidence that the G-tetraplex structures formed by telomeric sequences may hinder initial telomerase binding (Zahler et al., 1991). However, it has been proposed that G-tetraplex formation may actually facilitate the translocation step.

G-quartet stuctures may also have a role in telomere function. For example, it has been shown that a variety of proteins will preferentially bind to G-quartet structures (Williamson, 1994). Also, the interaction between guanine rich DNA strands may be involved in the association of chromosomes seen in cells in the presence of varying concentrations of Na^+ (Diaz & Lewis, 1975). The function of chromosomal association is unknown but it has been proposed that it is important in such functions as homologous pairing involved in meiosis (Sen & Gilbert, 1988). Recently, a yeast nuclease (*Kem*1p) was found to specifically recognize and cut only G-quartet structures (Liu & Gilbert, 1994). Deletion of this enzyme was shown to cause telomere shortening, cellular senescence, and blockage in the pachytene stage of meiosis in yeast (Bahler et al., 1994; Tishkoff et al., 1995; Liu et al., 1995).

Another possible function of telomeres has sparked a great deal of interest in cancer research. It has been recently proposed that telomere length may serve as a "mitotic clock" (Harley, 1995; Shay, 1995). Normal cells in which telomeres shorten to a critical length become senescent (Allsopp et al., 1992; Harley, 1991). In contrast cancer cells, being generally immortal, have an unlimited replicative capacity. Due to the findings that telomerase activity is present in a variety of tumor cells (Chadeneau et al., 1995; Counter et al., 1994; 1995; Kim et al., 1994), it appears that activation of telomerase is one link to cellular immortality. This makes inhibition of telomerase an ideal strategy for anti-cancer therapy. A number of nucleoside reverse transcriptase inhibitors do show anti-telomerase activity in human and *Tetrahymena* (Strahl & Blackburn, 1994, 1996).

In the quest for finding nucleotide analogues that inhibit telomerase, we have found two nucleotide analogues, 7-deaza-2′-deoxyguanosine triphosphate (7-deaza-dGTP)[1] and 7-deaza-2′-deoxyadenosine triphosphate (7-deaza-dATP) are potent inhibitors of telomerase activity. Our interest in these nucleotides stemmed from the fact that the N7 nitrogen of purine bases is required for Hoogsteen base-pairing involved in secondary structures formed by telomeric sequences.

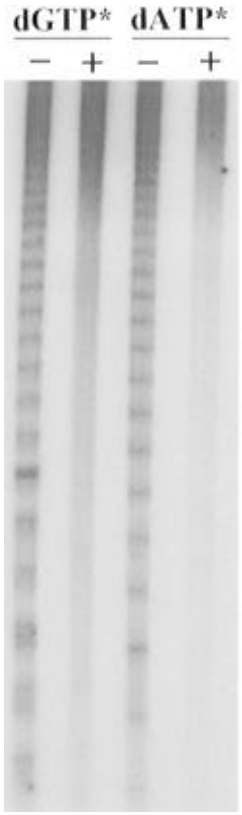

FIGURE 1: Telomerase activity with 1 mM dTTP, 1 μM $(TTAGGG)_3$, 20 μL of S100, 1.56 μM [α-^{32}P]dGTP, 1 mM dATP (dGTP*), or 3.12 μM [α-^{32}P]dATP, and 1 mM dGTP (dATP*). Reactions contained either no RNase A (−) or 0.125 μg/μL of RNase A (+).

MATERIALS AND METHODS

Materials. Oligonucleotide primers (Genosys), dNTP's including 7-deaza-dGTP and 7-deaza-dATP (Pharmacia), [α-^{32}P]dGTP and [α-^{32}P]dATP (Dupont NEN), RNase A, PMSF, pepstatin A, and leupeptin, and other chemicals (Sigma).

Telomerase Isolation (S100). Cells from the transformed human embryonic kidney 293 cell line (a kind gift from Dr. Greider, Cold Spring Harbor) were grown in 2 L of Joklik's modified MEM with 5% FBS to 1×10^6 cells/mL in a spinner flask. The cells were washed with phosphate-buffered saline (10 mM KPO_4, pH 7.5, 140 mM NaCl). The S100 extract was prepared by resuspending the cell pellet in Hypobuffer which contains 10 mM HEPES (pH 8.0), 3 mM KCl, 2 mM $MgCl_2$, 1 mM dithiothreitol, 10 units/mL RNAguard (Pharmacia), 0.1 mM PMSF, 10 μM pepstatin A, and 1 μM leupeptin. The cells were disrupted using a Dounce homogenizer. The homogenate was centrifuged at 100000*g* in a Beckman type 70.1 Ti rotor for 1 h. The supernatant was stored at −80 °C in 20% glycerol and 100 mM NaCl (Heintz, 1984; Morin, 1989).

