Phosphorylation of Arabinofuranosylthymine in Non-infected and Herpesvirus (TK+ and TK-) Infected Cells

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SUMMARY

The phosphorylation of arabinofuranosylthymine (araThd) has been studied both in non-infected cells and in those infected with herpes simplex virus (HSV-1, Lennette; HSV-1, IES and HSV-2, D-316). In these experiments, HSV strains were used which either contain (Lennette, TK+ and D-316 TK+) or lack (IES, TK-) the capacity to induce pyrimidine deoxyribonucleoside kinase. It was found that extracellularly administered araThd is phosphorylated to araTTP via araTMP and araTDP in both non-infected and in HSV-infected cells. The phosphorylating capacity is more than tenfold lower in non-infected cells than in infected cells. Interestingly, cells infected with the TK- strain have a tenfold higher phosphorylating capacity than normal, uninfected cells, a fact which might indicate that host cell deoxythymidine kinase is induced during HSV infection. AraTMP is incorporated into cellular DNA but not into HSV DNA. This finding is in contrast to observations with arabinofuranosyladenine, which is incorporated into both cellular and HSV DNA. In vitro experiments with HSV-induced DNA polymerase show that araTTP strongly inhibits the enzyme activity. Therefore we conclude that the inhibition of HSV DNA polymerase by araTTP (formed intracellularly from araThd) is the explanation for the observed antiviral activity of araThd.

INTRODUCTION

At the present stage of knowledge, experimental studies on therapy and control of herpesvirus infection, as well as transformation, must be restricted to prevention of transmission of the virus. One approach to blocking horizontal spread is by inhibiting virus growth. This can be achieved by compounds which inactivate key enzymes necessary for virus growth. However these compounds must at least be relatively selective in that they affect virus-infected cells more than uninfected host cells. Compounds which inhibit herpesvirus-induced enzymes, e.g. the DNA polymerases, would be suitable for this purpose. Recently it has been found that arabinofuranosyladenine is a highly selective inhibitor of herpes virus (review: Müller, 1979); this compound, as the triphosphate affects the herpesvirus induced DNA polymerase.

Another herpesvirus-induced enzyme, pyrimidine deoxyribonucleoside kinase, which appears to be essential for virus replication under certain conditions (Subak-Sharpe et al. 1975), was recently proposed as a target enzyme for compounds with antiviral potency (Gentry & Aswell, 1975; Miller et al. 1977). These authors found that the natural meta-
bolite of the sponge Cryptothlyta crypta (Bergmann & Feeney, 1950), 1-β-D-arabino-
furanosylthymine (araThd), is an inhibitor of herpes simplex virus (HSV) replication with
a high antiviral selectivity. This observation was explained by the finding (Kit et al. 1966)
that although araThd might not be a substrate for some mammalian cell deoxymethylidine
kinases (Kit et al. 1966), it could be phosphorylated by the HSV-induced pyrimidine
deoxyribonucleoside kinase with its broad substrate specificity (Jamieson et al. 1974). The
experimental proof for this assumption has been given by Aswell et al. 1977. However,
this explanation of the high anti-herpes activity attributed to araThd (Gentry & Aswell,
1975) must be considered with some reservation because (i) the HSV-induced pyrimidine
deoxyribonucleoside kinase is only indispensable for virus growth in serum-starved cells
but is not important for virus replication in actively growing culture cells (Jamieson et al.
1974) and (ii) araThd acts cytostatically in the uninfected L5178y mouse lymphoma cell
system at low concentrations (50% inhibition was caused by 9.8 μM; Müller et al. 1979a).

The purpose of the present studies was to elucidate the role of HSV-induced pyrimidine
deoxyribonucleoside kinase in the antiviral effect observed with araThd. It must be stated
that no study has provided any experimental data indicating that araThd acts by inhibition
of the pyrimidine deoxyribonucleoside kinase. Our experiments were performed in vivo with
non-infected as well as HSV-infected mammalian cells. Finally, the influence of araTTP on
isolated HSV-induced DNA polymerase has been determined.

