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3′-Deoxyribonucleotides Inhibit Eukaryotic DNA Primase[1]

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In order to elucidate the biological activities of cordycepin (3′-deoxyadenosine) and related 3′-deoxyribonucleosides on eukaryotic DNA replication, the inhibitory effects of triphosphate derivatives of 3′-deoxyadenosine (3′-dATP), 8-azido-3′-deoxyadenosine (8-N_3-3′-dATP), 3′-deoxyguanosine (3′-dGTP), 3′-deoxyuridine (3′-dUTP), 5-fluoro-3′-deoxyuridine (5-F-3′-dUTP), 3′-deoxycytidine (3′-dCTP), and 5-fluoro-3′-deoxycytidine (5-F-3′-dCTP) on DNA primase and replicative DNA polymerases α, δ, and ε purified from cherry salmon (*Oncorhynchus masou*) testes or calf thymus were examined. All analogs, except 8-N_3-3′-dATP, showed strong inhibitory effects on DNA primase, but none of them inhibited DNA polymerases α, δ, or ε. Kinetic analysis revealed that the inhibition modes of them were competitive with respect to the incorporation of natural substrate that had the corresponding base moiety and non-competitive with respect to other substrates. Based on the kinetic data, we compared the affinities of 3′-dNTPs between DNA primase and RNA polymerases I and II, since 3′-dNTPs also inhibit eukaryotic RNA polymerases. Although the K_i values for DNA primase were much larger than those for RNA polymerases, the K_i/K_m values, which indicate the affinity of the analog to the enzyme, were very similar.

Key words: cherry salmon, DNA polymerase α family, DNA primase, 3′-deoxyribonucleotides, synthetic inhibitor.

Cordycepin is a nucleoside analog which was isolated from the mold *Cordyceps militaris* (*1, 2*). The structure of cordycepin was determined as 3′-deoxyadenosine. This compound is cytotoxic and produces a number of effects, including inhibition of RNA biosynthesis. We have previously reported that the triphosphate derivative of 3′-deoxyadenosine (3′-dATP) and related 3′-deoxyribonucleotide analogs strongly inhibited RNA polymerases I and II purified from cherry salmon (*Oncorhynchus masou*) liver or from *Dictyostelium discoideum* cells (*3-5*). Furthermore, cordycepin inhibits DNA synthesis in cultured cells, but DNA polymerase activity in the extract from these cells shows no inhibition by the triphosphate (*1*). It is suspected that RNA synthesis-dependent DNA synthesis might be the target of inhibition by 3′-deoxyribonucleotide *in vivo*. However, there is no evidence for this.

Eukaryotic DNA primase activity was found in association with DNA polymerase α (*6-8*). This enzyme synthesizes a short RNA oligomer, the so-called RNA primer. Since DNA polymerases cannot initiate DNA synthesis without a primer, DNA primase has an important role in DNA replication. As mentioned above, this enzyme is a candidate for the target of inhibition by 3′-deoxyribonucleotide. In this context, we examined the inhibitory effects of several 3′-deoxyribonucleotides on DNA primase and DNA polymerases purified from cherry salmon testes or calf thymus. Here we show that the 3′-deoxyribonucleotide inhibits eukaryotic DNA primase, whereas replicative DNA polymerases are not affected by this analog (*9*).

MATERIALS AND METHODS

Materials—Unlabeled deoxyribonucleoside 5′-triphosphates (dNTPs) and ribonucleoside 5′-triphosphates (NTPs) were purchased from Yamasa Shoyu (Choshi). Radioactive compounds ([*methyl*-^{3}H]dTTP, [2,8-^{3}H]-ATP, [8-^{3}H]GTP, [5-^{3}H]UTP, [5-^{3}H]CTP, [α-^{32}P]ATP, and [α-^{32}P]GTP) were obtained from New England Nuclear (USA). Other chemicals used here were of analytical grade.