Telomerase Assay. The standard telomerase assay mixture (40 μL total) was composed of 50 mM Tris-acetate pH 8.5, 50 mM potassium acetate, 5 mM β-mercaptoethanol, 1 mM spermidine, 1 mM $MgCl_2$, 0.5−2 mM dATP, 0.5−2 mM dTTP, 1.56 μM [α-^{32}P]dGTP (800 Ci/mmol), 1 μM oligonucleotide primer, $(TTAGGG)_3$, and 20 μL of telomerase

[1] Abbreviations: bp; Tris-HCl, Tris(hydroxymethyl)aminomentane hydrochloride; MEM, minimal essential medium; HEPES, *N*-[2-hydroxyethyl]piperazine-*N*′-[2-ethansulfonic acid]; PMSF, phenylmethylsulfonic acid; EDTA, ethylenediaminetetraacetate; dGTP, 2′-deoxyguanosine-5′-triphosphate; dATP, 2′-deoxyadenosine-5′-triphosphate; TTP, 2′-deoxythymidine-5′-triphosphate; dG, 2′-deoxyguanosine; dA, 2′-deoxyadenosine; dC, 2′-deoxycytidine.

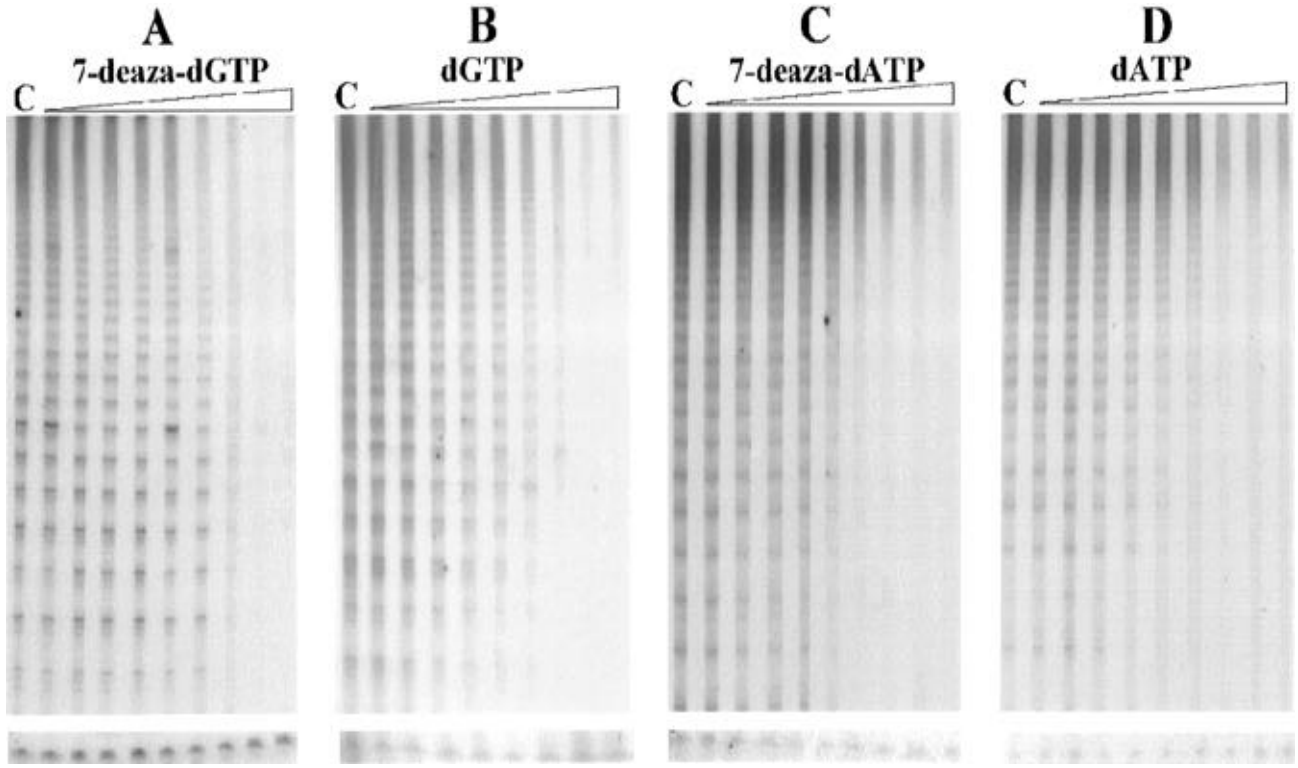

FIGURE 2: Inhibition of telomerase ladder with 7-deaza-dGTP (A) or dGTP (B) in the presence of 1.56 μM [α-^{32}P]dGTP containing 1 mM dTTP, 1 mM dATP, 1 μM $(TTAGGG)_3$, 20 μL of S100, and, from left to right, 0 (C), 1, 2.5, 5, 7.5, 10, 25, 50, 75, and 100 μM 7-deaza-dGTP or dGTP. Inhibition of telomerase ladder with 7-deaza-dATP (C) or dATP (D) in the presence of 3.12 μM [α-^{32}P]dATP, containing 1 mM dTTP, 1 mM dGTP, 1 μM $(TTAGGG)_3$, 20 μL of S100, and from left to right, 0 (C), 1, 2.5, 5, 7.5, 10, 25, 50, 75, and 100 μM 7-deaza-dATP or dATP. The panels below each gel contain the ^{32}P 5′ end-labeled $(TTAGGG)_3$ added before ethanol precipitation for use as an internal standard in the quantitation of telomerase activity as described in Materials and Methods.

S100. The reaction mixture was incubated for 1 h at 30 °C followed by the addition of 50 μL of stop solution (10 mM Tris-HCl, pH 7.5, 20 mM EDTA, and 100 μg/mL RNase A) and incubation for 15 min at 37 °C. The mixture was deproteinated by incubation at 37 °C in the presence of 50 μL of a 0.3 mg/mL proteinase K in 10 mM Tris-HCl (pH 7.5) and 0.5% SDS. The proteinase K mixture also contained a ^{32}P 5′ end-labeled oligonucleotide, $(TTAGGG)_3$, as an internal standard. Addition of 50 μL of the proteinase K mixture will also deliver an