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ACCELERATED COMMUNICATION

Differential Inhibitory Effects of Several Pyrimidine 2′,3′-Dideoxynucleoside 5′-Triphosphates on the Activities of Reverse Transcriptase and Various Cellular DNA Polymerases

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SUMMARY

Several analogues of 2′,3′-dideoxythymidine 5′-triphosphate [i.e., 3′-azido-2′, 3′-dideoxythymidine 5′-triphosphate(AzddTTP), 2′,3′-didehydro-2′,3′-dideoxythymidine 5′-triphosphate (ddeTTP), α,β-methylene 3′-azido-2′,3′-dideoxythymidine 5′-diphosphate, α,β-methylene 3′-azido-2′,3′-dideoxythymidine 5′-triphosphate, and β,γ-methylene 3′-azido-2′,3′-dideoxythymidine 5′-triphosphate] and 2′,3′-didehydro-2′,3′-dideoxycytidine 5′-triphosphate (ddeCTP) have been evaluated for their inhibitory effects on murine retroviral reverse transcriptase and various other DNA polymerases, including DNA polymerases α, β, and γ, terminal deoxynucleotidyl transferase, and DNA polymerase I. None of the compounds inhibited the activity of DNA polymerase α under the reaction conditions employed. When Mg^{2+} was replaced by Mn^{2+}, however, DNA polymerase α was strongly inhibited only by ddeTTP. DNA polymerase β activity was inhibited only by ddeTTP and ddeCTP. All the compounds, except for ddeCTP, inhibited DNA polymerase γ activity, ddeTTP being a particularly strong inhibitor of γ-polymerase (K_i = 3.5 nM). Terminal deoxynucleotidyl transferase was only slightly inhibited by any of the compounds. AzddTTP was a potent inhibitor of reverse transcriptase (K_i = 42 nM), but it also inhibited the activities of DNA polymerase γ and DNA polymerase I.

Since the causative agent of AIDS has been identified as a retrovirus, now designated HIV, some antiviral agents have attracted special attention in the strategies for chemotherapy of AIDS, and various compounds have been found to be effective as antiretroviral agents (1–3). Most of these compounds appear to be targeted at the virus-associated reverse transcriptase. In fact, a number of reverse transcriptase inhibitors have been reported to inhibit HIV replication *in vitro*, i.e., suramin (4), HPA23 (5), phosphonoformate (6), AZT (7), and various 2′,3′-dideoxynucleosides (8) [for HPA23, the reported inhibitory effect only concerned HIV reverse transcriptase activity (5) and not HIV replication per se].

Both clinical and immunological improvements were noted upon a short term (6-week) treatment of AIDS patients with AZT (9). However, administration of AZT caused serious side effects, such as anemia and leucopenia (10). Many other 2′,3′-dideoxynucleoside analogues have been investigated for anti-HIV potency and selectivity, and it was found that not only AZT, but also 2′,3′-dideoxycytidine (8), 2′,3′-didehydro-2′,3′-dideoxycytidine (11–13), 2′,3′-dideoxythymidine (8, 13, 14), 2′,3′-didehydro-2′,3′-dideoxythymidine (13–15), 2′,3′-dideoxyadenosine (8, 16), ddDAPR (16), 2′,3′-dideoxyguanosine (17), 3′-azido-2′,3′-dideoxyguanosine (17–19), and several others (19, 20) demonstrate a high selectivity index as HIV inhibitors and, therefore, represent promising candidate drugs for the treatment of AIDS.

We have synthesized the 5′-triphosphate derivatives of some of these 2′,3′-dideoxynucleosides [i.e., ddeTTP, ddeCTP] as well as AzddTTP analogues with a phosphonate, instead of phoshate, group (21) and evaluated these compounds for their inhibitory effects on reverse transcriptase and cellular DNA polymerases. Such studies should increase our understanding

