Role of DNA Polymerase γ in Adenovirus DNA Replication. Mechanism of Inhibition by 2′,3′-Dideoxynucleoside 5′-Triphosphates†

Peter C. van der Vliet* and Marijke M. Kwant

ABSTRACT: In contrast to cellular or SV40 DNA replication, adenovirus type 5 (Ad5) or type 2 (Ad2) DNA synthesis in isolated nuclei is strongly inhibited by low concentrations of 2′,3′-dideoxythymidine 5′-triphosphate (ddTTP). On the basis of differential sensitivity of cellular DNA polymerases, a role of DNA polymerase γ in adenovirus DNA replication has been proposed. We have investigated the mechanism of inhibition of adenovirus DNA synthesis, using [α-^{32}P]ddTTP and other dNTP analogues. Both ddATP and ddGTP were as inhibitory as ddTTP, while ddCTP had an even stronger effect on adenovirus DNA replication. DNA polymerase α was resistant to all four ddNTP's, while DNA polymerase γ was very sensitive. The inhibition by ddTTP in isolated infected nuclei was slowly reversible. [α-^{32}P]ddTTP was incorporated into Ad5 DNA as a chain-terminating nucleotide, and the analogue could be used as a substrate by DNA polymerase γ. Under similar conditions, incorporation in cellular DNA or using DNA polymerase α was not observed. The nucleoside analogues ddA and ddC suppressed adenovirus DNA replication in intact cells and reduced plaque formation. These results provide further evidence for a function of DNA polymerase γ in adenovirus DNA synthesis.

Three different DNA-dependent DNA polymerases, designated α, β, and γ, are commonly found in mammalian cells. They can be easily separated by ion-exchange chromatography and display different enzymatic properties (Weissbach, 1979). DNA polymerase α, which is most abundant in growing cells, has been implicated in cellular DNA replication and in the replication of SV40 DNA. This is based primarily upon the presence of DNA polymerase α in replication complexes (Otto & Fanning, 1978), the sensitivity of DNA replication to the DNA polymerase α inhibitor aphidicolin (Ikegami et al., 1978; Krokan et al., 1979), and the resistance to 2′,3′-dideoxythymidine 5′-triphosphate (ddTTP),[1] a nucleotide analogue which inhibits both DNA polymerase β and γ, but not α (Edenberg et al., 1978; van der Vliet & Kwant, 1978; Waqar et al., 1978; Wist, 1979).

The role of DNA polymerase β is unclear. The enzyme may function in repair processes (Hübscher et al., 1979; Weissbach, 1979). DNA polymerase γ is present in nuclei as well as in mitochondria and is most likely responsible for mitochondrial DNA replication (Hübscher et al., 1979; Weissbach, 1979). Its role in nuclei is still obscure. DNA polymerase γ has been found in adenovirus DNA replication complexes, together with DNA polymerase α (Arens et al., 1977; Brison et al., 1977; Abboud & Horwitz, 1979). Recently, evidence for a function of DNA polymerase γ in adenovirus replication was obtained from the strong sensitivity to ddTTP of adenovirus DNA synthesis in isolated nuclei (van der Vliet & Kwant, 1978) and in replication complexes (Abboud & Horwitz, 1979). This is attractive in view of the similarities that exist between the nuclear and mitochondrial DNA polymerase γ (Bertazzoni et al., 1977; Hübscher et al., 1977) and between the mechanism of replication of mitochondrial DNA and adenovirus DNA. In contrast to cellular or SV40 DNA, both DNAs replicate by a displacement mechanism and contain an altered nucleosome structure (Tate & Philipson, 1979). Therefore, it has been suggested that DNA polymerase γ is responsible for displacement-type synthesis in nuclei (van der Vliet & Kwant, 1978; Weissbach, 1979).

However, the ddTTP inhibition studies do not exclude the analogue's inhibiting other components of the replication machinery rather than the DNA polymerase itself. Therefore, we have investigated the mechanism of inhibition in more detail. Our studies show that both in vivo and in vitro adenovirus DNA replication is sensitive to 2′,3′-dideoxynucleotides and that the inhibition by ddTTP is accompanied by incorporation of the analogue into DNA. Since only DNA polymerase γ accepts ddTTP as a substrate, these results confirm the role of DNA polymerase γ in adenovirus DNA chain growth.

Materials and Methods

Growth of Cells and Viruses. KB cells were grown in suspension in Eagle's minimum essential medium supplemented with 5% calf serum, 0.05% glutamine, and antibiotics. The doubling time was 24 h. The cells were mycoplasm free as tested via the standard agar plate growth procedure. At a density of 3–4 × 10^5 cells/mL, at the end of the logarithmic growth phase, the cells were infected with adenovirus type 5 (Ad5) (10–20 pfu/cell) and harvested at 16 h postinfection. Under these conditions, all cells were infected, and 90–95% of the newly synthesized DNA was viral. Plaque tests were performed on 293 cells (Graham & Van der Eb, 1977).