equal amount (1000−2000 cpm) of the oligonucleotide to each reaction tube. The DNA products were extracted with phenol:chloroform:isoamyl alcohol and ethanol precipitated. The [α-^{32}P]dGTP-labeled telomerase products were separated by electrophoresis on an 8% polyacrylamide sequencing gel and detected by X-ray film (Xomat, Kodak) autoradiography. Inhibition studies were performed by addition of varying concentrations of nucleotide analogue to the standard telomerase assay mixture. Alternative telomerase assays in the presence of radioactive dATP contained 3.12 μM [α-^{32}P]dATP (800 Ci/mmol) and 1 mM each of TTP and dGTP. Studies in which 7-deaza-dGTP (0.1−2 mM) or 7-deaza-dATP (0.1−2 mM) replace their native nucleotides, dGTP and dATP respectively, were performed to determine if the 7-deaza nucleotides are substrates.

Determination of the IC_{50} Values: Inhibitor Concentration That Inhibits 50% of [α-^{32}P]-Labeled Telomerase Ladder. Telomerase activity for each concentration of added nucleotide analogue was obtained by densitometric analysis (ImageQuant, Molecular Dynamics) of the autoradiographs. The telomerase activity in each gel lane was normalized to the ^{32}P 5′ end-labeled oligonucleotide, $(TTAGGG)_3$ internal standard. Telomerase activity as a % of the control (Y) in the presence of each analogue concentration was plotted *vs* the logarithm of the nucleotide analogue concentration. The IC_{50} values were determined by a computer program that fits all of the data to the following four-parameter logistic equation (EZ-ED50 software, Perrella Scientific Inc.):

$$Y = \frac{A_{max} - A_{min}}{[1 + (X/IC_{50})^n]} + A_{min}$$

where the parameter A_{max} is the maximal telomerase activity of the control, the parameter A_{min} is the telomerase activity in the presence of the highest inhibitor concentration, Y is the observed % telomerase activity, the parameter X is the inhibitor concentration, and n determines the shape of the curve (Chen et al., 1993). The IC_{50} value of each nucleotide was determined from 10−20 data points obtained from two to four separate experiments.