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ABBREVIATIONS: AIDS, acquired immunodeficiency syndrome; HIV, human immunodeficiency virus; suramin, hexasodium *sym*-bis(*m*-aminobenzoyl-*m*-amino-*p*-methylbenzoyl-I-naphthylamino-4,6,8-trisulfonate)carbamide; HPA23, heteropolyanion 23(21-tungsto-9-antimoniate ammonium salt); AZT (AzddThd) and AzddTTP, 3′-azido-2′,3′-dideoxythymidine and its 5′-triphosphate; $AzddTDP_{\alpha\to\beta}$, α,β-methylene AzddTDP; $AzddTTP_{\alpha\to\beta}$, α,β-methylene AzddTTP; $AzddTTP_{\beta\to\gamma}$, β,γ-methylene AzddTTP; ddeCTP, 2′,3′-didehydro-2′,3′-dideoxycytidine 5′-triphosphate; ddeTTP, 2′,3′-didehydro-2′,3′-dideoxythymidine 5′-triphosphate; ddDAPR, 2,6-diamino-9-(2,3-dideoxy-β-D-glycero-pentofuranosyl)purine, (2,6-diaminopurine-2′,3′-dideoxyriboside); dNTP, 2′-deoxynucleoside 5′-triphosphate; TdT, terminal deoxynucleotidyl transferase.

of how these anti-HIV agents act at the molecular level and, thus, help in the design of new congeners with improved antiviral potential.

Materials and Methods

Chemicals. The sources of the chemicals used in this work were as follows: [^{3}H]dNTPs from the Radiochemical Centre (Amersham, England); unlabeled nucleotides, poly(rA), poly(rI), oligo(dA), oligo(dC), and oligo(dT) from P-L Biochemicals, Inc. (Milwaukee, WI); activated calf thymus DNA from Worthington Biochemical Corp. (Freehold, NJ); and DEAE-cellulose paper disk (DE81, ϕ23 mm) from Whatman Ltd. (Springfield Mill, England).

Analogues of 2′,3′-dideoxynucleoside 5′-triphosphates. AzddTTP, ddeTTP, and ddeCTP were prepared from the monophosphates as described by Hoard and Ott (22) (yield, 60%).

AzddTDP$_{\alpha\to\beta}$ was prepared from AzddThd and methylene diphosphonic acid in the presence of dicyclohexylcarbodiimide (23), followed by an acetic anhydride treatment in dry pyridine (24). The product was purified on a DEAE cellulose column, followed by an XAD-2 column, and precipitated as the sodium salt in acetone (without methanol) (yield, 25%).

AzddTTP$_{\beta\to\gamma}$ was synthesized from AzddTMP (1 mmol) and methylene diphosphonic acid (monotributyl ammonium salt) (5 mmol) in the presence of carbonyl diimidazole (5 mmol) in anhydrous dimethyl formamide. The product was purified on DEAE cellulose and precipitated as the sodium salt in acetone (yield, 26%).

AzddTTP$_{\alpha\to\beta}$ was synthesized by condensation of AzddTDP$_{\alpha\to\beta}$ with di(tributylammonium)phosphate in the presence of carbonyl diimidazole, purified by DEAE cellulose column, and precipitated as the sodium salt. Structural formulae of these test compounds are depicted in Fig. 1.

Enzymes. DNA polymerases α, β, and γ were purified from KBIII cells, as previously described for DNA polymerase α (25), β (26), and γ (27) with some modifications. DNA polymerases α and β were also purified from mouse myeloma MOPC104E cells as described for human DNA polymerases (25, 26). TdT was purified from calf thymus, as described previously (28). Rauscher murine leukemia virus was obtained from the culture medium of an established virus-producing cell line, R-17 (29), and reverse transcriptase was purified according to the method described earlier (30). A highly purified preparation of *Escherichia coli* DNA polymerase I was purchased from P-L Biochemicals.

Assay for DNA polymerases. The template·primers used for assay of DNA polymerases were as follows: activated calf thymus DNA for DNA polymerases α and β and DNA polymerase I; $(rA)_n\cdot(dT)_{12-18}$ for DNA polymerases β and γ and reverse transcriptase; $(rI)_n\cdot(dC)_{12-18}$ for reverse transcriptase; and $(dA)_{12-18}$ for TdT. All assay conditions summarized in Table 1 were optimized with respect to the ratios and concentrations of all the template·primers used as well as to pH and divalent and monovalent cation concentrations. Different concentrations of [^{3}H]dNTPs or template·primers and dideoxynucleotide inhibitors were used in experiments designed to determine the K_m and K_i values. All incubations (50 μl) were carried out at 37° for 30 min, and the reaction was stopped by adding 20 μl of 0.2 M EDTA and immersing the mixture in ice. Then, 50 μl of the mixture was transferred onto a DE81 filter paper disk and radioactivity was counted as previously described (31).