Nucleoside and Nucleotide Analogs—3′-Deoxyadenosine and 3′-deoxyguanosine were kindly provided by Yamasa Shoyu. 3′-Deoxyuridine, 5′-fluoro-3′-deoxyuridine, 3′-deoxycytidine and 5′-fluoro-3′-deoxycytidine were synthesized from 3′-deoxyadenosine as reported previously (*10, 11*). 8-Azido-3′-deoxyadenosine was synthesized as described below; 3′-deoxyadenosine was converted to the 8-bromo derivative according to the reported method (*12*)

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Abbreviations: 3′-dNTPs, 3′-deoxyribonucleotides; 3′-dATP, 3′-deoxyadenosine 5′-triphosphate; 8-N_3-3′-dATP, 8-azido-3′-deoxyadenosine 5′-triphosphate; 3′-dGTP, 3′-deoxyguanosine 5′-triphosphate; 3′-dUTP, 3′-deoxyuridine 5′-triphosphate; 5-F-3′-dUTP, 5-fluoro-3′-deoxyuridine 5′-triphosphate; 3′-dCTP, 3′-deoxycytidine 5′-triphosphate; 5-F-3′-dCTP, 5-fluoro-3′-deoxycytidine 5′-triphosphate; araCTP, 1-β-D-arabinofuranosylcytosine 5′-triphosphate; 2-fluoro-araATP, 2-fluoro-9-β-D-arabinofuranosyladenine 5′-triphosphate; 2′-fluoro-araTTP, 1-β-D-(2-deoxy-2-fluoroarabinofuranosyl)thymine 5′-triphosphate.

and reacted with 60% aqueous hydrazine hydrate for 2 h at 95°C. The solvent was evaporated, and the residue was crystallized from water to afford 8-hydrazino-3′-deoxyadenosine with a yield of 88.9% from 8-bromo derivative. The 8-hydrazino derivative was dissolved in 1 N HCl, and aqueous sodium nitrite (2 eq.) was added. The mixture was stirred for 3 h at 0°C, then the pH was adjusted to 3 with a few drops of 10 N NaOH, and the precipitate was collected by filtration and crystallized from water. 8-Azido-3′-deoxyadenosine was obtained in a yield of 66.3% (*13*). Analytical data for 8-azido-3′-deoxyadenosine were as follows: melting point; 202–204°C (decomp.). ^{1}H-nmr (DMSO-d_6); δppm 8.80 (s, 1H, 2-H), 7.27 (s, 2H, 6-NH_2), 5.58 (d, 1H, 2′-OH, J=3.7 Hz), 5.53 (d, 1H, 1′-H, J=4.4 Hz), 5.13 (m, 1H, 2′-H), 4.25 (m, 1H, 4′-H), 3.6–3.5 (m, 1H, 5′-Ha), 3.5–3.4 (m, 1H, 5′-Hb), 2.42 (m, 1H, 3′-Ha), 1.96 (m, 1H, 3′-Hb). Mass spectra; m/z 292 (M^+), 266 ($M^+-N_2+H_2$), 176 (B^+), 150 ($B^+-N_2+H_2$). UV spectra (H_2O); λ_{max} 281 nm, λ_{min} 247 nm. Ir spectra (nujol); 2,150 cm^{-1} (-N_3). Anal. *calcd for* $C_{10}H_{12}N_8O_3$; C:41.10, H:4.14, N:38.34. *Found*: C:40.99, H:4.06, N:38.39.

All nucleoside analogs were converted to corresponding 5′-triphosphate derivatives according to the method of Yoshikawa *et al.* (*14*) and the phosphoroimidazolidate method (*15*) with some modifications (*16*). Synthetic methods for 3′-dNTPs in detail were described in the previous paper (*11*).

DNA Primase and DNA Polymerases α, δ, and ε—DNA primase-DNA polymerase α complex (designated as DNA polymerase α-primase) was purified from cherry salmon, *Oncorhynchus masou*, testes as described previously (*9, 16, 17*). Calf thymus DNA polymerases α (associated with DNA primase), δ, and ε were purified as described (*18, 19*). The final preparations showed specific activities of 110,000 units/mg for cherry salmon DNA polymerases α, 260,000 units/mg for calf thymus DNA polymerase α, 6,000 units/mg for DNA polymerase δ, and 1,000 units/mg for DNA polymerase ε, with activated salmon sperm DNA as a template-primer. One unit is defined as the amount of enzyme that catalyzes the incorporation of 1 nmol of deoxyribonucleotide into activated DNA at 37°C in 1 h. (Since DNA polymerases δ and ε prefer another template-primer than the activated DNA, the specific activities of both enzymes are estimated as lower than that of DNA polymerase α.)