RESULTS

Telomerase Activity in 293 Cells. Telomerase activity from human cells possesses a number of characteristics (Figure 1). First, in the presence of a G-rich human telomeric primer $(TTAGGG)_3$, TTP, dATP, and [α-^{32}P]dGTP, a ladder consisting of bands spaced six bases apart was formed (Morin, 1989). Second, since telomerase contains an RNA component, telomerase activity was obliterated in the presence of RNase A. It has been shown that the bands formed in the presence of excess cold nucleotides TTP and dATP and limiting [α-^{32}P]dGTP (1.56 μM) were indicative of a pause site at the first guanine in the repeating unit of

Table 1: Inhibition of Radiolabeled Human Telomerase Ladder by 7-Deaza-dGTP, 7-Deaza-dATP, dGTP, and dATP

inhibitor	[α-^{32}P]dNTP (μM)	non-radioactive nucleotides (mM)	IC_{50} (μM)[a]
7-deaza-dGTP	dGTP* (1.56 μM)	TTP and dATP (1 mM)	11
dGTP	dGTP* (1.56 μM)	TTP and dATP (1 mM)	5
7-deaza-dATP	dATP* (3.12 μM)	TTP and dGTP (1 mM)	8
dATP	dATP* (3.12 μM)	TTP and dGTP (1 mM)	10
7-deaza-dGTP	dATP* (3.12 μM)	TTP and dGTP (1 mM)	56
7-deaza-dATP	dGTP* (1.56 μM)	TTP and dATP (1 mM)	59

[a] IC_{50} values were obtained according to Materials and Methods.

TTAGGG (Morin, 1989). We have shown that this pause site can shift to bands two bases smaller in the presence of excess TTP and dGTP and limiting concentrations of [α-^{32}P]-dATP (Figure 1, lane 3) which would be the second thymine. The telomerase ladder was difficult to obtain with concentrations less than 3.12 μM of [α-^{32}P]dATP. In addition, we were unable to generate a telomerase ladder in the presence of limiting radioactive dTTP. The dependence of the location of the pause sites on the concentration of nucleotides has also been demonstrated with *Tetrahymena* telomerase (Greider & Blackburn, 1987).

Inhibition of Telomerase by 7-Deaza-dGTP and 7-Deaza-dATP in the Presence of Limiting Amounts of dGTP and dATP. Figure 2A and 2C illustrate the effect of 7-deaza-dGTP and 7-deaza-dATP on the level of telomerase activity. Inhibition of telomerase activity was indicated by a reduction in the intensity of the bands with increasing concentrations of inhibitor. The degree of inhibition was determined by measuring the intensity of the bands in the telomerase ladder with densitometry. The intensities in each lane were normalized to an internal standard, ^{32}P 5′ end-labeled $(TTAGGG)_3$, to compensate for differences in processing of the products and gel loading (the panels below each particular gel). Intensities in all lanes were expressed as a percent of the control to eliminate variability due to differences in film exposure and enzyme preparations. The concentration of inhibitor that results in a 50% reduction in telomerase activity (IC_{50}) was determined according to the method outlined in Materials and Methods (IC_{50} values are in Table 1). The similarity of their IC_{50} values reveals that 7-deaza-dGTP is just as efficient as cold dGTP in inhibiting the formation of [α-^{32}P]dGTP-labeled telomerase ladders (Figure 2A,B, Table 1). Both 7-deaza-dATP and cold dATP have a similar capacity to inhibit the formation of [α-^{32}P]-dATP-labeled products (Figure 2C,D, Table 1).

Inhibition of Telomerase Activity by 7-Deaza-dGTP and 7-Deaza-dATP in the Presence of Excess Amounts of dGTP and dATP. 7-Deaza-dGTP and 7-deaza-dATP can inhibit telomerase activity even in excess (1 mM) dGTP or dATP, respectively (Figure 3A and 3B). The IC_{50} value of 7-deaza-