Results

Effects of various pyrimidine 2′,3′-dideoxynucleotide analogues on the activities of murine retroviral reverse transcriptase and DNA polymerases. The effects of various pyrimidine 2′,3′-dideoxynucleotide analogues on the activities of reverse transcriptase and DNA polymerases were examined under the assay conditions described in Materials and Methods. As shown in Fig. 2, all the test compounds were found to inhibit

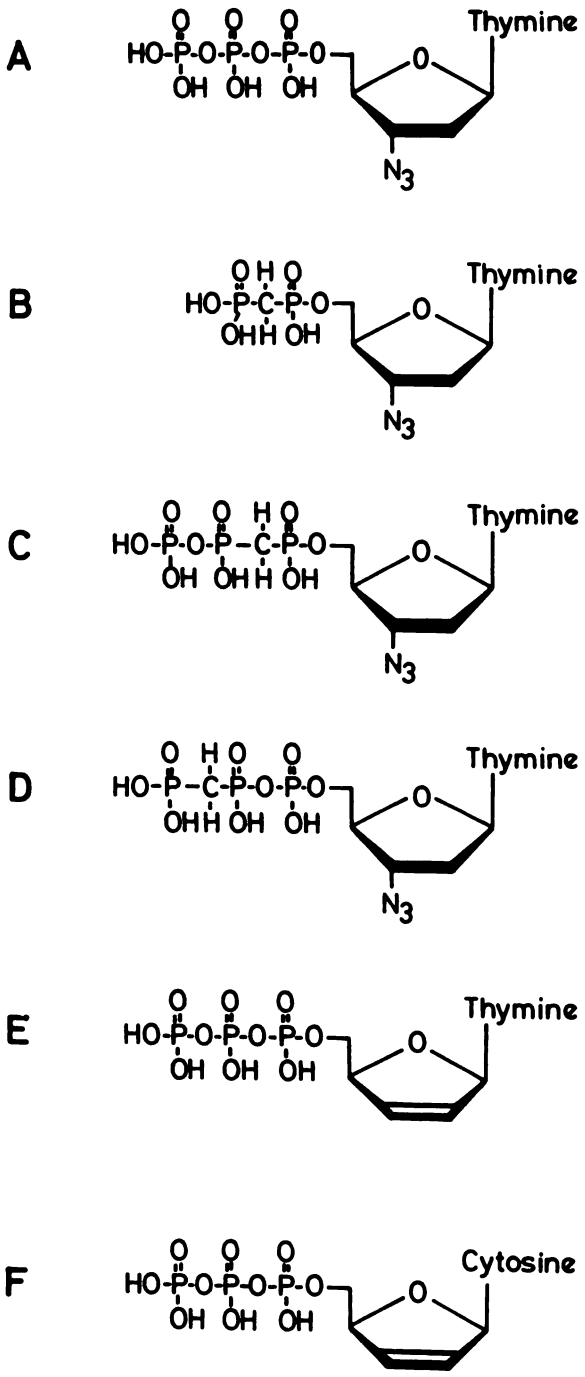

Fig. 1. Structural formulae of various pyrimidine 2′,3′-dideoxynucleotide analogues evaluated in the present study. A, AzddTTP; B, AzddTDP$_{\alpha\to\beta}$; C, AzddTTP$_{\alpha\to\beta}$; D, AzddTTP$_{\beta\to\gamma}$; E, ddeTTP; F, ddeCTP.

the activity of reverse transcriptase and that of several cellular DNA polymerases except for DNA polymerase α. DNA polymerase α was resistant to inhibition by any of these drugs at concentrations up to 50 μM.

AzddTTP (Fig. 2A) was strongly inhibitory to both reverse transcriptase and DNA polymerase γ but not DNA polymerases α and β. The reverse transcriptase and γ-polymerase activities were inhibited by more than 80% and 90%, respectively, in the presence of 2 μM AzddTTP, whereas the α- and β-polymerase activities were virtually not inhibited. TdT and DNA polymerase I showed only modest sensitivity to AzddTTP. The activ-

TABLE 1

Assay conditions for various DNA polymerases

All reaction mixtures contained 15% (v/v) glycerol and 5 mM dithiothreitol.