Assay of DNA Primase and DNA Polymerases—DNA primase activity was generally determined as a RNA synthesis-dependent DNA synthesis activity with the mixture (25 μl) containing 100 μg/ml M13mp18 single-stranded DNA, 50 mM Tris-HCl (pH 8.0), 4 mM $MgCl_2$, 1 mM dithiothreitol, 400 μg/ml bovine serum albumin, 100 μM NTP (corresponding to the analog), 400 μM each of the other three NTPs, 100 μM each of dATP, dGTP, and dCTP, 25 μM [^{3}H]dTTP (500 cpm/pmol), the indicated amount of 3′-deoxyribonucleotide analog and 1 unit (as DNA polymerase α activity) of DNA polymerase α-primase. Incubation was performed at 30°C for 30 min. When DNA primase activity was specifically measured as a RNA synthesis activity, [^{3}H]dTTP was replaced by [^{3}H]NTP (corresponding to the analog, 100 cpm/pmol).

The activities of DNA polymerases α, δ, and ε were measured in 25 μl of the mixture containing 100 μg/ml activated salmon sperm DNA, 50 mM Tris-HCl (pH 8.0), 4 mM $MgCl_2$, 1 mM dithiothreitol, 400 μg/ml bovine serum albumin, 100 μM each of three dNTPs, 25 μM [^{3}H]dNTP (corresponding to the analog, 400 cpm/pmol), the indicated amount of analog and 1 unit of DNA polymerase α, 0.6 units of DNA polymerase δ or 0.1 units of DNA polymerase ε. For DNA polymerase δ assay, 50 ng of mouse proliferating cell nuclear antigen (PCNA) expressed in *Escherichia coli* with a recombinant plasmid carrying PCNA-cDNA (*19, 20*) was added. Incubation was carried out at 37°C for 30 min.

After incubation, the reaction mixture was chilled in ice-water and transferred to DEAE-cellulose paper discs (Whatman DE81). The discs were washed with 5% Na_2-

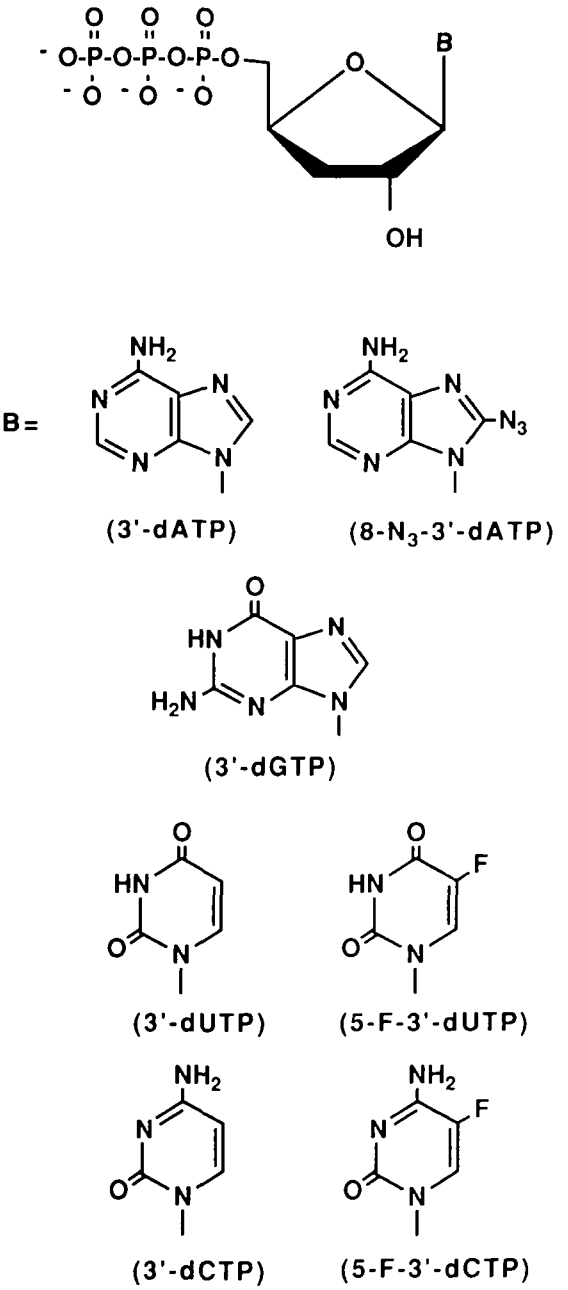

Fig. 1. Chemical structures of various 3′-deoxyribonucleotides.

HPO_4 (six times), water (twice), ethanol (twice) and ether, and dried. The remaining radioactivity was measured with a toluene scintillator.