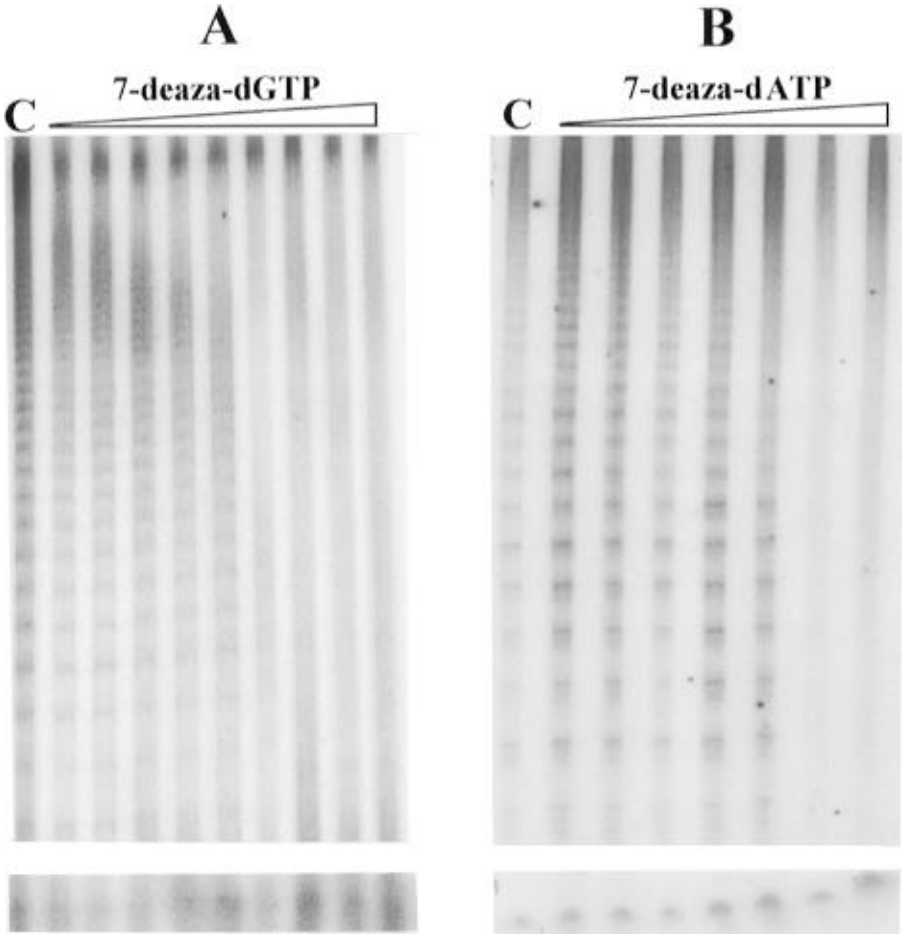

FIGURE 3: (A) Inhibition of telomerase ladder with 7-deaza-dGTP in the presence of 3.12 μM [α-^{32}P]dATP, containing 1 mM dTTP, 1 mM dGTP, 1 μM $(TTAGGG)_3$, 20 μL of S100, and, from left to right, 0 (C), 0.01, 0.025, 0.05, 0.075, 0.1, 0.25, 0.5, 0.75, 1.0, and 2.0 mM 7-deaza-dGTP. (B) Inhibition of telomerase ladder with 7-deaza-dATP in the presence of 1.56 μM [α-^{32}P]dGTP containing 1 mM dTTP, 1 mM dATP, 1 μM $(TTAGGG)_3$, 20 μL of S100, and, from left to right, 0 (C), 0.01, 0.025, 0.05, 0.075, 0.1, 0.25, 0.5, 0.75, 1.0, and 2.0 mM 7-deaza-dATP. The panels below each gel contain the ^{32}P 5' end-labeled $(TTAGGG)_3$ added before ethanol precipitation for use as an internal standard in the quantitation of telomerase activity as described in Materials and Methods.