METHODS

Compounds. 3H-dGTP (sp. act. 13.4 Ci/mmol) was obtained from The Radiochemical
Centre, Amersham (England); unlabelled deoxyribonucleoside triphosphates, micrococcal
nuclease, spleen phosphodiesterase and alkaline phosphatase from Boehringer, Mannheim
(Germany); GF/C filters from Whatman-Hormuth and Vetter, Heidelberg (Germany);
Nonidet NP-40 from Deutsche Shell, Hamburg (Germany); DEAE-Sephadex A-25 from
Deutsche Pharmacia, Freiburg (Germany); cellulose F (thin layer) plates from E. Merck,
Darmstadt (Germany); aquasol from NEN, Dreichenhain (Germany). Herring sperm
DNA, isolated according to Zahn et al. (1962), and araThd were gifts of H. Mack, Illertissen
(Germany).

Cells. Monolayer cultures of primary rabbit kidney cells (PRK) and baby hamster kidney
cells (BHK) were prepared as described previously (Bittlingmaier et al. 1977). The cells
were used 24 h after seeding. The cultures contained about 5% cells in S-phase (Bittlingmaier
et al. 1977).

Viruses. The HSV-1 strains, Lennette and IES, and the HSV-2 strain D-316 were used
(Bittlingmaier et al. 1977). The IES strain completely lacks pyrimidine deoxyribonucleoside
kinase activity (TK−; Falke, 1965); this was checked after each passage. The strains
Lennette and D-316 possess kinase-inducing activity (TK+; Dundaroff & Falke, 1972). In
the experiments reported below the m.o.i. was 4 to 10 p.f.u./cell. Ninety minutes after
infection the cultures were washed to remove the non-adsorbed virus and fresh medium
containing 5% calf serum was added.

The experiments to determine the extent of phosphorylation of araThd, were carried
out in Petri dishes containing about 3.5 × 10⁸ BHK cells/dish; the incorporation studies
were performed with PRK cells propagated and infected in Roux bottles (1.5 × 10⁷ cells/bottle).

Preparation of 3H-araThd and araTTP. AraThd was labelled by catalytic exchange in
solution with tritium gas, followed by a purification step on Norit (Müller et al. 1979a).
The specific radioactivity was found to be 8.6 Ci/mmol (concentration 12.5 mM). The
AraThd in herpesvirus-infected cells

material was chromatographically pure as checked by the procedure described by Pizer & Cohen (1960).

The method for obtaining araTTP used in this study has been described in detail (Müller et al. 1979b). Briefly, araThd was enzymically phosphorylated to araTMP; subsequently araTTP was chemically prepared from araTMP by the dicyclohexylcarbodiimide method.

The araTTP preparation was chromatographically pure, as determined by thin-layer (cellulose plates) chromatography with the solvent system: isobutyric acid (66), concentrated NH$_4$OH (1), water (33). The R$_p$ values in this system were 0·18 for araTTP, 0·22 for dTTP and 0·02 for UTP.

**Intracellular phosphorylation of $^3$H-araThd.** The experiments were performed in Petri dishes, each containing either non-infected or HSV-infected BHK cells (about $3·5 \times 10^6$ cells/dish) and 10 ml medium plus serum. At 3 or 8 h post infection (p.i.) 10 µl $^3$H-araThd (final concentration: 12·5 µM) were added to one Petri dish and incubation continued for 60 min at 37 °C. After incubation, the cells were harvested and subsequently washed three times with saline by centrifugation (3000 g; 3 min; 2 °C). The cellular pellet was suspended in 5 ml of 60% aqueous methanol (60 °C) and homogenized by 20 strokes in a Dounce homogenizer with a tight-fitting pestle. The methanol-insoluble material was removed by centrifugation (10000 g; 10 min; 2 °C) and the resulting extract was evaporated to dryness. The residue was analysed for araThd and its phosphorylated derivatives as described by Arendes et al. (1977) and Müller et al. (1977a) using a DEAE-Sephadex column and elution with a NH$_4$HCO$_3$ gradient. The nucleoside araThd appeared during the wash with 0·05 M-NH$_4$CO$_3$ and the nucleotides at the following molarities of bicarbonate: araTMP, 0·18; araTDP, 0·24 and araTTP, 0·32. One Petri dish gave enough cell material for the determination of the phosphorylating capacity of one type of culture (non-infected or infected with different HSV strains) at one time.

