Differential Incorporation of Thymidylate Analogues into DNA by DNA Polymerase α and by DNA Polymerases Specified by Two Herpes Simplex Viruses

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SUMMARY

Several triphosphates (TP) of 5-substituted deoxyuridine (dU), like 5-ethyl (Et), 5-n-propyl (n-Pr), 5-iso-propyl (iso-Pr), 5-n-hexyl (n-Hx), and 5-trifluorothymidine (Fa-dT) were used as substrates for HeLa DNA polymerase α and for two herpes simplex virus (HSV)-coded DNA polymerases isolated from HeLa cells infected with HSV-1, strain C42 (wild-type), or its mutant resistant to phosphonoformate (PFAr). All polymerases were purified up to the DNA-cellulose column step and they showed comparable specific activities. The incorporation into DNA studied with all the alkyl analogues of dUTP is several times higher with the virus enzymes than with DNA polymerase α. The DNA polymerase of the mutant virus incorporates dUTP analogues to a lower extent than the wild-type polymerase. The two virus enzymes also differ in the Kₘ and Vₘₐₓ values for different substrates, indicating that the mutation to PFAr has affected the structure of the virus DNA polymerase. Surprisingly, all three enzymes use Fa-dTTP as substrate for DNA synthesis to an equal but limited extent.

INTRODUCTION

Several nucleoside analogues have been used in the therapy of herpes simplex virus (HSV) infection (Gauri, 1979; Gauri & Walter, 1973; Gauri & Malorny, 1967; Kaufmann & Heidelberger, 1964; Kaufmann et al., 1962; Schabel, 1968). Some nucleoside analogues which are efficiently phosphorylated by cellular kinases and are incorporated into DNA in proliferating cells and tissues show cytotoxic effects in uninfected cells (Gauri et al., 1976; Kit, 1976). Other analogues with no or low cytotoxicity show specific antiviral activity due to their selective or increased uptake by HSV-infected cells. HSV-induced pyrimidine kinase phosphorylates these nucleoside analogues more efficiently than the corresponding cellular enzyme (Gauri & Walter, 1973; Cheng et al., 1979; Kit, 1976) resulting in a higher intracellular concentration of the phosphorylated analogues in infected cells. Additionally, herpes viruses code for their own DNA polymerase (Keir et al., 1966; Weissbach et al., 1973). The DNA polymerases of the host and virus differ markedly in their pH and salt concentration optima, and in their affinity to substrate analogues and inhibitors (Koch et al., 1981; Spadari & Weissbach, 1974; Weissbach, 1977).

The generation of herpesvirus resistance towards antiviral drugs from the nucleoside group and those belonging to phosphonates, imidazolidinones and urea derivatives is known (Gauri,
1979, 1981; Gauri et al., 1979). Surprisingly, some of the mutants show cross-resistance for phosphonoformate (PFA) and arabinofuranosyladenine (Ara-A), although they were propagated only in the presence of PFA (Gauri, 1981). Herpesvirus-infected cells become permeable to nucleoside triphosphates within 2 to 3 h after infection. Mock-infected cells remain impermeable to these compounds (Koch et al., 1981). The permeability of infected cells for nucleoside triphosphates is of special interest for those nucleotide analogues where the corresponding nucleoside is not phosphorylated to the triphosphate level either by the host cell kinases or by the corresponding virus-induced enzyme(s). Therefore, it should be possible to incorporate base analogues solely into DNA of infected cells by the addition of nucleoside analogue triphosphates. We report here on the differential incorporation of dTTP analogues into DNA by purified HeLa cell DNA polymerase α and two herpesvirus-coded polymerases, isolated from HeLa cells, one after infection with HSV-1 strain C42, and the other after infection with C42 PFA' mutant strain.

**Methods**

**Chemicals.** Unlabelled deoxyribonucleoside triphosphates and dithiothreitol (DTT) were purchased from Boehringer, Mannheim. Bovine serum albumin (BSA) was from Sigma. [3H]dGTP (7 Ci/mmol) and [3H]dTTP (42 Ci/mmol) were from Amersham International.