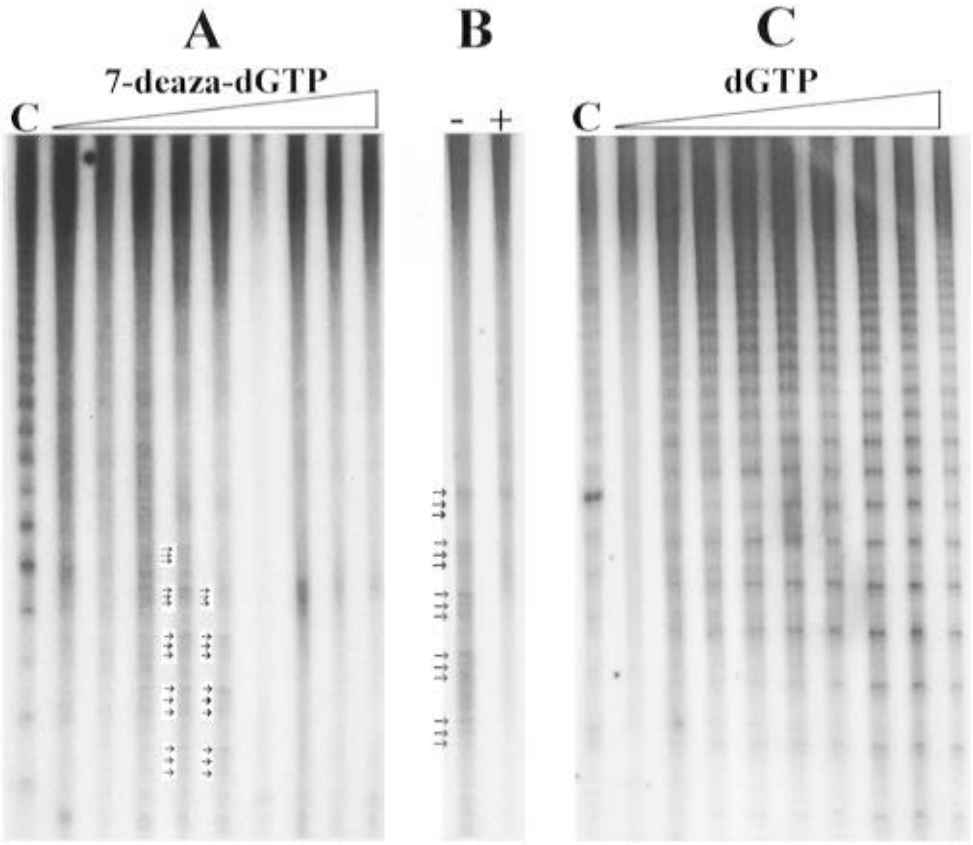

FIGURE 4: (A) 7-deaza-dGTP as a telomerase substrate with 3.12 μM [α-^{32}P]dATP, 1 mM dTTP, 1 μM $(TTAGGG)_3$, 20 μL of S100, and, from left to right, 1 mM dGTP (C), and 0, 0.25, 0. 5, 0.75, 1.0, 1.25, 1.5, 1.75, and 2.0 mM 7-deaza-dGTP. The arrows point to the triplet band most prominent at 0.75 and 1.0 mM 7-deaza-dGTP. (B) 7-Deaza-dGTP as a telomerase substrate with 3.12 μM [α-^{32}P]dATP, 1 mM dTTP, 1 μM $(TTAGGG)_3$, 20 μL of S100, 1 mM 7-deaza-dGTP without (−) or (+) with RNase A at 0.125 μg/μL. The arrows point out the triplet bands. (C) dGTP as a telomerase substrate with 3.12 μM [α-^{32}P]dATP, 1 mM dTTP, 1 μM $(TTAGGG)_3$, 20 μL of S100, and, from left to right, 1 mM dGTP (C) and 0, 0.25, 0. 5, 0.75, 1.0, 1.25, 1.5, 1.75, and 2.0 mM dGTP.

dGTP as a telomerase inhibitor in the presence of 1 mM dGTP, dTTP, and 3.12 μM [α-^{32}P]dATP was 56 μM. The IC_{50} of 7-deaza-dATP was 59 μM when in the presence of 1 mM dATP, TTP, and 1.56 μM [α-^{32}P]dGTP.

7-Deaza-dGTP and 7-Deaza-dATP are Telomerase Substrates. Replacing dGTP with 7-deaza-dGTP in a reaction containing 1 mM dTTP and 3.12 μM [α-^{32}P]dATP resulted in formation of radiolabeled products that are not formed when the enzyme is inactivated by RNase A (Figure 4A and 4B). There are important characteristics of this reaction which distinguish it from a reaction with the native dGTP. First, the total amount of products produced by 7-deaza-dGTP was much less than that produced with dGTP. Second, telomerase appears to be less processive with 7-deaza-dGTP. As the concentration of 7-deaza-dGTP increased, the products became increasingly shorter and the total amount of products decreased until there was no activity at >1.5 mM 7-deaza-dGTP. There is no significant change in activity when dGTP is used as a substrate in the reaction with concentrations varying from 0.25−2.0 mM (Figure 4C). Finally, telomerase paused at significantly more sites in the presence of 7-deaza-dGTP. At a concentration of 0.5 mM 7-deaza-dGTP, there was such a large number of bands pertaining to various pause sites that a predominant pause site could not be determined. When the 7-deaza-dGTP concentration is 0.75−1 mM, a repeating triplet of pause sites was distinguishable (Figure 4A, lanes 5 and 6, Figure 4B). These bands appeared to be two, three, and four bases smaller than that with dGTP (Figure 4A, control lane) corresponding to the guanines in the TTAGGG repeat.

Similar results were observed when dATP was replaced by 7-deaza-dATP with 1 mM TTP and 1.56 μM [α-^{32}P]dGTP (Figure 5A and 5B). The products were sensitive to RNase A, and processivity decreased with increasing 7-deaza-dATP concentrations. No telomerase activity was detected at >1.5 mM 7-deaza-dATP. However, the level of telomerase activity remained unchanged with 0.25−2.0 mM dATP as a substrate. Unlike what is observed with 7-deaza-dGTP, the presence of 7-deaza-dATP does not result in a change in pause sites compared to the reactions in the presence of dATP.