**Labelling of DNA by incubation with $^3$H-araThd.** Roux bottles containing about $1·5 \times 10^7$ non-infected or HSV-infected PRK cells in 50 ml culture fluid were supplemented with 50 µl $^3$H-araThd (final concentration 12·5 µM) at 3 or 8 h p.i. and the incubation continued for 60 min at 37 °C. After incubation the cells were harvested and the DNA was extracted.

**Extraction of DNA from cells.** The DNA was isolated from the cells by the Sarkosyl-Pronase method, purified by chloroform-butanol extraction and subsequently analysed by neutral isopycnic CsCl gradient centrifugation (Müller et al. 1977c). Centrifugation was performed in a Beckman L-2 50 B ultracentrifuge at 20 °C in a SW 40 rotor for 72 h at 120000 g; the vol. of each tube was 10 ml. Fractions (0·4 ml) were assayed for absorption at 260 nm and refractive index. A total of 50 µg DNA (corresponding to about $5·7 \times 10^6$ cells) was added to each tube. For determination of the radioactivity the entire fraction was added to 10 ml aquasol and counted.

**Digestion of $^3$H-araTMP DNA.** The DNA isolated from cells, which had been incubated with $^3$H-araThd, was enzymically hydrolysed to the nucleoside level with micrococal nuclease, spleen phosphodiesterase and alkaline phosphatase, as described elsewhere (Müller et al. 1975). Ten micrograms of araTMP DNA was digested per assay. The resulting digest (50 µl) was chromatographed on cellulose F plates. The running distance of the chromatogram was limited to 10 cm, using the isobutyric acid-ammonia solvent system of Pizer & Cohen (1960). To determine the radioactivity the chromatogram was processed as described in the ‘Miscellaneous’ section below.

**DNA polymerase preparations.** The HSV DNA polymerase was isolated from PRK cells infected with HSV-1 (strain Lennette). The cells were disrupted at 8 h p.i. and the polymerase was partially purified by sucrose density gradient centrifugation (Müller et al. 1978). To remove the sucrose, the enzyme preparation was dialysed overnight against 50 mm-K-
phosphate buffer (pH 7.5; 100 mM-(NH₄)₂SO₄; 1 mM-dithiothreitol and 0.2% Nonidet). The sp. act. of the enzyme preparation was found to be 39 nmol of deoxynucleotide incorporated into an acid-insoluble form per mg protein in 20 min at 37 °C; the protein concentration was 0.2 mg/ml.

The cellular DNA polymerase α (sp. act. 19 nmol of labelled substrate/mg protein, under the assay conditions used) and DNA polymerase β (6.3 nmol/mg protein) were isolated from rabbit kidney cells according to Rohde et al. (1975).

**Determination of DNA polymerase activity.** The reaction mixture (60 μl) for HSV-induced polymerase contained 50 μM-³H-dGTP (50 ctf/min/pmol) and 50 μmol each of dATP, dCTP and dTTP as substrates and activated DNA as template (Müller et al. 1978); 100 mM-(NH₄)₂SO₄ was included in the reaction mixture instead of 200 mM-KCl; 40 μg enzyme protein was added per assay.

The assay of cellular DNA polymerases α and β was essentially as described by Müller et al. 1977b. The substrate concentrations were adjusted to 50 μM and activated DNA was used as template. The reactions were carried out for 20 min at 37 °C (the reaction kinetics were linear during this incubation time); 50 μl portions were then processed as described (Müller et al. 1978).

**Miscellaneous.** Native DNA was activated according to Aposhian & Kornberg (1962). Protein was determined according to Lowry et al. (1951) and DNA according to Kissane & Robins (1958).