Reagents. Unlabeled ribo- and deoxyribonucleoside triphosphates were from Sigma. 2′,3′-Dideoxynucleosides and 2′,3′-dideoxynucleotide 5′-triphosphates were obtained from P-L Biochemicals. Nucleotide concentrations were determined spectrophotometrically. [^{3}H]dTTP (40 Ci/mmol) and [^{3}H]dGTP (12 Ci/mmol) were from the Radiochemical Centre (Amersham) and [α-^{32}P]ddTTP from ICN. For removal of contaminating [α-^{32}P]dTTP, the product was purified by chromatography on thin-layer cellulose sheets (Eastman Kodak) by using isobutyric acid–1 M NH_4OH–0.1 M EDTA (50:30:0.8) as solvent (Atkinson et al., 1969). The R_f of ddTTP was 0.47 as compared to 0.38 for dTTP. The [α-^{32}P]ddTTP was eluted with H_2O and dried under a nitrogen stream. Exonuclease III was purchased from Biolabs. Snake

† From the Laboratory for Physiological Chemistry, University of Utrecht, 3521 GG Utrecht, The Netherlands. *Received August 20, 1980.* This work was supported in part by the Netherlands Foundation for Chemical Research with financial aid from the Netherlands Organization for the Advancement of Pure Research.

[1] Abbreviations used: ddTTP, 2′,3′-dideoxynucleoside 5′-triphosphate; pfu, plaque-forming units; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid.

0006-2960/81/0420-2628$01.25/0

venom phosphodiesterase was obtained from Boehringer Mannheim and pancreatic deoxyribonuclease from Worthington.

Nuclear DNA Synthesis. Nuclei were isolated essentially as described (van der Vliet & Sussenbach, 1972). The nuclei (4×10^7/mL) were suspended in 0.05 M *N*-(2-hydroxyethyl)piperazine-*N'*-2-ethanesulfonate (Hepes), pH 7.8, 0.11 M NaCl, 1 mM $MgCl_2$, and 1 mM dithiothreitol and incubated at 32 °C for 30 min in a mixture containing [^{3}H]dTTP of [^{3}H]dGTP and ddNTP's as indicated. The final concentrations were 40 mM Hepes, pH 7.8, 55 mM NaCl, 45 mM KCl, 5 mM $MgCl_2$, 0.4 mM $CaCl_2$, 1 mM dithiothreitol, 2 mM ATP, 2 mM ethylene glycol tetraacetate, 1 mM phosphoenolpyruvate, 0.05 mM each of UTP, CTP, GTP, dATP, dGTP, and dCTP, and 1 unit/mL pyruvate kinase.

[^{3}H]dTTP, evaporated to remove ethanol, was present at 5 μM and 10 Ci/mmol. For calculations of the ratio of dideoxynucleotides to deoxynucleotides, the dNTP pool present in the nuclei (<1 μM) was neglected. The reaction was stopped by addition of a 10-fold excess of 0.01 M Tris-HCl, pH 8.1, and 0.01 M EDTA, and viral DNA was isolated as described (van der Vliet et al., 1977). Incorporation of radioactivity in Ad5 DNA was measured by spotting samples on Whatman 3MM filters followed by TCA precipitation. DNA synthesis in nuclei from uninfected cells was determined directly by Cl_3AcOH precipitation and filtration on Whatman GF-C glass fiber filters. The characterization of viral DNA by sucrose gradient centrifugation and CsCl equilibrium density centrifugation was performed as described (van der Vliet et al., 1977).

Nuclease Digestions. Viral DNA was digested with 10 U/mL exonuclease III in 67 mM Tris-HCl, pH 7.8, 4 mM $MgCl_2$, and 4 mM dithiothreitol at 37 °C. Samples of 25 μL were cooled and 100 μL of 0.1 M EDTA and 1% bovine serum albumin (BSA) were added followed by 0.5 mL of 10% Cl_3AcOH. Cl_3AcOH-soluble radioactivity was determined by filtration on GF-C filters. For characterization of the 5'-nucleotides, DNA was first digested with pancreatic DNAse (50 μg in 0.1 mL) for 2 h at 37 °C in 50 mM Tris-HCl, pH 7.5, 5 mM $MgCl_2$, and 0.05% BSA. The pH was raised to 9.0 with 0.05 M NaOH, and snake venom phosphodiesterase (12 μL, 1 mg/mL) was added.