DNA polymerase	Template·primer	Concentration of reaction components						
		Template·primer concentration[a]	Buffer[b]	pH	[^{3}H]labeled nucleotide concentration	Unlabeled nucleotide concentration	Divalent cation concentration	K^+ concentration
		μg/ml			μM	μM	mM	mM
α	Activated DNA	80	Tris	7.5	dTTP or dCTP, 10	Other three dNTPs, 10 each	Mg^{2+}, 4	0
β	Activated DNA	200	Tris	9	dTTP or dCTP, 10	Other three dNTPs, 10 each	Mg^{2+}, 10	30
	$(rA)_n \cdot (dT)_{12-18}$	10 (1:2)[c]	Tris	8.5	dTTP, 10		Mn^{2+}, 0.2	100
γ	$(rA)_n \cdot (dT)_{12-18}$	10 (10:1)	Tris	7.5	dTTP, 1		Mn^{2+}, 0.1	70
TdT	$(dA)_{12-18}$	6	KPi[d]	6.5	dGTP, 10		Mn^{2+}, 5	50
Reverse transcriptase	$(rA)_n \cdot (dT)_{12-18}$	2 (1:1)	Tris	8	dTTP, 10		Mn^{2+}, 0.2	130
	$(rI)_n \cdot (dC)_{12-18}$	25 (5:1)	Tris	8.5	dCTP, 25		Mn^{2+}, 0.5	100
Polymerase I	Activated DNA	2	Tris	7.5	dTTP or dCTP, 10	Other three dNTPs, 10 each	Mn^{2+}, 0.2	100

[a] Concentration with respect to the template in the case of synthetic homopolymers.
[b] All buffer concentrations are 50 mM.
[c] Numbers in parentheses are base ratios of template to primer.
[d] KPi, potassium phosphate.

ities of these enzymes were inhibited by approximately 60% in the presence of 10 μM AzddTTP.

When a methylene group was introduced between the α and β phosphorus atoms or the β and γ phosphorus atoms of the triphosphate moiety of AzddTTP, however, the inhibitory properties were, as a rule, much less pronounced as compared to those of the mother compound, AzddTTP. Not only DNA polymerases α and β, but also DNA polymerase I, were insensitive to inhibition by these AzddTTP derivatives (Fig. 2B-D). DNA polymerase γ, TdT and reverse transcriptase proved susceptible, albeit only moderately, to the inhibitory effects of these compounds. DNA polymerase γ was particularly sensitive to $AzddTTP_{\alpha\to\beta}$ (80% inhibition at 10 μM $AzddTTP_{\alpha\to\beta}$). The degree of inhibitory effect of $AzddTTP_{\alpha\to\beta}$ or $AzddTTP_{\beta\to\gamma}$ on TdT was comparable to that by AzddTTP, while the reverse transcriptase was much less sensitive to these compounds than AzddTTP (compare fig. 2A, C and D). the diphosphonate of AZT ($AzddTDP_{\alpha\to\beta}$) was only slightly inhibitory to TdT and reverse transcriptase (Fig. 2B).

ddeTTP was a strong inhibitor of reverse transcriptase, DNA polymerase I, and in particular DNA polymerase γ. The activity of the γ-polymerase was inhibited by 90% in the presence of 2 μM ddeTTP (Fig. 2E). The reverse transcriptase and DNA polymerase I activities were inhibited by 80% and 70%, respectively, in the presence of 5 μM ddeTTP. DNA polymerase β and TdT were moderately sensitive to ddeTTP. The β-polymerase was markedly inhibited by ddeTTP, whether activated DNA or $(rA)_n \cdot (dT)_{12-18}$ was used as the template·primer. A similar inhibitory effect on the β-polymerase was observed for ddeCTP if activated DNA served as the template·primer with dCTP as the labeled substrate (Fig. 2F). Also, ddeCTP was a potent inhibitor (70% inhibition at a concentration of 20 μM) of reverse transcriptase, if $(rI)_n \cdot (dC)_{12-18}$ was used as the template·primer. The drug was also effective in inhibiting the activities of TdT and DNA polymerase I (Fig. 2F). However, DNA polymerase γ was not inhibited by ddeCTP under the reaction conditions used with $(rA)_n \cdot (dT)_{12-18}$ as the template·primer (data not shown).