To identify araTTP thin-layer chromatography was performed in an ascending system on cellulose F plates with the following solvent mixture: isobutyric acid (66), concentrated NH₄OH(1) and water (33). To detect the radioactivity, the chromatograms (length: 10 cm) were developed and cut into strips (0.5 cm × 1.0 cm), each of which was eluted with 0.4 ml 0.1 N-HCl. For determination of the radioactivity 0.3 ml was added to 10 ml aquasol and counted in a scintillation counter.

RESULTS

**Phosphorylation of araThd in non-infected and HSV-infected cells**

The phosphorylation capacity of non-infected and HSV-infected BHK cells was determined at 3 and 8 h p.i. The reason for selecting these two incubation times was the observation from the literature that (i) at 3 h p.i. the HSV pyrimidine deoxyribonucleoside kinase starts to be induced and reaches a maximum around 8 h p.i. (Jamieson & Subak-Sharpe 1974); (ii) at 3 h p.i. the HSV-DNA polymerase is detectable and its activity reaches a maximum at 8 h p.i. (Müller et al. 1978); and (iii) the pattern of HSV DNA synthesis shows a close time-correlation with the appearance of the HSV DNA polymerase (Müller, 1978).

From the data summarized in Table 1 it is evident that in both non-infected and HSV-infected BHK cells araThd, supplied extracellularly, is phosphorylated to araTTP via araTMP and araTDP. In non-infected cells, only 5.2% of the total intracellular radioactivity derived from ³H-araThd is found in the triphosphate form. In contrast, in HSV-infected cells, around 50% of the intracellular radioactivity is found as araTTP if the pool is analysed 3 h p.i. The proportion of araTTP increases further if the HSV-infected cells are pulsed at 8 h p.i.; in this case up to 77% of the radioactivity is present as araTTP.

It should be noted that cells infected with the TK⁻ strain (HSV-1; IES) only take up the same amount of exogenous radioactivity as the controls, a figure which is about one half of that found in cells infected with the two TK⁺ strains. However, 66% of the precursor is phosphorylated to araTTP in the case of the HSV-1 (IES) infected cells while in the control only 5.2% of the endogenous radioactivity is present as araTTP.
AraThd in herpesvirus-infected cells

Table 1. Phosphorylation of $^3$H-araThd in non-infected and HSV-infected cells

<table>
<thead>
<tr>
<th>Cells</th>
<th>HSV type and strain†</th>
<th>Time (h) of addition of $^3$H-araThd (p.i.)</th>
<th>Distribution in the methanol-soluble fraction (per 10 µg DNA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-infected</td>
<td>—</td>
<td>3</td>
<td>araThd araTMP araTDP araTTP</td>
</tr>
<tr>
<td>HSV-1</td>
<td>—</td>
<td>8</td>
<td>2.7 1.8 0.67 0.034 0.14</td>
</tr>
<tr>
<td>Lennette (TK+)</td>
<td>8</td>
<td>3</td>
<td>2.6 1.9 0.45 0.036 0.12</td>
</tr>
<tr>
<td>HSV-1</td>
<td>8</td>
<td>3</td>
<td>5.2 1.2 0.24 0.53 2.5</td>
</tr>
<tr>
<td>IES (TK-)</td>
<td>8</td>
<td>8</td>
<td>6.2 0.8 0.19 0.73 4.1</td>
</tr>
<tr>
<td>HSV-2</td>
<td>8</td>
<td>3</td>
<td>2.5 0.8 0.16 0.20 1.3</td>
</tr>
<tr>
<td>D-316 (TK+)</td>
<td>8</td>
<td>8</td>
<td>3.0 0.3 0.13 0.31 2.1</td>
</tr>
</tbody>
</table>

* The cells of one Petri dish (3.5 x 10⁶) were incubated for 60 min with 12.5 µmol of $^3$H-araThd (sp. act. 18.9 x 10³ d/min/pmol) and subsequently broken and extracted with hot methanol.

† TK⁺, TK⁻ indicate the possession or lack of inducible pyrimidine deoxyribonucleoside kinase activity.