The enzyme was dialyzed before use to remove glycerol which disturbed the thin-layer chromatography of the reaction products. After incubation for 2 h at 37 °C, the nucleotide mixture was concentrated under a nitrogen stream and spotted on prewashed cellulose sheets (20 × 20 cm), and the chromatogram was developed in isobutyric acid–1 M NH_4OH–0.1 M EDTA (50:30:0.8). ddTMP marker was prepared by digestion of ddTTP with snake venom phosphodiesterase. The markers were visualized under UV, and the corresponding radioactive nucleotides were eluted and counted.

Isolation of DNA Polymerases α, β, and γ. DNA polymerases α and β from uninfected KB cells or Ad5-infected KB cells 16-h postinfection were purified by DEAE-cellulose chromatography as described (De Jong et al., 1977). DNA polymerase γ was isolated essentially according to Knopf et al. (1976) and purified by DEAE-cellulose, phosphocellulose, hydroxylapatite, and DNA cellulose chromatography. BSA (0.1 mg/mL) was included from the phosphocellulose step to prevent losses, and the ACA-22 hydroxylapatite chromatography step (Knopf et al., 1976) was omitted. We modified the hydroxylapatite chromatography by elution with a shallow gradient (0.1–0.3 M potassium phosphate) which improved the separation of DNA polymerases α and γ. DNA polymerase α was characterized by sedimentation at 8.8 S and sensitivity to *N*-ethylmaleimide (NEM) (95% inhibition at 1 mM NEM). DNA polymerase β sedimented at 3.4 S and was completely resistant to 5 mM NEM. DNA polymerase γ preferred poly(rA)·$(dT)_{12-18}$ 4-fold compared to activated DNA as template in the presence of phosphate. The enzyme was inhibited for 85% by 5 mM NEM and devoid of residual DNA polymerase α, as determined by the complete sensitivity to ddTTP under conditions where DNA polymerase α is resistant (see Results).

DNA Polymerase Assays. DNA polymerase α was assayed with 20 μg of activated calf thymus DNA in a volume of 0.1 mL containing 10 mM Tris-HCl, pH 8.5, 10 mM $MgCl_2$, 1 mM dithiothreitol 50 μM each of dATP, dCTP, and dGTP, 5 μM [^{3}H]dTTP (2 Ci/mmol), and 20 μL of enzyme. Cl_3AcOH-insoluble radioactivity was determined as described (van der Vliet & Kwant, 1978). One unit is defined as the amount catalyzing the incorporation of 1 pmol of TMP in DNA in 30 min at 37 °C. DNA polymerase β was tested in the presence of 5 mM NEM. The DNA polymerase γ reaction mixture contained in 0.1 mL 50 μg of BSA, 50 mM Tris-HCl, pH 7.5, 0.5 mM $MnCl_2$, 100 mM KCl, 2.5 mM dithiothreitol, 2.5 μg of poly(rA)·(dT_{12-18}), and 1 μM [^{3}H]-dTTP (10 Ci/mmol). The template was reannealed for 5 min at 70 °C in 50 mM Tris-HCl, pH 7.5, and 100 mM KCl before each experiment. Incubation was at 30 °C, and Cl_3AcOH-insoluble radioactivity was determined as for DNA polymerase α. One unit is defined as 1 pmol of dTTP incorporated in 30 min at 30 °C. When assayed with activated DNA as template, DNA polymerase γ was incubated in a reaction mixture containing, in 0.1 mL, 10 μg of activated calf thymus DNA, 50 μg of BSA, 50 mM Tris-HCl, pH 8.5, 7.5 mM $MgCl_2$, 0.5 mM dithiothreitol, 50 μM each of dATP, dCTP, and dGTP, and 1 μM [^{3}H]dTTP (10 Ci/mmol). In all tests, an incubation at 0 °C under identical conditions served as a control.

Results

Adenovirus DNA Synthesis Is Inhibited by All Four 2',3'-Dideoxynucleoside 5'-Triphosphates. Isolated nuclei from adenovirus-infected KB cells are capable of elongation of preexisting DNA replication intermediates in all stages of replication (van der Vliet & Sussenbach, 1972; Winnacker, 1975). To study the effect of various ddNTP's, nuclei from infected or uninfected cells were incubated with increasing concentrations of the analogues, and DNA synthesis was measured (Figure 1). All four ddNTP's reduced viral DNA synthesis at low concentrations, while an effect on cellular DNA synthesis was only observed at much higher ddNTP to dNTP ratios. The concentration required for 50% inhibition for Ad5 DNA synthesis was identical for ddATP, ddTTP, and ddGTP, respectively, while ddCTP inhibited about 3-fold stronger (see Table I). Similar results were obtained with Ad2 infected KB cells (not shown).