Analysis of the mode of inhibition and determination of kinetic constants. All the inhibitors examined in this study were pyrimidine nucleotide analogues and, therefore, they were expected to compete with the corresponding natural substrates dTTP or dCTP for incorporation into DNA. The kinetics of inhibition was analyzed for all DNA polymerases (except for the α-polymerase) by changing the concentration of either the template·primer or the triphosphate substrate. Typical examples are shown in Fig. 3 and the results are summarized in Table 2.

An example is ddeTTP, which inhibited the activity of DNA polymerase γ competitively with respect to dTTP (Fig. 3A) but noncompetitively with respect to the template·primer [$(rA)_n \cdot (dT)_{12-18}$] (data not shown). The K_m value of dTTP was determined to be 0.63 μM and the K_i of ddeTTP was estimated by Dixon plotting to be 3.5 nM. Similarly, the mode of inhibition of the activity of reverse transcriptase by ddeCTP was competitive with respect to dCTP (Fig. 3B) and the K_i of ddeCTP was 0.27 μM, which was much lower than the K_m of dCTP (9.1 μM) (Table 2). All K_m and K_i values determined for various DNA polymerases are listed in Table 2.

Effect of manganese ion on the inhibition of DNA polymerases α and β by 2′,3′-dideoxynucleotide analogues. ddNTPs have been shown to inhibit the activity of DNA polymerases β and γ but not that of DNA polymerase α, the latter being assayed with activated DNA in the presence of Mg^{2+} (32–34). When Mg^{2+} was replaced by Mn^{2+} in the reaction mixture, however, α-polymerase was strongly inhibited by ddNTPs (35, 36).

By analogy with these results, replacement of the divalent cation from Mg^{2+} to Mn^{2+} is expected to have a differential effect on the inhibition of α- and β-polymerases by ddNTPs. Indeed, a slightly higher extent of inhibition of β-polymerase activity by AzddTTP, ddeTTP, and ddeCTP was observed in the presence of 0.2 mM Mn^{2+} than in the presence of 10 mM