**Nucleotide analogues.** The detail of the synthesis of 5-alkyl deoxyuridines and their corresponding monophosphates has been described previously (Gauri et al., 1969; Walter & Gauri, 1975). Triphosphates were synthesized according to the method of Walter & Gauri (1975) and Moffatt (1964). Subsequently, details of the synthesis were published by Sagi et al. (1980). The purity of all analogue triphosphates was determined by high pressure liquid chromatography by Dr P. Kabus in our laboratory. Contamination by di- and monophosphates was lower than 20%.

**Preparation of activated DNA.** Activated calf thymus DNA was prepared as described previously (Pedrali-Noy & Weissbach, 1977).

**Purification of DNA polymerase.** DNA polymerase α was purified from HeLa cells as described by Spadari et al. (1974) and then further purified on single-stranded DNA–cellulose columns (Litman, 1968). The eluted DNA polymerase α had a specific activity of 22,000 U/mg. The HSV-coded virus DNA polymerases were isolated from HeLa cells infected with either HSV wild-type strain C42 or a mutant strain resistant to PFA, phosphonoacetate (PAA) and Ara-A, and were purified also up to the single-stranded DNA–cellulose column step. Their specific activity was 15,000 U/mg.

**Assay of DNA polymerase.** HeLa cell DNA polymerase α was assayed in 20 mM-potassium phosphate pH 7.2, 10 mM-MgCl₂, 1 mM-DTT, 300 μg/ml BSA, 255 μg/ml activated calf thymus DNA, 100 μM each of dATP, dCTP and [3H]dGTP (50 ct/min/pmol), and 50 μM-dTTP. In the assay for the two virus DNA polymerases potassium phosphate buffer was replaced by 50 mM-Tris–HCl pH 8.3; 250 mM-KCl was included (Weissbach, 1977) and [3H]dGTP was present at 150 ct/min/pmol. DNA polymerase was present in a concentration of 1 to 2 U per 0.3 ml assay. One unit of DNA polymerase is defined as 1 nmol of total deoxynucleotide incorporated into acid-precipitable material in 60 min at 37 °C.

The overall rate of DNA synthesis was determined by following the kinetics of incorporation of [3H]dGTP into acid-insoluble material in 0.3 to 0.5 ml assays. Samples (80 μl) were taken at the times indicated, spotted on 2.5 cm GF/C filters and batch-washed with trichloroacetic acid, pyrophosphate and alcohol as described by Bollum (1959). The background level of radioactivity on filters was always less than 200 ct/min (2 pmol of incorporated [3H]dGTP). Assays were modified, as indicated in the figure legends, by variation of the content of individual nucleoside triphosphates. The rate of [3H]dGTP incorporation in the presence of 5, 10, 20, 50, 100 or 200 μM of dTTP or dTTP analogues
Thymidylate analogue incorporation by DNA polymerase was determined, and the data obtained were used to compute $K_m$ values by drawing classical Lineweaver–Burk diagrams.

RESULTS

DNA synthesis was determined either by the incorporation of $[^3H]$dGTP or $[^3H]$dTTP. In one set of experiments (Fig. 1 and Fig. 2a, b), $[^3H]$dGTP (100 $\mu$M) was supplied together with optimal concentrations of dATP and dCTP (100 $\mu$M). In the absence of dTTP there is a low background incorporation of $[^3H]$dGTP into DNA (Weissbach et al., 1973). Inactivation of the enzyme abolishes this background incorporation. Therefore, incorporation of $[^3H]$dGTP into DNA obtained in the presence of 50 $\mu$M-dTTP was set as 100%, and in the absence of dTTP as 0%. In the other set of experiments $[^3H]$dTTP (0.05 $\mu$M) was supplied together with the other three nucleoside triphosphates (each 100 $\mu$M) either without dTTP, with 50 $\mu$M-dTTP, or with one of the listed analogue triphosphates at 50 $\mu$M.