Analysis of Primer RNA Synthesized by DNA Primase in the Presence of Analog—To analyze the product synthesized by DNA primase, we used two template systems.

One was the natural template, M13mp18 single-stranded DNA, and the other was a synthetic homopolymer, poly(dC). For the M13 DNA system, the reaction mixture (50 μl) contained 100 μg/ml M13mp18 single-stranded DNA, 50 mM Tris-HCl (pH 8.0), 4 mM $MgCl_2$, 1 mM dithiothreitol, 400 μg/ml bovine serum albumin, 400 μM each of GTP, CTP, and UTP, 100 μM [α-^{32}P]ATP (1,000 cpm/pmol), 100 μM each of dATP, dGTP, dCTP, and dTTP, the indicated amount of 3′-dATP, and 10 units of DNA polymerase α-primase. When poly(dC) was used, M13 DNA, [α-^{32}P]ATP, and 3′-dATP were replaced with 100 μg/ml poly(dC), 100 μM [α-^{32}P]GTP (1,000 cpm/pmol), and the indicated amount of 3′-dGTP, respectively, and ATP, CTP, UTP, dATP, dCTP, and dTTP were omitted. Incubation was carried out at 30°C for 30 min. After incubation, the mixture was extracted with phenol and chloroform, and precipitated with ethanol. The precipitate was dried and dissolved in 20 μl of 10 mM Tris-HCl (pH 7.5) containing 4 mM $MgCl_2$, and digested with excess of deoxyribonuclease I (Takara Shuzo, Kyoto). After phenol and chloroform treatment, the mixture was precipitated again with ethanol. The precipitate was dried and dissolved in 5 μl of the loading solution (89 mM Tris, 89 mM borate, 2.5 mM EDTA, 15% glycerol, 0.1% bromophenol blue, and 0.1% xylene cyanol), and electrophoresed on 20% polyacrylamide gel containing 7 M urea. The radio-labeled product was detected by autoradiography with Kodak X-ray film and an intensifying screen.

RESULTS

Inhibitory Effects of 3′-Deoxyribonucleotides on DNA Primase and DNA Polymerases α, δ, and ε—We first examined the effects of 3′-deoxyribonucleotides on DNA primase and DNA polymerases α, δ, and ε. The chemical structures of seven kinds of 3′-deoxyribonucleotide analogs used in this study are shown in Fig. 1. DNA polymerase α, δ, and ε activities were measured with the activated salmon sperm DNA as a template-primer, and DNA primase activity was determined as a primer RNA synthesis-dependent DNA synthesis activity with the unprimed M13mp18 single-stranded DNA. The activity without inhibitor was taken as 100%, and the residual activity in the presence of various concentrations of inhibitor was measured as percent. Figure 2 shows the results. None of the 3′-deoxyribonucleotide analogs inhibited DNA polymerase α purified from cherry salmon testes (Fig. 2A).