DISCUSSION

The compounds 7-deaza-dGTP and 7-deaza-dATP have been found to be potent inhibitors of telomerase activity in the S100 extracts of 293 cells. The level of inhibition of the radioactive native substrate by these compounds is comparable to that caused by the cold native substrate. Although the results in Figure 2 suggest that both 7-deaza-dGTP and 7-deaza-dATP compete well with the natural substrates, dGTP and dATP, a reduction of radiolabeled products due to inhibition by or incorporation of nonradiolabeled 7-deaza nucleotides cannot be distiguished. However, in the presence of high concentrations of the cold native nucleotide (1−2 mM, Figure 3), the 7-deaza nucleotides are able to inhibit telomerase activity with IC_{50} values of less than 100 μM. In this case, a reduction of radiolabeled products demonstrates an inhibition of telomerase activity.

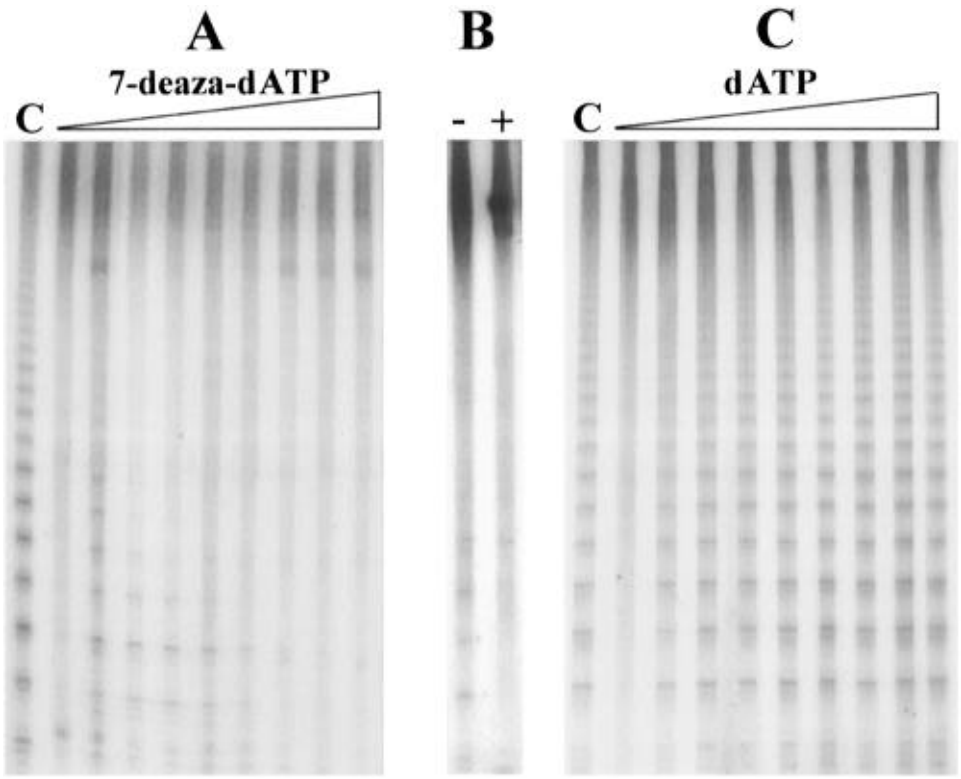

FIGURE 5: (A) 7-Deaza-dATP as a telomerase substrate with 1.56 μM [α-^{32}P]dGTP, 1 mM dTTP, 1 μM $(TTAGGG)_3$, 20 μL of S100, and from left to right, 1 mM dATP (C) and 0, 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75, and 2.0 mM 7-deaza-dATP. (B) 7-Deaza-dATP as a telomerase substrate with 1.56 μM [α-^{32}P]dGTP, 1 mM dTTP, 1 μM $(TTAGGG)_3$, 20 μL of S100, 1 mM 7-deaza-dATP without (−) or with (+) RNase A at 0.125 μg/μL. (C) dATP as a telomerase substrate with 1.56 μM [α-^{32}P]dGTP, 1 mM dTTP, 1 μM $(TTAGGG)_3$, 20 μL of S100, and, from left to right, 1 mM dATP (C) and 0, 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75, and 2.0 mM dATP.