Kinetics of DNA synthesis in the absence and presence of nucleotide analogues

DNA polymerase $\alpha$

Fig. 1 shows the rate of DNA synthesis by polymerase $\alpha$ using $[^3H]$dGTP as label. All analogues studied were incorporated to a lower extent than dTTP. In the absence of dTTP and with EtdUTP as substrate DNA synthesis proceeded at a rate 45% of DNA synthesis in the presence of dTTP. An increase in the chain length of the 5-alkyl substituent progressively decreased the incorporation of the analogues into DNA. In the presence of either n-HxdUTP or iso-PrdUTP DNA synthesis, as measured by $[^3H]$dGTP incorporation (Fig. 1), proceeded at the same low rate of only 12%. Arabinosylthymine-5′-triphosphate (Ara-TTP) suppressed the incorporation of $[^3H]$dGTP far below the level obtained in the absence of dTTP, indicating that Ara-TTP is an inhibitor of DNA polymerase activity. Substitution of dTTP by F$_3$-dTTP reduced the rate of DNA synthesis to 61%. The experiments with $[^3H]$dTTP revealed that all studied analogues clearly inhibited the incorporation of the labelled natural substrate in DNA by DNA polymerase $\alpha$, although to a much lower extent than the same quantity of unlabelled dTTP (data not shown).

HSV DNA polymerases

Kinetic studies of the incorporation of $[^3H]$dGTP in the presence of various analogues by both the wild-type and PFA$^r$ mutant polymerases are shown in Fig. 2(a, b). Again, all analogues were incorporated to a lower extent than dTTP, and an increase in the length of the alkyl side-chain progressively diminished their substrate efficiency. There were some similarities and differences in both the kinetics and in the total amount of DNA synthesized by the two enzymes in the presence of various 5-alkyl dUTP analogues. In these experiments, dTTP and all other analogues were supplied at 50 $\mu$M, which is well above the $K_m$ for dTTP, but does not take into account that the $K_m$ for some dTTP analogues is higher. Nevertheless, we thought it worthwhile to use this condition first, since the efficacy of anti-herpesvirus drugs in vivo is usually studied at similar concentrations. Experiments with $[^3H]$dTTP as label yielded results which were in accordance with the above data obtained with $[^3H]$dGTP (data not shown). Both virus enzymes were inhibited to a comparable extent by Ara-TTP under these conditions.

Differential incorporation of thymidylate analogues into DNA by DNA polymerase $\alpha$, Escherichia coli DNA polymerase, and two HSV DNA polymerases

A comparison of the data obtained with DNA polymerase $\alpha$ (Fig. 1) and the virus enzymes (Fig. 2) revealed that all alkyl dUTP analogues were more readily incorporated by the two
Fig. 1. Effect of dTTP analogues on in vitro DNA synthesis by HeLa cell DNA polymerase α. In the presence of 100 μM each of dATP, dCTP and labelled [3H]dGTP, DNA synthesis reached a basal level (○) which was set at 0% (and subtracted from the other incorporation data). When 50 μM-dTTP were added, DNA synthesis increased to a level (●) which was set as 100%. The following analogues were added instead of dTTP in a concentration of 50 μM: 5-F3-dTTP (△), 5-EtdUTP (▲), 5-n-PrdUTP (■), 5-iso-PrdUTP (●) and 5-n-HxdUTP (▽). Addition of Ara-TTP (▽) lead to a decrease of DNA synthesis below the 0% level. Addition of 1 U DNA polymerase in 400 μl led to the incorporation of 31.3 pmol [3H]dGTP in 45 min at 37 °C in an 80 μl sample (total incorporation in 400 μl by 1 U DNA polymerase is 625 pmol nucleoside triphosphates).

Fig. 2. Effect of dTTP analogues on in vitro DNA synthesis by DNA polymerases coded by (a) HSV-1 C42 (wild-type) and (b) its mutant strain resistant to PFA. In the presence of 100 μM each of dATP, dCTP and [3H]dGTP, DNA synthesis reached a basal level (○) which was set as 0%. When 50 μM-dTTP was added, DNA synthesis increased to a level (●) which was set as 100%. The following analogues were added instead of dTTP in a concentration of 50 μM: 5-EtdUTP (▲), 5-n-PrdUTP (■), 5-F3-dTTP (△), 5-n-HxdUTP (▽), 5-iso-PrdUTP (●) and Ara-TTP (▽). (a) and (b) should be compared with Fig. 1 showing similar experiments with HeLa cell DNA polymerase α. Two U DNA polymerase led to an incorporation of 1015 and 1793 pmol nucleoside triphosphates in 60 min.

virus polymerases (Fig. 3). In contrast, when DNA synthesis was measured by 3H-labelled guanylate incorporation in the presence of F3-dTTP, it was slightly higher with DNA polymerase α than with the virus enzymes. EtdUTP served as a substrate for DNA
Thymidylate analogue incorporation by DNA polymerase