To estimate the absolute concentration of araTTP in HSV infected cells a rough conversion from pmol/10 µg DNA into pmol/10⁶ cells can be performed on the basis of a DNA content of a rabbit kidney cell of 8.7 x 10⁻¹⁸ g (Sober & Harte, 1968). Using this figure in the case of HSV-2 (D-316) 4.9 pmol of araTTP/10 µg DNA corresponds to about 4.3 pmol of araTTP/10⁶ cells. This value is relatively small in comparison with the dTTP pool size in mammalian cells, e.g. L5178y in which 32 pmol/10⁶ cells were found (Lowe et al. 1977).

**Analysis of araTTP formed in vivo**

Since some microorganisms contain an enzyme system which causes an enzymic epimerization of the 2’-hydroxyl group of ribose (Anderson & Suhadolnick, 1973), it was necessary to determine whether the phosphorylated derivate of araThd is indeed araTTP or whether it has been metabolically converted to dTTP or even to UTP. Therefore the isolated triphosphate, formed intracellularly in non-infected as well as in HSV-1-(Lennette) infected BHK cells, was analysed by thin-layer chromatography using araTTP, dTTP and UTP as reference compounds. The results (Fig. 1) clearly show that the radioactive material, eluted from the ion exchange column at the araTTP elution point, has an R₂ value corresponding to that of araTTP.

**Incorporation of araThd into DNA**

From a previous study (Müller et al. 1979a) it is known that exogenously supplied $^3$H-araThd is incorporated into the DNA of non-infected L5178y mouse lymphoma cells during DNA synthesis. In the present experiments it was also shown that in HSV-infected cells, the cellular DNA was labelled after incubation with $^3$H-araThd. The cellular DNA was separated from the HSV DNA by CsCl density gradient centrifugation (Fig. 2); the cellular DNA peak appears in the buoyant density range 1.68 to 1.69 g/ml and the HSV DNA peak in the range 1.72 to 1.73 g/ml [the maxima of the HSV DNA peaks are as follows: HSV-1 (Lennette), 1.725; HSV-1 (IES), 1.728 and HSV-2 (D-316) 1.721 g/ml]. The sedimentation characteristics of the HSV DNA were checked by marker runs with standard HSV DNA as previously described (Müller et al. 1973). On average, the cellular DNA contained a radioactivity of 1000 d/min/10 µg DNA in the controls and 450 d/min/10 µg DNA in the case of HSV-infected PRK cells (Table 2). This decrease in the specific radioactivity may reflect the known inhibition of cellular DNA synthesis by HSV infection (Müller et al. 1977c).
Fig. 1. Characterization of the araTTP product, formed in non-infected (●—●) and HSV-1 (Lennette)-infected BHK cells (○—○). The cells were harvested and extracted with hot methanol at 8 h p.i.; then the extract was analysed by ion exchange chromatography. Samples (500 µl) were taken from the araTTP fraction (1.5 ml) and evaporated to dryness in a rotary evaporator. The residue was dissolved in 50 µl distilled water and chromatographed on thin layer plates. The radioactivity of 0.5 × 1.0 cm spots was determined. The bars (■) mark the R_f values of the authentic compounds araTTP, dTTP and UTP. Further details are given under Methods.

Fig. 2. Separation of cellular DNA from HSV DNA by CsCl density gradient centrifugation. HSV-2 (D-316) infected PRK cells were incubated at 3 or 8 h p.i. with 3H-araThd for 1 h. The DNA was then isolated and centrifuged in a CsCl gradient; fractions were collected and the buoyant density (×—×) and absorbance were determined. For the determination of the radioactivity the fractions were added to 10 ml Aquasol and then counted. The results of two separate experiments are shown in this figure; 3 h incubation experiment: ——, absorbance; ●—●, radioactivity; and 8 h incubation experiment: - - -, absorbance; ○—○, radioactivity. For further details see Methods.
**AraThd in herpesvirus-infected cells**

Table 2. Incorporation of $^3$H-araTMP into DNA from non-infected and HSV-infected PRK cells*