On the other hand, all analogs, except 8-N_3-3′-dATP,

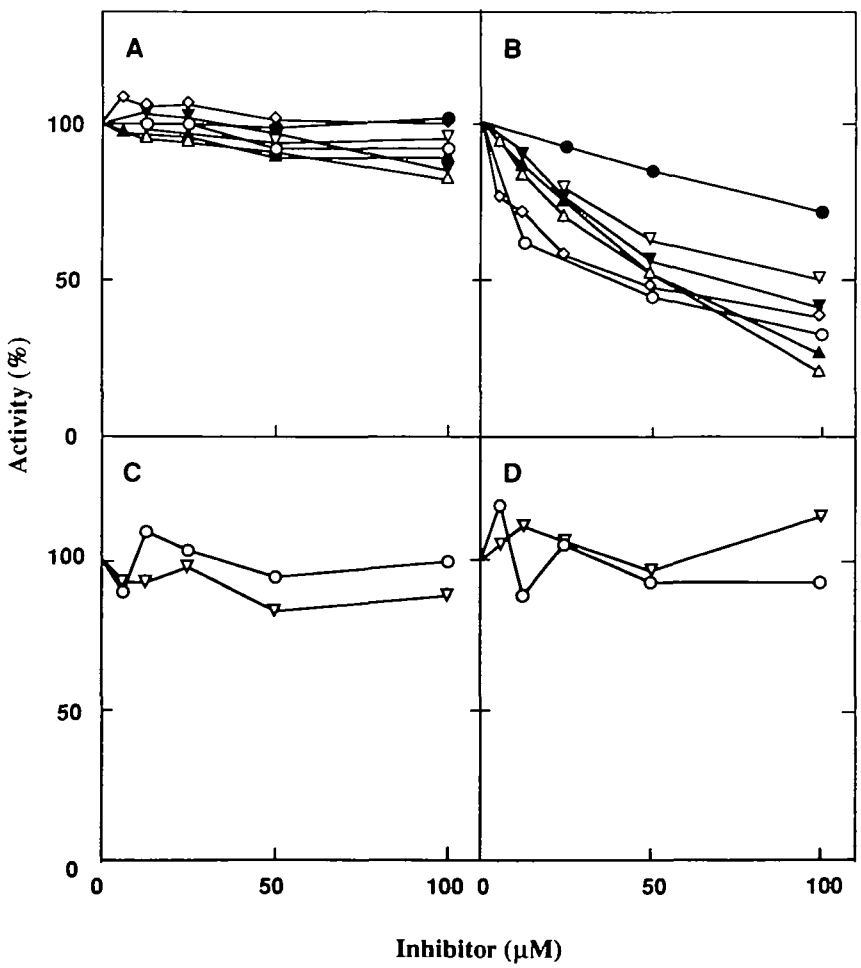

Fig. 2. **Inhibitory effects of 3′-deoxyribonucleotides on DNA polymerase α (A), primer RNA synthesis-dependent DNA synthesis (B), DNA polymerase δ (C), and DNA polymerase ε (D).** The activities of DNA polymerases α, δ, and ε were measured with activated DNA, and the activity of primer RNA synthesis-dependent DNA synthesis was determined with M13mp18 single-stranded DNA as described in "MATERIALS AND METHODS." The activity without analog was taken as 100%, and the residual activities (%) with several concentrations of 3′-dATP (○), 8-N_3-3′-dATP (●), 3′-dGTP (◇), 3′-dUTP (△), 5-F-3′-dUTP (▲), 3′-dCTP (▽), and 5-F-3′-dCTP (▼) were measured.

showed the inhibitory effects upon primer RNA synthesis-dependent DNA synthesis (Fig. 2B). We also examined the effect of these compounds on the calf thymus enzyme. The result was very similar to that with the cherry salmon enzyme (data not shown). These results indicate that the 3′-dNTPs inhibit the primer RNA synthesis, but not DNA chain elongation. To determine whether 3′-deoxyribonucleotides really affect DNA primase activity, we also directly measured DNA primase activity in the presence of 3′-deoxyribonucleotide analogs. The resulting inhibition curves are shown in Fig. 3. As expected, DNA primase activity was inhibited by 3′-deoxyribonucleotides. The activities of DNA polymerases δ and ε from calf thymus were not affected by 3′-dNTPs (Fig. 2, C and D). Thus, the inhibitory effect of 3′-deoxyribonucleoside on DNA replication in the cell might be due to the inhibition of DNA primase by the 5′-triphosphate derivatives.

Mode of Inhibition and Determination of Kinetic Parameters—The mode of inhibition of DNA primase by 3′-dNTPs, except 8-N_3-3′-dATP, was analyzed using Lineweaver-Burk plots. In this experiment, ribonucleotide substrate corresponding to the analog was labeled with tritium. As a typical example, Fig. 4 shows plots of data for the inhibition of DNA primase by 3′-dATP (Fig. 4A) and 3′-dGTP (Fig. 4B). In both plots, the inhibition mode was competitive with respect to the incorporation of ATP or GTP, respectively. All 3′-deoxyribonucleotides were essentially competitive with respect to the natural substrate that had the corresponding base moiety. In the case of different base moieties between the substrate and analog, such as UTP and 3′-dATP, the inhibition mode was non-competitive (Fig. 4C).

Based on the results shown above, kinetic parameters (K_i values) of the 3′-dNTPs were estimated by replots of apparent K_m value *versus* concentration of inhibitor, and