![Graph showing DNA synthesis with different thymidylate analogues](image)

Fig. 3. DNA synthesis in the presence of the indicated thymidylate analogues as a percentage of DNA synthesis in the presence of dTTP. The calculation of percentage incorporation was determined after subtraction of the incorporation of [3H]dGTP in the absence of dTTP (0%, see Fig. 1) and dTTP analogues. The columns were designated as follows: α, DNA synthesis by DNA polymerase α; w, DNA polymerase derived from HSV-1 C42 (wild-type); m, DNA polymerase derived from its mutant strain resistant to PFA. The values are the average of 10 determinations comparable to those shown in Fig. 1 and Fig. 2 (α, b). Bars at the top of the columns show 68% confidence limits.

Table 1. Incorporation of [14C]EtdUTP by cellular and virus DNA polymerase*

<table>
<thead>
<tr>
<th>DNA polymerase from</th>
<th>[14C]EtdUTP incorporation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50 μM</td>
</tr>
<tr>
<td>HeLa cells (α)</td>
<td>38</td>
</tr>
<tr>
<td>HSV-1 C42 (wt)</td>
<td>100</td>
</tr>
<tr>
<td>HSV-1 C42 (PFA')</td>
<td>78</td>
</tr>
</tbody>
</table>

* The enzymic activities of the listed DNA polymerases were determined by rate of incorporation of [3H]dTTP (50 ct/min/pmol). In parallel, 14C-labelled EtdUTP was supplied instead of dTTP. Samples were taken at 0, 30, 60, 120 and 180 or 240 min and trichloroacetic acid-precipitable counts determined. Incorporation by HSV-1 strain C42 DNA polymerase at a [14C]EtdUTP concentration of 50 μM was expected to be the highest (Fig. 3) and was set at 100%.

polymerase in the absence of dTTP, but total DNA synthesis amounted to only 45% of that found with dTTP as substrate. Both the virus enzymes utilized EtdUTP almost as well as dTTP (95 and 91% respectively). The increased incorporation of EtdUTP into DNA by virus DNA polymerases, as compared to that of DNA polymerase α, was also found in studies with [2-14C]EtdUTP (Table 1).

All other alkyl dUTP analogues were less efficient than dTTP as substrates for the HSV polymerases. Again, an increase in the length of the 5-alkyl side-chain progressively diminished the rate of DNA synthesis. The difference in the rate of incorporation of dUTP analogues into DNA by the host and virus enzymes increased with the length and branching
Table 2. Relative rate of DNA synthesis*  

<table>
<thead>
<tr>
<th>Substrate</th>
<th>DNA polymerase† from HeLa cells infected with HSV-1 C42 (wt)</th>
<th>DNA polymerase† from HSV-1 C42 (PFA')</th>
<th>DNA polymeraseI† from E. coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>dATP + dCTP + [3H]dGTP</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>+ dTTP</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>+ EtddUTP</td>
<td>45</td>
<td>95</td>
<td>91</td>
</tr>
<tr>
<td>+ n-PrddUTP</td>
<td>31</td>
<td>74</td>
<td>64</td>
</tr>
<tr>
<td>+ n-HxddUTP</td>
<td>12</td>
<td>56</td>
<td>46</td>
</tr>
<tr>
<td>+ iso-PrddUTP</td>
<td>12</td>
<td>50</td>
<td>37</td>
</tr>
<tr>
<td>+ F3-dTTP</td>
<td>61</td>
<td>55</td>
<td>50</td>
</tr>
</tbody>
</table>

* DNA synthesis in the presence of dTTP was set as 100%. The calculated rates of DNA synthesis in the presence of the indicated substrates were obtained after subtraction of [3H]dTTP incorporation in the presence of only dGTP, dATP and dCTP.

† Cellular and virus polymerases were assayed as described in Methods. DNA polymerase I from E. coli MRE 600 (Kornberg polymerase) was purchased from Boehringer, Mannheim. The incubation mixture contained 66.6 mM-potassium phosphate buffer pH 7.4, 6.6 mM-MgCl2, 1 mM-2-mercaptoethanol, 65 µg/ml activated calf thymus DNA and 33.3 nM each of dATP, dCTP and [3H]dGTP (50 ct/min/pmol) and 0 or 33.3 nM of dTTP or analogue. Incubation and measurement of DNA synthesis were performed as described in Methods.