7-Deaza-dGTP and 7-deaza-dATP can replace the native purine nucleotides dGTP and dATP, respectively, but are somewhat poor telomerase substrates. Not only is the total activity weak, but shorter ladders are formed when the 7-deaza analogues replace their native nucleotides. In comparison, 7-deaza purine nucleotides are effective substrates for a variety of other DNA polymerases (Mizusawa et al., 1986; McConlogue et al., 1988; Seela & Roling, 1991). This raises a question as to why N7 is essential for telomerase activity. The reason for this is not known at this time, although three distinct possibilities can be imagined. One interesting possibility involves the propensity of G-rich DNA sequences to form secondary structures involving G-quartets. N7 is involved in G-quartet and hairpin structures. Replacing dG with dI in various telomeric sequences disrupted G-tetraplex formation but had no inhibitory effect on telomerase *in vitro* (Henderson et al., 1990). Furthermore, it has been shown that G-quartet structures may actually inhibit the initiation of telomerase activity (possibly by prevention of binding of telomerase to the oligonucleotide). However, it is possible that G-tetraplex structures can facilitate the translocation step (Zahler et al., 1991). Incorporation of the 7-deaza nucleotides can prevent formation of these structures making the translocation step more difficult. The result of this would be formation of shorter telomerase products. It is important to note that the telomerase ladder formed in the presence of 7-deaza-dATP are also prematurely shortened. While it is possible that N7 of adenine may also be involved in the formation of stable G-tetraplex structures, there is conflicting evidence to that effect (Murchie & Lilley, 1994; Balagutumoorthy & Brahmachari, 1994). Thus, it remains to be seen if addition of one 7-deaza-2′-deoxyadenine per TTAGGG repeat along the growing chain is sufficient to disrupt G-quartet structures or hairpin formation.

Another possible situation is that incorporation of 7-deaza nucleotides destabilizes the DNA−telomerase RNA duplex or causes a conformational change resulting in complete dissociation of the growing strand from telomerase. It has been shown that replacing dG with 7-deaza-dG in alternating d(G-C) or d(C-G) oligomers does not change the conformation from B DNA but does result in a decrease in melting temperatures (Seela & Driller, 1989). Replacing dA with 7-deaza-dA in homooligomers dA·dT also results in a decrease in stability. However, alternating d(A-T) oligomers where dA is replaced by 7-deaza-dA are slightly more stable than d(A-T) oligomers. Replacing dA with 7-deaza-dA in homooligomers dA·dT also results in a decrease in stability. However, alternating d(7-deazaA-T) oligomers are slightly more stable than d(A-T) oligomers (Seela & Thomas, 1995). In addition, replacing dA with 7-deaza-dA within poly dA tracts reduces the degree of bending (Seela et al., 1989). Dissociation of the enzyme from the growing strand may account for decrease in processivity of telomerase as the 7-deaza nucleotides are incorporated. Furthermore, a change in stability or conformational change caused by addition of one or more 7-deaza-dG's per TTAGGG repeat may cause telomerase to dissociate during the elongation step. This could be the cause of the shift and increase in number of pause sites observed in the presence of 7-deaza-dGTP but not 7-deaza-dATP.

A third possible situation involves substrate inhibition. The substrate inhibition seen at higher 7-deaza-dGTP and 7-deaza-dATP concentrations has been observed for a variety of other enzymes (Dixon & Webb, 1979). Substrate inhibi-

tion is also seen with *Tetrahymena* telomerase in 10 mM dGTP (and 2.5 μM [α-^{32}P]TTP) which is 1000−10000-fold greater than the 1−10 μM dGTP required for optimum activity (Collins & Greider, 1995). Substrate inhibition could be due to two mechanisms (Dixon & Webb, 1979). In the first mechanism, the nucleotides can bind to the telomerase active site to form other ineffective telomerase−nucleotide complexes which inhibit telomerase activity. The less effective binding modes have a higher K_m than the correct mode so that inhibition becomes more apparent only when higher concentrations of substrate is present. In the second mechanism, the nucleotides can bind to a site other than the active site which causes a conformational change resulting in inhibition. Like the first circumstance, this binding would have a lower affinity than the active site binding so that inhibition becomes more apparent with increasing substrate concentrations. When the 7-deaza nucleotide competes with the radioactive nucleotide at limiting concentrations, 1−3 μM, the observed IC_{50} value most likely reflects the direct competition between the cold and labeled nucleotide. However, the observed inhibition of telomerase activity by the 7-deaza nucleotides in the presence of excess concentrations of the nonradioactive native nucleotide may be due to substrate inhibition. Due to the fact that the details of human telomerase structure is unknown at this time, it is difficult to speculate how the substitution of a carbon for N7 is responsible for the observed substrate inhibition.

One of the most important observations of this study is that the 7-deaza nucleotides are poor substrates for human telomerase. These nucleotides not only present a novel mode of telomerase inhibition but they also have the potential of being used as a tool for the study of the role of DNA secondary structure in telomerase mechanism.

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