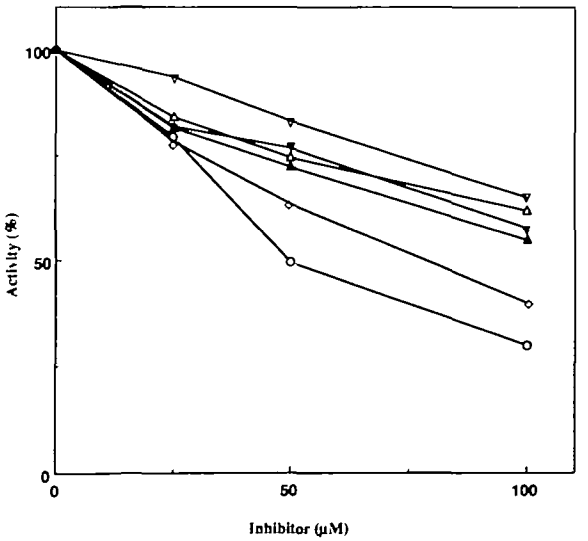

Fig. 3. **Inhibitory effects of 3′-deoxyribonucleotides on DNA primase.** The activity of DNA primase was determined with M13mp18 single-stranded DNA as described in "MATERIALS AND METHODS." The activity without any analog was taken as 100%, and the residual activities (%) with several concentrations of 3′-dATP (○), 3′-dGTP (◇), 3′-dUTP (△), 5-F-3′-dUTP (▲), 3′-dCTP (▽), or 5-F-dCTP (▼) were measured.

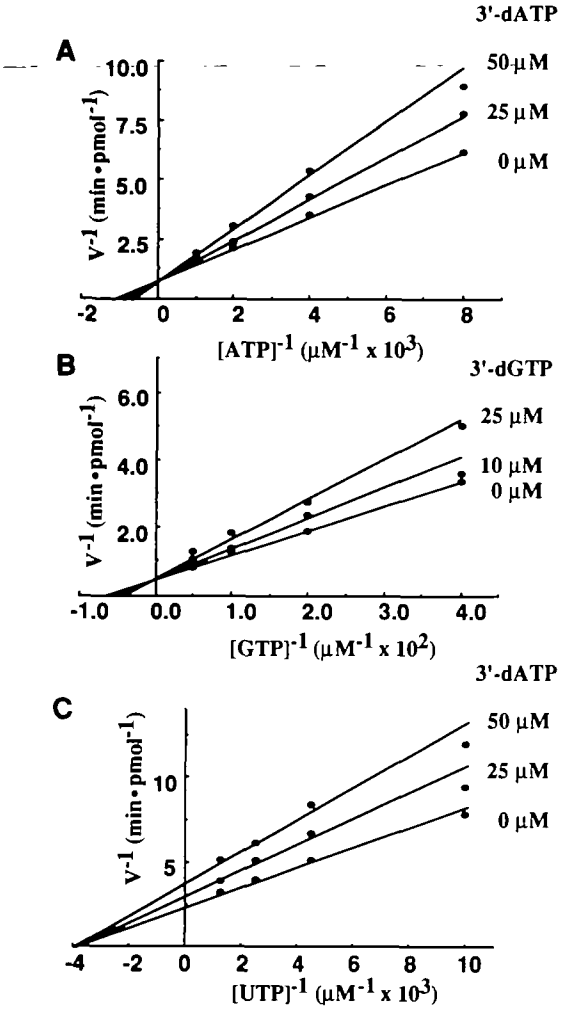

Fig. 4. **Lineweaver-Burk plots for the inhibition of DNA primase by 3′-deoxyribonucleotides DNA primase activity was measured in the absence or presence of the indicated amount of 3′-dATP (A, C) or 3′-dGTP (B) with various concentrations of [³H]ATP (A), [³H]GTP (B), or [³H]UTP as described in "MATERIALS AND METHODS."** The Lineweaver-Burk plot was obtained by double-reciprocal plotting of substrate concentration *versus* amount of incorporated nucleotides.

TABLE I. **Kinetic parameters of various 3′-deoxyribonucleotides.**

Compound	DNA primase		RNA polymerase I		RNA polymerase II	
	K_i (μM)	K_i/K_m	K_i (μM)	K_i/K_m	K_i (μM)	K_i/K_m
ATP	800 (K_m)		34 (K_m)[a]		30 (K_m)[a]	
3′-dATP	70	0.094	3	0.084	3	0.10
GTP	143 (K_m)					
3′-dGTP	43	0.30				
UTP	250 (K_m)		8 (K_m)[b]		4 (K_m)[b]	
3′-dUTP	84	0.34	2	0.25	1	0.25
5-F-3′-dUTP	80	0.32				
CTP	200 (K_m)		8.3 (K_m)[c]		6.2 (K_m)[c]	
3′-dCTP	85	0.43	4.0	0.48	3.0	0.48
5-F-3′-dCTP	77	0.39				

[a]From cherry salmon liver (Ref. 3). [b]From cherry salmon liver (Ref. 5). [c]From *Dictyosterium discoideum* cells (Ref. 4).