Similarly, the two virus polymerases differed in their rates of DNA synthesis when n-PrddUTP, iso-PrddUTP or n-HxddUTP replaced dTTP. The difference in the incorporation rates was significant for iso-PrddUTP, n-HxddUTP and n-PrddUTP with a probability of error of lower than 1, 2 or 5% respectively. This is also indicative of a structural change in the virus enzyme due to mutation.

Substrate properties of the dTTP analogues were also studied for E. coli polymerase I. The results were recorded and compared with those of the HSV-encoded and cellular polymerases in Table 2. It is interesting to note that the E. coli enzyme and the DNA polymerase α used the alkyl analogues with similar low efficiency, whereas the substrate efficiency for HSV enzymes was considerably higher. Sagi et al. (1980) have reported on a comparative analysis of DNA polymerase α and of E. coli polymerase I in the presence of alkyl analogues of dTTP. Our results are in complete agreement with one exception relating to n-HxddUTP. In our hands, the E. coli and the HeLa enzyme used n-HxddUTP with the same low efficiency, while Sagi et al. (1980) reported a twofold higher incorporation of n-HxddUTP by polymerase α.

Kₘ values of dTTP and analogues for cellular and virus enzymes

The Kₘ values for the individual polymerases, as summarized in Table 3, often did not show marked differences. However, the differences were consistent for dTTP and several analogues and are therefore described below. The values for dTTP for polymerase α have previously been determined to be 6 µM (Bollum, 1975), while analysis under our present conditions indicates it to be 4.5 µM. HSV-1 C42 wild-type polymerase had a higher affinity for dTTP with a Kₘ of 3 µM. The Kₘ value for the C42 PFA' enzyme was 6 µM, which is approx. twofold higher than that of the wild-type. However, the intracellular dTTP concentration in HeLa cells is 12 to 16 µM (Pedrali-Noy et al., 1980), which is in the range of the Kₘ values of all three polymerases.

The relation of the Kₘ values for the dTTP analogues for the three enzymes followed the pattern seen with dTTP. The HSV-1 C42 wild-type enzyme showed the lowest Kₘ for all substrates. Interestingly, the cellular enzyme had by far the lowest affinity for iso-PrddUTP.
Thymidylate analogue incorporation by DNA polymerase

Fig. 4. Inhibition of HeLa cell DNA polymerase α by n-HxdUTP, dATP, dCTP and [³H]dGTP were present at 100 µM, and n-HxdUTP was added at the concentrations indicated. HeLa cell DNA polymerase α was present at 2 U/0.3 ml. Each point is the average of two determinations. Analysis of the rate of incorporation yields a $K_m$ of 12 µM for n-HxdUTP.

Fig. 5. Inhibition of HeLa cell DNA polymerase α (○), HSV wild-type (●) and PFA-resistant mutant (□)-encoded DNA polymerases by Ara-TTP. In the presence of 100 µM each of dATP, dCTP and [³H]dGTP and 50 µM-dTTP, Ara-TTP was added at the concentrations indicated. $V_{max}$ is 134 (○), 63 (●), or 64 (□) pmol incorporated [³H]dGTP/h. The concentrations of Ara-TTP leading to a 50% inhibition ($K_t$) were 8.3 µM (○), 62.5 µM (●) and 87 µM (□). Each point is the average of two determinations.

Table 3. $K_m$ values of dTTP and its analogues for cellular and virus enzymes*

<table>
<thead>
<tr>
<th>Substrate</th>
<th>DNA polymerase from HeLa cells infected with</th>
<th>DNA polymerase from HSV-1 C42</th>
<th>DNA polymerase from HSV-1 C42</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HeLa cell polymerase α</td>
<td>(wt)</td>
<td>(PFA')</td>
</tr>
<tr>
<td>dTTP</td>
<td>4.5</td>
<td>2.9</td>
<td>6.2</td>
</tr>
<tr>
<td>EtldUTP</td>
<td>11.0</td>
<td>5.2</td>
<td>9.5</td>
</tr>
<tr>
<td>n-PrdUTP</td>
<td>50.0</td>
<td>37.0</td>
<td>170.0</td>
</tr>
<tr>
<td>iso-PrdUTP</td>
<td>210.0</td>
<td>7.4</td>
<td>24.0</td>
</tr>
<tr>
<td>n-HxdUTP</td>
<td>12.0</td>
<td>9.5</td>
<td>16.0</td>
</tr>
<tr>
<td>F₃-dTTP</td>
<td>12.0</td>
<td>6.9</td>
<td>26.0</td>
</tr>
</tbody>
</table>