The inhibition by ddTTP can be suppressed by an excess of dTTP (van der Vliet & Kwant, 1978). For studies on whether the inhibiting effect was completely reversible, nuclei from Ad5-infected KB cells were preincubated with an excess (150 μM) of ddTTP, in the absence of radioactive label. The nuclei were then washed extensively to remove ddTTP and further incubated in the presence of [^{3}H]dGTP and dTTP. The results in Figure 2 show that initially the rate of DNA synthesis in the preincubated nuclei lags behind the control. After 60 min of incubation, the same maximal level of incorporation is reached. This result might be explained by incorporation of ddTMP as a chain-terminating nucleotide

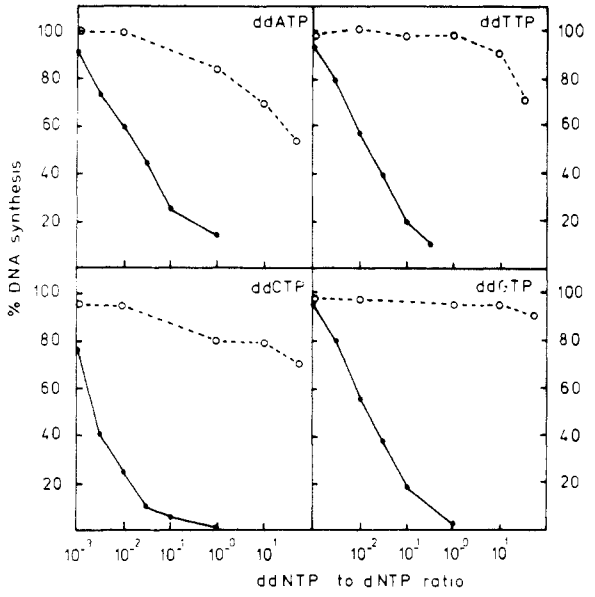

FIGURE 1: Effect of 2′,3′-dideoxynucleoside 5′-triphosphates on adenovirus and cellular DNA syntheses in isolated nuclei. DNA synthesis was assayed in a reaction mixture containing 50 μM unlabeled dNTP's, 5 μM [^{3}H]dTTP, and increasing concentrations of ddATP, ddCTP, or ddGTP. When inhibition by ddTTP was studied, 5 μM [^{3}H]dGTP was present as radioactive label, and 50 μM unlabeled dTTP was added. The nuclei were incubated for 30 min, and the inhibitors were present from the beginning of the incubation. (○) Cellular DNA, 100% = 5656 cpm/10^6 nuclei; (●) Ad5 DNA, 100% = 22844 cpm/10^6 nuclei.

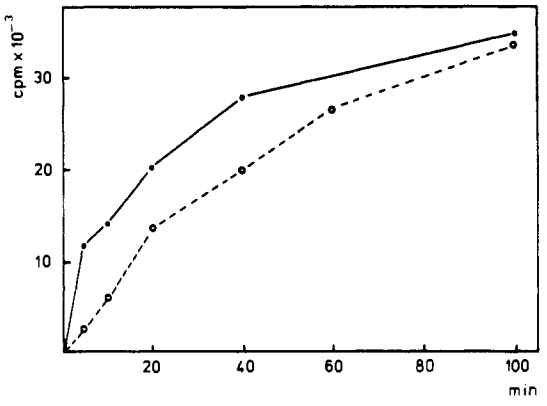

FIGURE 2: Inhibition of Ad5 DNA synthesis by ddTTP is slowly reversible. Nuclei from infected cells were incubated for 30 min at 32 °C in the presence of 50 μM dTTP and 150 μM ddTTP, without radioactive label. The nuclei were freed from ddTTP by extensive washing at 4 °C and then incubated in a complete reaction mixture containing [^{3}H]dGTP, without inhibitor. Control nuclei were treated identically but without ddTTP. DNA synthesis was then measured as a function of time. (●) Control nuclei; (○) nuclei preincubated with ddTTP.

Table I: Concentration of ddNTP (μM) Which Is Required To Give 50% Inhibition (dNTP Concentration 50 μM)

	nuclear DNA synthesis		DNA polymerase		
	Ad5	cellular	α[a]	β	γ
ddTTP	0.7	>500	1750	50	3 (0.2)[c]
ddATP	0.7	>500	>500	nd[b]	3
ddCTP	0.2	>500	>500	nd	3
ddGTP	0.7	>500	>500	nd	4

[a] These values were found for DNA polymerase α from both uninfected and Ad5-infected KB cells, 16 h postinfection. [b] nd, not determined. [c] Assayed with poly(rA)·(dT$_{12-18}$) as template.

during preincubation, followed by a slow removal of the blocking nucleotide before elongation can proceed.