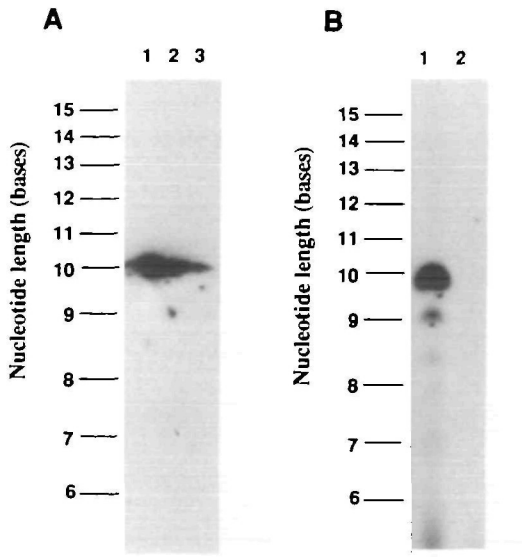

Fig. 5. **Analysis of product RNA synthesized by DNA primase.** The DNA primase reaction was performed with M13mp18 single-stranded DNA (A) or poly(dC) (B) for template with [α-^{32}P]ATP (A) or [α-^{32}P]GTP in the absence (lane 1 of each panels) or presence of 50 μM (A, lane 2), 100 μM 3′-dATP (A, lane 3) or 100 μM 3′-dGTP (B, lane 2) as described in "MATERIALS AND METHODS." After treatment with deoxyribonuclease I, the ^{32}P-labeled product RNA was subjected to 20% polyacrylamide gel electrophoresis in the presence of 7 M urea, and detected by autoradiography with X-ray film. Size markers was prepared by alkaline hydrolysis of 5′-^{32}P-labeled polyadenylic acid.

the results are summarized in Table I. Since DNA primase could be categorized as DNA-dependent RNA polymerase, kinetic parameters for RNA polymerases I and II (*3-5*) are also shown in this table for comparison. The K_i values of all 3′-dNTP analogs on DNA primase were smaller than the K_m value of ribonucleotide substrates. For example, the K_i value of 3′-dATP was 70 μM, whereas the K_m value for ATP was 800 μM, and the K_i/K_m value, a smaller value of which indicates great affinity to the enzyme, was 0.094.

Thus, 3′-deoxyribonucleotides have strong affinity to DNA primase. No remarkable difference of K_i value was seen between 3′-dUTP and 5-F-3′-dUTP or between 3′-dCTP and 5-F-3′-dCTP. Compared with RNA polymerases, the K_i values of 3′-dNTPs for DNA primase were much larger than those for RNA polymerases, but, the K_i/K_m values were very similar.

Effect of 3′-dATP on Primer RNA Synthesis by DNA Primase—We then asked whether 3′-dNTP caused chain termination by being incorporated in the primer RNA. The product RNA synthesized by DNA primase on M13mp18 single-stranded DNA in the absence or presence of 3′-dATP was analyzed. As can be seen in Fig. 5A, DNA primase synthesized RNA oligomer of about 10 nucleotides in length without inhibitor (Fig. 5A, lane 1). However, when the reaction was performed with 50 or 100 μM 3′-dATP, the amount of 10-nucleotide RNA decreased (Fig. 5A, lanes 2 and 3).

The 10-nucleotide bands seen in Fig. 5A were quantitatively analyzed with a densitometric scanner. When the intensity of the band without inhibitor (Fig. 5A, lane 1) was taken as 100%, those of the bands with 50 and 100 mM 3′-dATP (lane 2 and 3) were 49 and 26%, respectively. Similar results were obtained when poly(dC) was used as a template (Fig. 5B). In both the M13 DNA and poly(dC) systems, the accumulation of RNA fragments shorter than 10 nucleotides was not observed in the presence of inhibitor, suggesting that the 3′-dNTPs could not cause chain termination.