* $K_m$ values (µM) were determined by drawing classical Lineweaver–Burk diagrams for the listed triphosphates. The counts incorporated into DNA in the absence of the listed triphosphate but in the presence of 100 µM each of dATP, dCTP and [³H]dGTP were subtracted from the [³H]dGTP incorporation found in the presence of 5, 10, 20, 50, 100 or 200 µM-dTTP or one of its analogues and 100 µM each of dATP, dCTP and [³H]dGTP at the individual time points up to 60 min.

DNA polymerase α also showed by far the lowest incorporation of [³H]dGTP in the presence of iso-PrdUTP, which agrees well with the high $K_m$ value. Although for n-PrdUTP and F₃-dTTP the largest differences of $K_m$ values were observed between the mutant and wild-type enzymes, the incorporation of [³H]dGTP into DNA by both enzymes was practically identical (Fig. 2, 3). These apparently contradictory results are not surprising if one considers that DNA synthesis is a multi-step enzymic process and that the substrate binding is not the sole rate-limiting factor.
The virus enzymes exhibited comparable $K_m$ values for n-HxdUTP and iso-PrdUTP. In contrast, the corresponding $K_m$ values for polymerase $\alpha$ differed for these substrates by a factor of 17. Nevertheless, DNA polymerase $\alpha$ incorporated $[^{3}H]dGTP$ in the presence of both analogues at the same rate. n-HxdUTP is not only a substrate but also an inhibitor of DNA polymerase $\alpha$. This conclusion is further supported by the determination of the 50% inhibition by n-HxdUTP which amounted to 235 $\mu$mol in the presence of the other natural triphosphates. Concentrations of n-HxdUTP above 20 $\mu$M progressively inhibited the incorporation of $[^{3}H]dGTP$ in the absence of dTTP into DNA by polymerase $\alpha$ (Fig. 4). The $K_m$ values for both virus enzymes were relatively similar. Despite this, the wild-type polymerase, but not the mutant enzyme, was inhibited by n-HxdUTP at a concentration over 100 $\mu$M. This is another interesting functional difference between the two virus polymerases. There is no evidence that one of the other tested alkyl dUTP analogues of $F_3$-dTTP shows such inhibition on cellular or virus polymerases in concentrations up to 200 $\mu$M.

All three enzymes incorporated $[^{14}C]EtdUTP$ into DNA (Table I). The data obtained with $[^{14}C]EtdUTP$ at a concentration of 50 $\mu$M indicate that EtdUTP was a better substrate for DNA polymerase encoded by HSV wild-type than for the mutant enzyme. Both virus polymerases incorporated EtdUTP considerably better into DNA than DNA polymerase $\alpha$ at both concentrations of 50 and 200 $\mu$M $[^{14}C]EtdUTP$. Thus, the labelling studies indicate that DNA polymerase $\alpha$ has the lowest, and that the wild-type virus enzyme the highest, affinity towards EtdUTP in accord with the kinetics of DNA synthesis in the presence of unlabelled EtdUTP.

In summary, the $K_m$ values were inversely proportional to the $[^{3}H]dGTP$ incorporation data with the following exceptions: n-HxdUTP had a lower $K_m$ value than expected for polymerase $\alpha$; n-PrdUTP had a higher $K_m$ value for both wild-type and mutant polymerases; iso-PrdUTP had a lower $K_m$ value for the wild-type enzyme than expected from the $[^{3}H]dGTP$ incorporation data. In the presence of $F_3$-dTTP mutant enzyme synthesized DNA at a higher rate than expected from its relatively high $K_m$ value. For all substrates, the mutant polymerase showed higher $K_m$ values than the wild-type polymerase. The largest difference in the $K_m$ values was found for n-PrdUTP (1:4·6), followed by $F_3$-dTTP (1:3·8), iso-PrdUTP (1:3·2), dTTP (1:2·1), EtdUTP (1:1·8), and n-HxdUTP (1:1·7).