<table>
<thead>
<tr>
<th>Cells</th>
<th>HSV type and strain</th>
<th>Time (h) of addition of $^3$H-araThd (p.i.)</th>
<th>Amount (µg)</th>
<th>Radioactivity (d/min × 10$^{-5}$)</th>
<th>Amount (µg)</th>
<th>Radioactivity (d/min × 10$^{-5}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-infected</td>
<td></td>
<td>3</td>
<td>47</td>
<td>48</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8</td>
<td>45</td>
<td>52</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>HSV-1</td>
<td></td>
<td>3</td>
<td>45</td>
<td>31</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Lennette (TK$^+$)</td>
<td></td>
<td>8</td>
<td>39</td>
<td>25</td>
<td>7.8</td>
<td>&lt; 0.1</td>
</tr>
<tr>
<td>HSV-1</td>
<td></td>
<td>3</td>
<td>42</td>
<td>13</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>IES (TK$^-$)</td>
<td></td>
<td>8</td>
<td>40</td>
<td>9</td>
<td>4.9</td>
<td>&lt; 0.1</td>
</tr>
<tr>
<td>HSV-2</td>
<td></td>
<td>3</td>
<td>49</td>
<td>36</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>D-316 (TK$^+$)</td>
<td></td>
<td>8</td>
<td>31</td>
<td>23</td>
<td>9.7</td>
<td>&lt; 0.1</td>
</tr>
</tbody>
</table>

* The DNA was isolated and cellular DNA was separated from virus DNA by CsCl density gradient centrifugation. The cellular DNA peak is in the buoyant density range 1.66 to 1.705 g/ml and the HSV DNA peak between 1.715 and 1.74 g/ml. The total amounts of the two DNA species are given as well as the total acid-insoluble radioactivity in each. The acid-insoluble radioactivity was determined as described previously (Müller et al. 1971). Other details as for Table 1.

In contrast to the incorporation into cellular DNA of radioactivity derived from $^3$H-araThd no acid-insoluble radioactivity could be detected in HSV DNA even 8 h p.i.; the period of maximum HSV DNA synthesis (Müller, 1978); see Table 2. These data show that, under the conditions used, DNA synthesis in HSV-infected cells is not completely suppressed even during the period of maximum HSV DNA synthesis.

The chemical nature of the radioactivity in cellular DNA was checked chromatographically after enzymic digestion to the nucleoside level. In these experiments more than 80% of the incorporated radioactivity was found at the $R_f$ value of araThd (Fig. 3). This finding is a strong indication that in the intact cell system araThd is indeed incorporated into cellular DNA.

**Effect of araTTP on DNA polymerases**

In Fig. 4 the response of the partially purified DNA polymerases to increasing concentrations of araTTP is shown. The experiments were performed in the presence of 50 µmol of the four normal deoxyribonucleoside triphosphate substrates. The data were plotted as concentration of araTTP (µM) versus percentage response. The concentration for 50% inhibition ($I_{50}$) was estimated by extrapolation. The DNA polymerase $\alpha$ and the HSV DNA polymerase were clearly the most sensitive enzymes ($I_{50} = 9$ µM and 13 µM respectively); in contrast, the $\beta$-polymerase was inhibited less by araTTP ($I_{50} = 38$ µM).

**DISCUSSION**

Since the discovery of arabinofuranosyladenine as an anti-herpesvirus antibiotic with high selectivity (review: Müller, 1979) a systematic search for other arabinosylnucleosides with specific anti-virus activity started. The rationale for the selection of arabinosylnucleosides for this screening programme is the finding that they occur in a different glycosidic conformation from naturally occurring nucleosides (Miles et al. 1977). From the knowledge that it is not the arabinosylnucleosides but the arabinosylnucleotides (e.g. Plunkett & Cohen, 1975) which are the active metabolites, the HSV-induced DNA polymerase seems to be a promising target enzyme for a specific inhibition of virus growth.