DISCUSSION

In the present paper, we have demonstrated that 3′-deoxyribonucleotides, except 8-N_3-3′-dATP, inhibit eukaryotic DNA primase (Fig. 2B, 3). The activities of DNA polymerases α, δ, and ϵ were not affected by these analogs (Fig. 2, A, C, and D). This result indicates that 3′-dNTP is a specific inhibitor of DNA primase among the several nucleic acid polymerases which are required for genomic DNA replication. Thus, 3′-dNTP could be a useful tool for studying the exact role of DNA primase during DNA replication. Arabinonucleotides such as araCTP also strongly inhibit DNA primase from calf thymus or leukemia cells (*21-24*). However, arabinonucleotides are also known to inhibit DNA polymerase α strongly (*25-27*). On the other hand, Yagura *et al.* (*28*) have reported that 2′-azido-2′-deoxycytidine 5′-triphosphate, which is an inhibitor of *Escherichia coli* DNA primase (*29*) and which inhibited DNA replication in polyoma-infected mammalian cells (*30*), inhibited mouse DNA primase.

At present, it is not known whether this analog affects the activity of DNA polymerase δ or ϵ.

The effect of 8-N_3-3′-dATP was much weaker than that of 3′-dATP (Fig. 1B), although Bodner and Bambara used 8-azidoadenosine 5′-triphosphate as a photo-affinity labeling reagent for DNA primase from calf thymus (*31*). Woody *et al.* (*32*) have reported that 8-azidopurine nucleotides could not be substrates for template-directed polymerases, such as RNA polymerase of *E. coli*. In other words, nucleic acid polymerase might recognize purine nucleotide substrates having only *anti*-conformation. The 8-azidopurine nucleotide preferentially shows *syn*-conformation due to steric hindrance by the 8-substituent. Therefore, DNA primase, like other polymerases, could not recognize 8-N_3-3′-dATP. On the other hand, substitution of hydrogen to fluoride at the 5-position of a pyrimidine nucleotide analog had no effect on the affinity for DNA primase. In contrast, Parker and Cheng (*23*) reported that arabinonucleotides containing fluoride (2-fluoro-araATP and 2′-fluoro araTTP) showed stronger inhibitory effects on DNA primase than arabinonucleotides themselves. Although the nature of the effect of fluoride substitution is unclear, these differences could provide interesting information about the recognition mode of substrate nucleotides by DNA primase.

We also examined the mechanism of inhibition of DNA primase by 3′-dNTPs. As reported (*33*), DNA primase synthesized RNA oligomer of 10 nucleotides in length on M13mp18 single-stranded DNA or a poly(dC) template in the absence of inhibitor (Fig. 5, A and B). When the reaction was performed with 3′-dATP or 3′-dGTP, the amount of 10-nucleotide oligomer remarkably decreased. However, the accumulation of short RNA fragments of less than 10 nucleotides was not observed. This indicates that the mechanism of inhibition by 3′-dNTP could not be chain

termination through incorporation into primer RNA. In the primer RNA synthesis by DNA primase, the first nucleotide incorporated is ATP or GTP in a ratio of 3 : 1 (*34*). Thus, the main mechanism of inhibition by 3′-dATP or 3′-dGTP might be interference with initiation by competing with the natural substrate. A similar mechanism was reported for the inhibition of primase by arabinonucleotides (*21*). Yoshida *et al.* proposed that the mechanism of inhibition by arabinonucleotide is interference with initiation.

All 3′-dNTPs having natural bases showed the smaller K_i values than the K_m values of the natural substrates (Table I).

The 3′-deoxyribonucleotides also inhibit eukaryotic RNA polymerases (*3-5*). The K_i values of 3′-dNTPs for RNA polymerases I and II were much smaller than those for DNA primase (Table I). However, K_i/K_m values were very similar among these enzymes. This indicates that the affinity of 3′-dNTP for DNA primase could be the same as that for RNA polymerase. As noted above, DNA primase might be categorized as DNA-dependent RNA polymerase, so, it is reasonable that the substrate recognition site of DNA primase may resemble that of RNA polymerase. Interestingly, DNA primase is inhibited by arabinonucleotide,whereas RNA polymerase is not. Therefore, the recognition of the sugar moiety on nucleotides by DNA primase could be more flexible than that by RNA polymerase.

In conclusion, we have shown that the various 3′-dNTPs inhibited DNA primase. Although the inhibitory effect is specific for DNA primase among several replication polymerases, it is not applicable for studying the role of DNA primase at the cell level since 3′-dNTP also affects the activity of RNA polymerase. To develop a specific inhibitor for DNA primase, further study of the inhibitory effects of sugar-modified nucleotide analogs, such as an analog bearing an azido or amino group at the 2′- or 3′-position, on DNA primase is being undertaken (*35*).

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