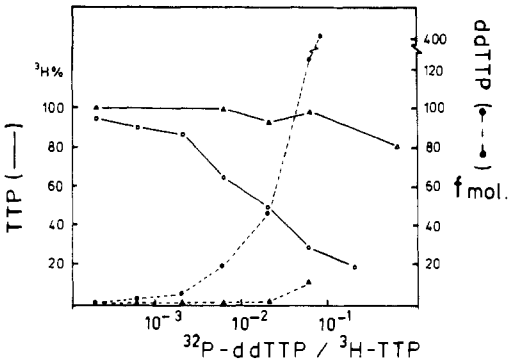

FIGURE 3: Incorporation of ^{32}[P]ddTTP into Ad5 DNA. Nuclei from Ad5-infected (●, ○) or uninfected KB cells (▲, △) were incubated with a reaction mixture containing [^{3}H]dTTP (2 μM, 10 Ci/mmol) and increasing amounts of [^{32}P]ddTTP (155 Ci/mmol). After 30 min, DNA synthesis and ^{32}P incorporation were measured as described under Materials and Methods. 100% = 12 pmol/5 × 10^6 nuclei for infected cells and 1.4 pmol/5 × 10^6 nuclei for uninfected cells.

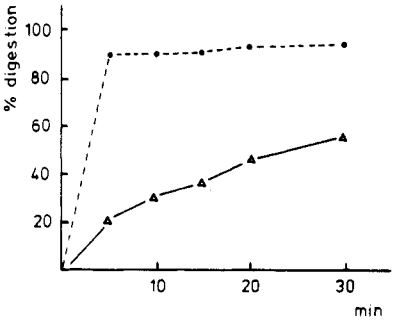

FIGURE 4: Exonuclease III digestion of Ad5 DNA labeled with [^{3}H]dTTP and [^{32}P]ddTTP. Ad5 DNA was synthesized in nuclei in the presence of [^{32}P]ddTTP and [^{3}H]dTTP. The ratio of ddTTP to dTTP was 6 × 10^{-2}. After phenol extraction and ethanol precipitation, the DNA was digested for various periods of time with exonuclease III, and the percentage of acid-soluble radioactivity was determined. (△) [^{3}H]; (●) [^{32}P].

Incorporation of ddTTP into Ad5 DNA. for a direct investigation of the possible incorporation of ddTMP into Ad5 DNA, nuclei from Ad5-infected KB cells were incubated with [^{3}H]dTTP and increasing concentrations of [α-^{32}P]ddTTP and DNA synthesis were studied. A gradual decrease of viral DNA replication is observed at increasing inhibitor concentrations, and ^{32}P radioactivity is incorporated into Ad5 DNA (Figure 3). At 50% inhibition of DNA synthesis, 0.05 pmol of ddTTP and 60 pmol of dTTP were incorporated. At 90% inhibition, ddTTP incorporation had not yet reached a plateau. In contrast to adenovirus DNA, no significant ^{32}P radioactivity is observed in cellular DNA (Figure 3).

The ^{32}P and ^{3}H radioactivity cosedimented in neutral sucrose gradients (not shown). Since we could not exclude the possibility that a small percentage of [^{32}P]ddTTP is converted during incubation into [^{32}P]dTTP and then incorporated, we isolated the viral DNA and digested it with pancreatic DNase followed by snake venom phosphodiesterase to produce 5′-deoxynucleotides. Analysis of the reaction product by thin-layer chromatography showed that 91% of the ^{32}P radioactivity was found in the position of ddTMP and only 7% on the dTMP position. The double-labeled DNA was also digested with exonuclease III. As shown in Figure 4, the [^{32}P]ddTMP was released much faster than the majority of [^{3}H]dTMP, indicating that the analogue is present at or near the 3′ ends of the nascent DNA chains.

Effect of ddNTP's on Isolated DNA Polymerases. DNA polymerases β and γ from CV1 cells (Edenberg et al., 1978), HeLa cells (Waqar et al., 1978; Wist, 1979), or KB cells (van

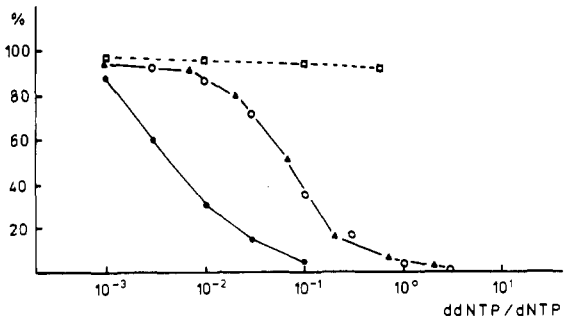

FIGURE 5: Influence of template on the inhibition of DNA polymerase γ by ddATP or ddTTP. DNA polymerase γ (5.5 units) was assayed in the presence of increasing concentrations of ddATP (○, □) or ddTTP (●, ▲) by using activated DNA (○, ▲) or poly(rA)·(dT_{12-18}) (○, □) as template. The values obtained without inhibitor were set at 100%.