### Inhibition of cellular and virus enzymes by Ara-TTP

In the presence of Ara-TTP $[^{3}H]dGTP$ incorporation by virus and cellular polymerases decreased below the level seen when only dATP, dCTP and dGTP were present. In this respect, Ara-TTP inhibited all polymerases to a greater extent than the alkyl deoxy analogues tested. We suggest that Ara-TTP is not merely a chain terminator but an effective inhibitor of virus and cellular polymerases.

### DISCUSSION

DNA polymerase $\alpha$ and HSV-coded DNA polymerases were isolated from HeLa cells before and after HSV infection and purified up to the DNA-cellulose column step (Litman, 1968). They showed comparable specific activity with $[^{3}H]dGTP$ or $[^{3}H]dTTP$ as label. Our results indicate that nucleosides like n-HxdU, which are not phosphorylated to triphosphates by host or by virus-induced nucleoside kinases (Bilello et al., 1981) serve as substrates for HSV and/or cellular DNA polymerases when supplied as nucleoside triphosphates. In general, the herpesvirus-specified polymerases utilize all dTTP-alkyl analogues as substrates with higher efficiencies than the cellular DNA polymerase $\alpha$. The cellular DNA polymerase $\beta$ shows similar properties in this regard as our results indicate for polymerase $\alpha$ (Sagi et al., 1980). This observation might open a new way for specific interference with HSV-directed
DNA synthesis, since HSV-infected cells become permeable to nucleotides within 2 to 3 h after infection, whereas mock-infected cells remain impermeable to these compounds (Koch et al., 1981). This view is supported by the observation that certain nucleoside analogue monophosphates show a higher anti-HSV efficacy in experimental rabbit keratitis than the corresponding nucleosides (Gauri, 1979).

Substrate efficiency of dTTP analogues decreased with increase and branching of the 5-alkyl substituents. The difference in the rate of incorporation of dUTP analogues into DNA by the host and virus enzymes increased with the length of the alkyl side-chain from 1:2.2 (EtdUTP) and 1:2.4 (n-PrdUTP) to 1:4.2 (iso-PrdUTP) and 1:4.5 (n-HxdUTP) (Fig. 3). In contrast, the rate of DNA synthesis by all the three polymerases was reduced to the same extent in the presence of F3-dTFP. The polymerases specified by HSV wild-type and a strain resistant to PFA differed considerably in some instances in the $K_m$ values for dTTP and its analogues.

For all substrates, the mutant polymerases showed higher $K_m$ values than the wild-type polymerase. In addition, the incorporation of [14C]EtdUTP into DNA was lower with the mutant than with the wild-type enzyme (Table 1). The largest difference in the $K_m$ values for the virus enzymes was found for n-PrdUTP (1:4.6), followed by F3-dTTP (1:3.8, iso-PrdUTP (1:3.2), dTTP (1:2.1), EtdUTP (1:1.8) and n-HxdUTP (1:1.7), indicating that the mutation altered both $V_{max}$ and $K_m$ of the virus polymerase. This supports the conclusion of others than the mutation has directly affected the virus DNA polymerase, since the DNA polymerase specified by the mutant strain shows 50% inhibition by PFA only in concentrations 40 times higher than with the wild-type enzyme (Erikson & Öberg, 1979). Our observation with Ara-TTP is interesting. It inhibited all polymerases. Even when all four natural triphosphates were present, DNA polymerase $\alpha$ was inhibited to 50% by 8 $\mu$M-Ara-TTP (Fig. 5). Under these conditions, 8- and 11-fold higher concentrations of Ara-TTP were required to inhibit 50% of the activity of the HSV-1 wild-type enzyme and the PFA-resistant mutant polymerase respectively (Fig. 5).

Our results of a comparative analysis of DNA polymerase and E. coli polymerase I are in complete agreement with previous data from Sagi et al. (1980), except for n-HxdUTP. This difference remains unexplained especially in the light of our finding that n-HxdUTP inhibits the DNA polymerase $\alpha$ from the HeLa cells when present in concentrations over 20 $\mu$M.

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