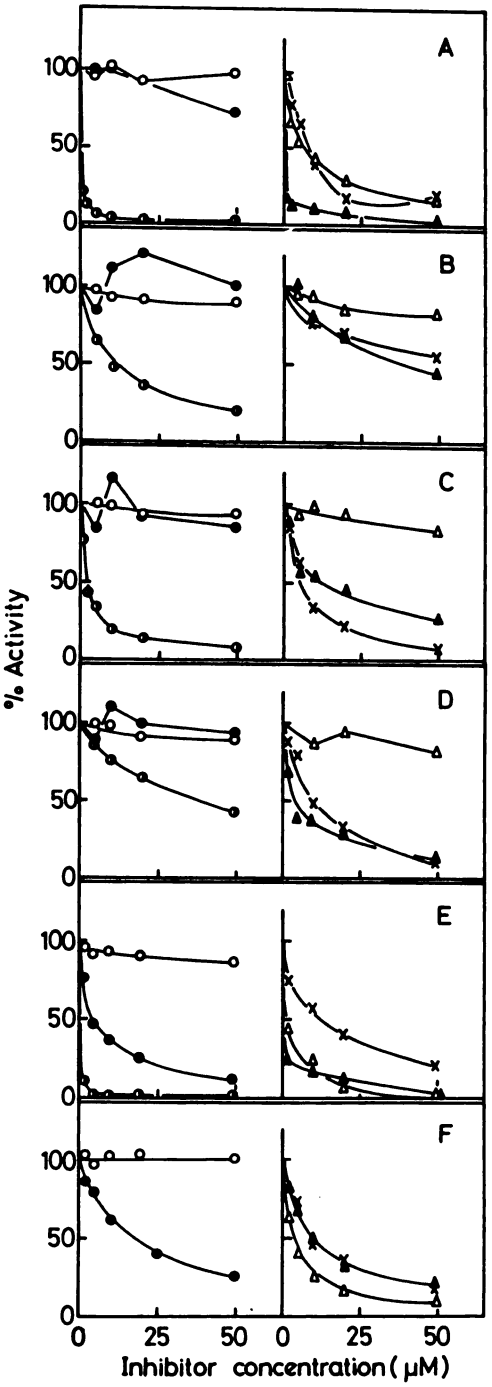

Fig. 2. Effects of various pyrimidine 2′,3′-dideoxynucleotide analogues on the activities of murine retroviral reverse transcriptase and various human DNA polymerases. Reactions were carried out under the conditions described in Materials and Methods, in the presence of various concentrations of the pyrimidine analogues as indicated. ○, DNA polymerases α; ●, β with $(rA)_n \cdot (dT)_{12-18}$ in A–E and activated DNA in F; and ◑, γ; ×, TdT; △, DNA polymerase I; and ▲; reverse transcriptase with $(rA)_n \cdot (dT)_{12-18}$ in A–E and $(rI)_n \cdot (dC)_{12-18}$ in F. Only in F, mouse DNA polymerase β was used with activated DNA as the template·primer and dCTP as the labeled substrate (●). Also, ^{3}H-labeled dCTP was used in F for the assay of DNA polymerases α and I. A, AzddTTP; B, $AzddTDP_{\alpha\to\beta}$; C, $AzddTTP_{\alpha\to\beta}$; D, $AzddTTP_{\beta\to\gamma}$; E, ddeTTP; F, ddeCTP. The specific radio-activities (cpm/pmol) of [^{3}H]dTTP were, in all cases, 1000 (○), 400 (●), 6000 (◑), 200 (△), and 400 (▲) in A–E, and that of [^{3}H]dGTP was 400 (×) in A–F. The specific radio-activities (cpm/pmol) of [^{3}H]dCTP in F were 1000 (○, ●, ▲) and 200 (△). The 100% values (pmol) were 36.3 (○), 11.3 (●), 19.8 (◑), 113.0 (△), and 25.0 (▲) in A–E; 19.7 (×) in A–F; and 14.6 (○), 11.6 (●), 118.8 (△), and 5.2 (▲) in F.

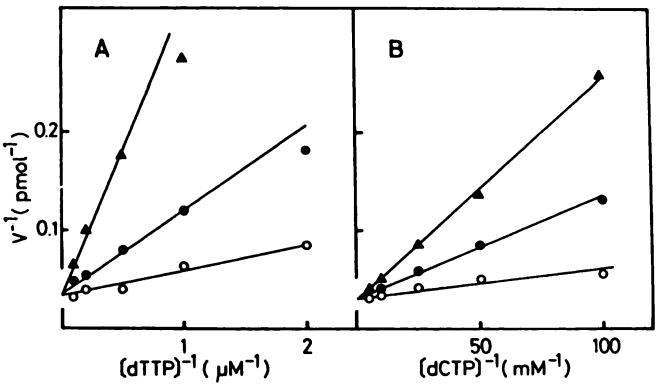

Fig. 3. Analysis of inhibition of murine retroviral reverse transcriptase and human DNA polymerase γ by 2′,3′-didehydro-2′,3′-dideoxypyrimidine 5′-triphosphates. Reactions were carried out under the conditions described in Materials and Methods, except that various concentrations of dTTP (6000 cpm/pmol) (A) or dCTP (200 cpm/pmol) (B) were used as indicated in the figure in the presence of 0 (○), 0.01 (●), and 0.03 μM (▲) ddeTTP (A) or 0 (○), 0.4 (●), and 1.0 μM (▲) ddeCTP (B). The enzymes used were DNA polymerase γ with $(rA)_n \cdot (dT)_{12-18}$ (A) and reverse transcriptase with $(rI)_n \cdot (dC)_{12-18}$ as the template·primer (B). The figure represents double reciprocal plots.

Mg^{2+} (Fig. 4, B, D, and F). K_i values of ddeTTP and ddeCTP with Mn^{2+} were determined in another set of kinetic experiments (data not shown) to be 12.5 μM and 11.1 μM, respectively, which were 2 times lower than those determined in the presence of Mg^{2+} (21.6 μM for ddeTTP and 21.5 μM for ddeCTP) (Table 3). On the other hand, DNA polymerase α was very strongly inhibited by ddeTTP in the presence of 0.1 mM Mn^{2+}, whereas 0.1 mM Mn^{2+} had little, if any, influence on the inhibition of α-polymerase by AzddTTP and ddeCTP (Fig. 4, A, C, and E). The K_i of ddeTTP for DNA polymerase α was determined to be 1.6 μM, which is similar to the K_m of dTTP in the presence of Mn^{2+} (1.7 μM) (Table 3).