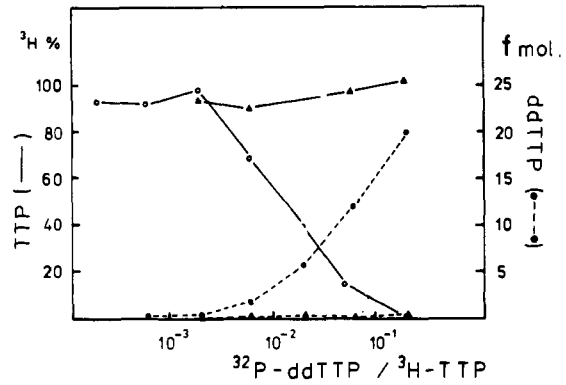

FIGURE 6: ddTTP used as substrate by DNA polymerase γ. DNA polymerase α (△, ▲) or DNA polymerase γ (○, ●) activity was monitored under standard conditions in the presence of increasing concentrations of [^{32}P]ddTTP (155 Ci/mmol). DNA polymerase γ was assayed with poly(rA)·(dT_{12-18}) as template. 100% = 12 U for DNA polymerase α and 2.5 U for DNA polymerase γ.

der Vliet & Kwant, 1978) are sensitive to ddTTP, while DNA polymerase α is resistant. We have extended these results for DNA polymerases α and γ of KB cells to ddCTP, ddGTP, and ddATP (Table I). The two DNA polymerases could discriminate between all nucleotide analogues. No major change in sensitivity was observed for DNA polymerases α or γ from Ad5-infected cells.

In an experiment designed to examine the influence of template on the inhibiting potency of DNA polymerase γ, the effects of ddATP and ddTTP were compared in the presence of activated DNA or the synthetic template poly(rA)·(dT_{12-18}). The results, given in Figure 5, show that, although ddATP and ddTTP are equally inhibitory with activated DNA, synthesis with poly(rA)·(dT_{12-18}) was even more sensitive to ddTTP and completely resistant to ddATP.

This indicates that incorporation of the analogues is required for inhibition. Direct proof for incorporation came from experiments in which [α-^{32}P]ddTTP was used as inhibitor in the presence of [^{3}H]dTTP. As in the case of Ad5 DNA synthesis, DNA polymerase γ incorporated ddTTP, while DNA polymerase α did not (Figure 6).

Inhibition of Viral DNA Synthesis by 2′,3′-Dideoxynucleosides in Intact Cells. In view of the results obtained in isolated nuclei, we explored the effects of various 2′,3′-dideoxynucleosides on viral and cellular DNA synthese in intact cells. Ad5-infected KB cells or logarithmically growing uninfected cells were exposed to 2′,3′-dideoxythymidine (ddT) or 2′,3′-dideoxyadenosine (ddA), and after 15 min, [^{3}H]thymidine was added and DNA synthesis was measured during a 1-h period. Figure 7A shows that ddT caused a slight inhibition of both viral and cellular DNA syntheses, in contrast to the results obtained in vitro with ddTTP. However, ddA inhibits Ad5 DNA synthesis to a much greater extent than cellular DNA synthesis, with 50% inhibition at 8.5 mM. Also, 2′,3′-dideoxycytidine discriminated between the two types of DNA synthesis, but 50% inhibition of Ad5 DNA synthesis occurred at higher concentrations (70 mM) (results not shown).