Stimulated by the finding of Kit et al. (1966) that araThd is not a substrate for some mammalian cell deoxycytidine kinases, Gentry & Aswell (1975) and Müller et al. (1977)
Fig. 3. Determination of the chemical nature of the incorporated radioactivity in DNA from PRK cells, infected with HSV-2 (D-316), after incubation with \(^3\)H-araThd (the label was added 8 h p.i.). Cellular DNA (10 \(\mu\)g), obtained after CsCl density gradient centrifugation (see Fig. 2), was digested and chromatographed as described in Methods. The bars (■) mark the \(R_f\) values of reference compounds.

Fig. 4. Inhibition of cellular and HSV DNA polymerases by araTTP. DNA polymerase \(\alpha\) (●—●) and \(\beta\) (○—○) were isolated from non-infected rabbit kidney cells; HSV DNA polymerase was isolated from HSV-1 (strain Lennette) infected PRK cells (×—×).
provided evidence that araThd is phosphorylated by the HSV-induced pyrimidine deoxy-
nucleoside kinase. While Gentry & Aswell (1975) assumed araThd to be a highly selective
anti-HSV compound, Miller et al. (1977) published data which indicated that araThd
inhibits dThd incorporation into the DNA of both HSV-infected cells and non-infected
cells. In addition, our earlier data demonstrate that araThd acts as a cytostatic agent in
non-infected L5178y cells (Müller et al. 1979a). With this experimental background, it was
necessary to clarify the substrate specificity of mammalian deoxythymidine kinase and
HSV deoxypyrimidine kinase with respect to araThd. We found that in both non-infected
and HSV-infected cells, araThd is phosphorylated to araTTP via araTMP and araTDP.
The phosphorylating capacity was highest with TK+ HSV strains and lower with TK−
HSV strains. However, the amount of araTTP formed with the TK− strain was still about
tenfold higher than in non-infected cells. This observation is the first indication that the
host-cell deoxythymidine kinase may be induced after HSV infection. The induction of this
enzyme during DNA synthesis is well documented only in the case of uninfected mamm-
alian cells (Roodman et al. 1976). The increase of the phosphorylating capacity in cells
infected with HSV TK+ strains during the period of HSV DNA synthesis (comparison of the
values determined 3 and 8 h p.i.), confirms the finding of Jamieson & Subak-Sharpe (1974)
that the deoxypyrimidine kinase is highly active during HSV DNA synthesis.

The data in the present study and in a preceding investigation (Müller et al. 1979a)
clearly indicate that araThd is phosphorylated not only in HSV infected cells but also in
non-infected cells. This finding, which is in contrast to the conclusions drawn by Gentry
& Powell (1975) and by Miller et al. (1977), implies that the HSV-specific kinase is not
essential for the antiviral function of araThd.

During HSV DNA synthesis exogenously applied 3H-araThd is incorporated into cellular
dNA but not into HSV DNA. This finding is in contrast to those published for arabino-
furanosyladenine which is incorporated to a large extent into HSV DNA (Müller et al.
1977c).

From the experiments described here, we have no reason to assume that the anti-HSV
activity of araThd is due to an inhibitory influence on HSV deoxypyrimidine kinase. On the
contrary, our experiments with the isolated HSV DNA polymerase indicate that a strong
inhibitory effect is exerted by araTTP. In dose response experiments, 50% inhibition of the
activity of this enzyme is achieved by 9 μM-araTTP at a substrate concentration of 50 μM.
This might indicate a high affinity of araTTP towards HSV DNA polymerase; however,
this conclusion should be interpreted with some caution, until it can be supplemented by
kinetic data. Nevertheless, at the present stage, we conclude that the inhibition of HSV
DNA polymerase by araTTP is the explanation for the antiviral activity of araThd. Similar
findings concerning the inhibition of DNA polymerase α and β and of HSV DNA poly-
merase by araATP are already published (Müller et al. 1978). Predictions about the anti-
HSV selectivity of araThd may be confirmed when exact kinetic data about the substrate
specificity of the two HSV enzymes towards araThd and its phosphorylated derivatives
become available and when more information on the influence of araThd on the growth of
TK+ and TK− HSV strains is obtained. Experiments along these lines are in progress.

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