Discussion

All the dideoxynucleotide analogues examined in this study have proven to be effective in inhibiting the activities of reverse transcriptase and various DNA polymerases except DNA polymerase α (Fig. 2). AzddTTP was the strongest inhibitor of the reverse transcriptase, inasmuch as it showed the lowest K_i value (42 nM). Introduction of a methylene group between the α- and β- or β- and γ-positions of the phosphates in AzddTTP or AzddTDP reduced the inhibitory potency on most of the DNA polymerases. The K_i values of $AzddTTP_{\alpha\to\beta}$ (15.4 μM) and $AzddTTP_{\beta\to\gamma}$ (4.5 μM) were similar to the K_i values of ddTTP (9.3 μM) and the K_m value of dTTP (15 μM) (Table 2). Thus, AzddTTP is a more potent inhibitor of the reverse transcriptase than are other AzddTTP derivatives. Both ddeTTP and ddeCTP showed a similar degree of inhibition against the reverse transcriptase, with similar K_i values (0.33 μM and 0.27 μM, respectively; Table 2). It should be noted that the K_i of ddeTTP (0.33 μM) was much less than that of ddTTP (9.3 μM).

DNA polymerase γ was strongly inhibited by all compounds except $AzddTTP_{\beta\to\gamma}$ and ddeCTP. The K_i values of ddTTP (3.0 nM) and ddeTTP (3.5 nM) were less than those of AzddTTP, $AzddTDP_{\alpha\to\beta}$, and $AzddTTP_{\alpha\to\beta}$ and the K_m value of dTTP (0.63 μM) (Table 2). However, the highly purified preparation of DNA polymerase γ was unable to utilize activated calf thymus DNA or $(rI)_n \cdot (dC)_{12-18}$ as the template·primer, and,

TABLE 2

Characterization of inhibition of DNA polymerases by 2′,3′-dideoxynucleotide analogues

DNA polymerase	Template·primer	Variable substrate ([^{3}H]dNTP)	K_m for [^{3}H]dNTP	K_i						
				ddTTP[a]	AzddTTP	AzddTDP$_{\alpha\to\beta}$	AzddTTP$_{\alpha\to\beta}$	AzddTTP$_{\beta\to\gamma}$	ddeTTP	ddeCTP
			μM				μM			
α	Activated DNA	dTTP	1.8[a]	NI[b]	NI	NI	NI	NI	NI	NI
β	Activated DNA	dTTP	22.2	1.6	NI	NI	NI	NI	21.6	
		dCTP	26.4							21.5
	$(rA)_n\cdot(dT)_{12-18}$	dTTP	143	1.1	SI[c]	NI	NI	NI	3	
γ	$(rA)_n\cdot(dT)_{12-18}$	dTTP	0.63	0.003	0.1	3.4	0.75	SI	0.0035	NI
TdT	$(dA)_{12-18}$	dGTP	10		1	SI	1	1	2.5	1
Reverse transcriptase	$(rA)_n\cdot(dT)_{12-18}$	dTTP	15	9.3	0.042	SI	15.4	4.5	0.33	
	$(rI)_n\cdot(dC)_{12-18}$	dCTP	9.1							0.27
Polymerase I	Activated DNA	dTTP	0.56		0.50	NI	NI	NI	0.13	
		dCTP	1.34							0.30

[a] Taken from our previous paper (37). ddTTP, 2′,3′-dideoxythymidine 5′-triphosphate.
[b] NI, not inhibitory.
[c] SI, slightly inhibitory.