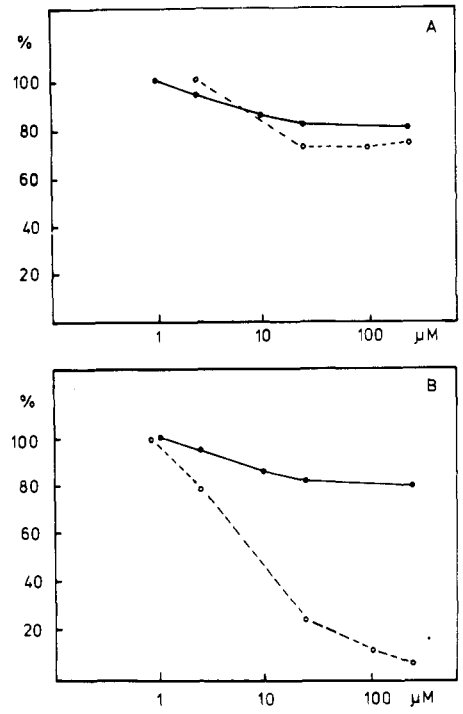

FIGURE 7: Effect of 2′,3′-dideoxythymidine (A) and 2′,3′-dideoxyadenosine (B) on Ad5 or cellular DNA synthesis in intact cells. To 2×10^6 Ad5 infected (○) or uninfected (●) KB cells was added 2′,3′-dideoxythymidine or 2′,3′-dideoxyadenosine at the indicated concentrations, and, after 15 min, DNA synthesis was determined for 1 h in the presence of 1 μCi/mL [^{3}H]thymidine. 100% = 43 373 cpm for uninfected cells and 209 255 cpm for infected cells. The viral nature of the DNA was established by CsCl equilibrium centrifugation in the presence of ^{14}C-marker Ad5 DNA.

The variable results with different 2′,3′-dideoxynucleosides are most likely explained by differences in the rate of intracellular phosphorylation. In contrast to thymidine, dideoxythimidine is only very slowly phosphorylated (Krokan et al., 1979). The phosphorylated rates of ddA or ddC have not been measured. Both ddA and ddC caused a 10-fold reduction in virus yield when tested at 50 μM in a plaque assay (H. J. Laanen, personal communication). Since concentrations of 100 μM or higher were cytotoxic, we did not investigate this effect further.

Discussion

The replication of adenovirus DNA differs in many respects from the replication of cellular DNA or the DNA from SV40, notably in the mechanism of replication, the lack of a tight coupling to protein synthesis, and the strong sensitivity to ddTTP of adenovirus DNA chain elongation, both in isolated nuclei and in replication complexes (van der Vliet & Kwant, 1978; Abboud & Horwitz, 1979). The data presented in this paper make it plausible that the ddTTP inhibition can be explained by the involvement of DNA polymerase γ since this enzyme is not only the most sensitive to all 2′,3′-dideoxynucleoside triphosphates but can also recognize and incorporate ddTTP. The incorporation of ddTTP in adenovirus DNA is clearly demonstrated. Other explanations, like a modification of DNA polymerase α to a ddTTP-sensitive enzyme during

adenovirus infection, are not very likely since DNA polymerase α from infected cells was as resistant to ddTTP as the enzyme from uninfected cells. However, we cannot completely exclude the presence of another, as yet undetected, enzyme with properties similar to DNA polymerase γ which could be responsible for Ad5 DNA synthesis, such as the poly(dC)-dependent DNA polymerase activity found in regenerating rat liver or Ehrlich ascites tumor cells (De Recondo et al., 1973; Yagura et al., 1980). Although incorporation of ddTMP can be demonstrated, it seems that the inhibition of viral DNA replication is caused primarily by a competitive effect with dTTP and that only at higher analogue concentrations chain termination by the incorporated ddTTP becomes important (see Figure 3 and 6). Such an effect was also observed with *Escherichia coli* DNA polymerase I. This enzyme requires high concentrations, at least 10 times that of the corresponding nucleotide, for incorporation of the analogue (Atkinson et al., 1969). Both DNA polymerases α and γ have been detected in adenovirus replication complexes extracted from isolated nuclei. It has not been established whether the DNA polymerase α found in these complexes is also functional in viral DNA synthesis. The results presented here do not exclude such an additional role of DNA polymerase α, either in a reaction requiring two DNA polymerases in different steps or in a coordinated complex of DNA polymerases α and γ. A role of DNA polymerase α has been suggested from the inhibition of adenovirus DNA replication by phosphonoacetic acid (Frenkel, 1978) and aphidicolin, a specific inhibitor of this enzyme (Krokan et al., 1978; Longiaru et al., 1979). However, a 300–400-fold difference exists between the sensitivity of Ad5 and cellular DNA replication to aphidicolin. Moreover, the inhibition of Ad5 DNA synthesis by this drug does not respond to changes in dCTP concentration, while purified DNA polymerase α is strongly influenced by such changes (Kwant & van der Vliet, 1980). These results suggest that DNA polymerase α is not involved in adenovirus DNA synthesis. Apart from adenovirus replication, displacement synthesis in eukaryotic cells has been observed in mitochondrial DNA (Robberson et al., 1972) and in the DNA of adeno-associated virus (Berns et al., 1978). It is noteworthy that, in all three cases, a DNA polymerase of the γ type is involved (Handa & Carter, 1979). Although the function of the nuclear DNA polymerase γ in uninfected cells is not known, the suggestion has been raised that this enzyme is responsible for displacement synthesis. This process might explain the presence of long single-stranded DNA in human cells (Bjursall et al., 1979). During adenovirus infection, a small increase, from 1.5% to 3.1% of the total DNA polymerase activity, was observed for DNA polymerase γ (J. Droog, unpublished observations). Recent information about the specific activity and processive reaction mechanism of DNA polymerase γ from chick embryo cells (Yamaguchi et al., 1980) provides further evidence for a role of this enzyme in adenovirus displacement synthesis.