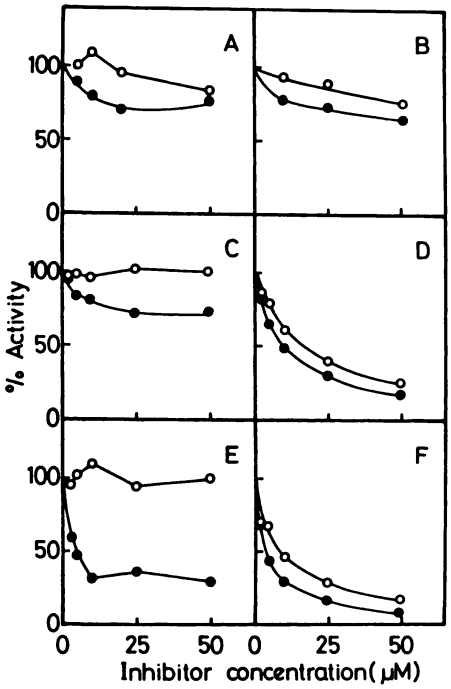

Fig. 4. Effect of manganese ion on the inhibition of mouse DNA polymerases α and β by 2′,3′-dideoxynucleotide analogues. Reactions of DNA polymerases α (A, C, and E) and β (B, D, and F) were carried out under the conditions described in Materials and Methods with activated DNA as the template·primer in the presence of magnesium ion (4 and 10 mM for DNA polymerase α and β, respectively) (○) or manganese ion (0.1 and 0.2 mM for DNA polymerase α and β, respectively) (●). The 2′,3′-dideoxynucleotide analogues tested were AzddTTP (A and B), ddeCTP (C and D), and ddeTTP (E and F) and their concentrations are indicated in the figure. The specific radioactivities of [^{3}H]dTTP (A, B, E, and F) and [^{3}H]dCTP (C and D) were, in all cases, 1000 cpm/pmol. The 100% values (pmol) were 11.2 (○) and 38.7 (●) in A and E; 31.9 (○) and 15.3 (●) in B and F; 5.2 (○) and 18.1 (●) in C; and 11.6 (○) and 18.0 (●) in D.

therefore, it was impossible to evaluate the inhibitory effects of ddCTP and ddeCTP on the γ-polymerase in the presence of their normal counterpart, dCTP, as substrate. In another laboratory (38) ddCTP has proven to be inhibitory (K_i, 0.016 μM) to the γ-polymerase when assayed with dCTP as substrate and activated calf thymus DNA as the template·primer. With $(rA)_n\cdot(dT)_{12-18}$ as the template·primer, ddeCTP did not prove to be inhibitory to DNA polymerase γ (Table 2).

TABLE 3

Effect of manganese ion on the inhibition of mouse DNA polymerases α and β by AzddTTP, ddeTTP, and ddeCTP

DNA polymerase	K_m		K_i		
	dTTP	dCTP	AzddTTP	ddeTTP	ddeCTP
	μM			μM	
α with Mg^{2+}	1.8	ND[a]	NI[b]	NI	NI
with Mn^{2+}	1.7	ND	SI[c]	1.6	SI
β with Mg^{2+}	22.2	26.4	SI	21.6	21.5
with Mn^{2+}	17.1	37.5	SI	12.5	11.1

[a] ND, not determined.
[b] NI, not inhibitory.
[c] SI, slightly inhibitory.

None of the test compounds were inhibitory to DNA polymerase α under the reaction conditions used (with Mg^{2+}) (Table 2). Upon replacement of Mg^{2+} by Mn^{2+}, however, DNA polymerase α was very strongly inhibited by ddeTTP (K_i, 1.6 μM) but not by ddeCTP (Fig. 4). Thus, under certain conditions [DNA polymerase γ activity measured in the presence of $(rA)_n\cdot(dT)_{12-18}$ as template·primer; DNA polymerase α activity measured in the presence of Mn^{2+} instead of Mg^{2+}], ddeTTP was inhibitory to the DNA polymerase activity whereas ddeCTP was not. Whether these differential effects on DNA polymerase activity may reflect a differential cytotoxicity of the compounds is an interesting question that remains to be addressed.

The inhibitory effects of the 2′,3′-dideoxynucleotides on DNA synthesis may also be explained by their being incorporated into the growing polynucleotide, thus terminating the DNA chain growth. Our previous studies on ddTTP (35), ddATP, ddCTP, ddGTP (36), and AzddTTP (39) have, however, shown that the inhibition by these compounds was mainly due to direct inhibition of the enzyme activity, suggesting the presence of the similar inhibition mechanism in the case of the newly synthesized compounds examined in the present study.

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