Acknowledgments

We thank Dr. H. J. Laanen for performing virus titrations and Drs. H. S. Jansz and J. S. Sussenbach for critical reading of the manuscript.

References

Abboud, M. M., & Horwitz, M. S. (1979) *Nucleic Acids Res. 6*, 1025–1039.

Arens, M., Yamashita, T., Padmanabhan, R., Tsurno, T., & Green, M. (1977) *J. Biol. Chem. 252*, 7947–7954.

Atkinson, M. R., Deutscher, M. P., Kornberg, A., Russell, A. F., & Moffatt, J. G. (1969) *Biochemistry 8*, 4897–4904.

Berns, K. I., Hauswirth, M. H., Fife, K. H., & Lusby, E. (1978) *Cold Spring Harbor Symp. Quant. Biol. 43*, 781–787.

Bertazzoni, U., Scovassi, A. I., & Brun, G. M. (1977) *Eur. J. Biochem. 81*, 237–248.

Bjursall, G., Gussander, E., & Lindahl, T. (1979) *Nature (London) 280*, 420–423.

Brison, O., Kedinger, C., & Wilhelm, J. (1977) *J. Virol. 24*, 423–435.

De Jong, A., van der Vliet, P. C., & Jansz, H. S. (1977) *Biochim. Biophys. Acta 476*, 156–165.

De Recondo, A. M., Lepesant, J. A., Fichot, O., Grasset, L., Rossignol, J. M., & Cazzilis, M. (1973) *J. Biol. Chem. 248*, 131–137.

Edenberg, H. J., Anderson, S., & Pamphilis, M. L. (1978) *J. Biol. Chem. 253*, 3273–3280.

Frenkel, G. D. (1978) *J. Virol. 25*, 459–463.

Graham, F. L., & Van der Eb, A. J. (1977) *J. Gen. Virol. 36*, 59–72.

Handa, H., & Carter, B. J. (1979) *J. Biol. Chem. 254*, 6603–6610.

Hübscher, U., Kuenzle, C. C., & Spadari, S. (1977) *Eur. J. Biochem. 81*, 249–258.

Hübscher, U., Kuenzle, C. C., & Spadari, S. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 2316–2320.

Ikegami, S., Taguchi, T., Ohashi, M., Oguro, M., Nagano, H., & Mano, Y. (1978) *Nature (London) 275*, 458–460.

Knopf, K.-W., Yamada, M., & Weissbach, A. (1976) *Biochemistry 15*, 4540–4548.

Krokan, H., Schaffer, P., & De Pamphilis, M. L. (1979) *Biochemistry 18*, 4431–4443.

Kwant, M. M., & van der Vliet, P. C. (1980) *Nucleic Acids Res. 8*, 3993–4008.

Longiaru, M., Ikeda, J. E., Jarkovsky, Z., Horwitz, S. B., & Horwitz, M. S. (1979) *Nucleic Acids Res. 6*, 3369–3386.

Otto, B., & Fanning, E. (1978) *Nucleic Acids Res. 5*, 1715–1728.

Robberson, D. L., Kasamatsu, H., & Vinograd, J. (1972) *Proc. Natl. Acad. Sci. U.S.A. 69*, 737–741.

Tate, V. E., & Philipson, L. (1979) *Nucleic Acids Res. 6*, 2769–2785.

van der Vliet, P. C., & Sussenbach, J. S. (1972) *Eur. J. Biochem. 30*, 584–592.

van der Vliet, P. C., & Kwant, M. M. (1978) *Nature (London) 276*, 532–534.

van der Vliet, P. C., Zandberg, J., & Jansz, H. S. (1977) *Virology 80*, 98–110.

Waqar, M. A., Evans, M. J., & Huberman, J. A. (1978) *Nucleic Acids Res. 5*, 1933–1946.

Weissbach, A. (1979) *Arch. Biochem. Biophys. 198*, 386–396.

Winnacker, E. L. (1975) *J. Virol. 15*, 744–758.

Wist, E. (1979) *Biochim. Biophys. Acta 562*, 62–69.

Yagura, T., Kozu, T., & Seno, T. (1980) *Biochem. Biophys. Res. Commun. 92*, 1289–1296.

Yamaguchi, M., Matsukage, A., & Takahashi, T. (1980) *Nature (London) 285*, 45–47.