Deoxyribonucleoside Triphosphate Pools in Herpes Simplex Type 1 Infected Cells

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SUMMARY

Deoxyribonucleoside triphosphate pools were analysed in both exponentially growing and serum starved wild type BHK C13 cells and in a derivative of this cell line which lacks both thymidine kinase and deoxycytidine kinase activities, before and after infection with herpes simplex virus. Serum starved BHK cells had low levels of all four deoxyribonucleoside triphosphates. In exponentially growing cells all pools were expanded, the pool of dCTP being largest and dGTP the smallest. The dATP and dTTP pools were of intermediate sizes. In exponentially growing deoxypyrimidine kinase free cells the pools, with respect to level and distribution, were the same as those observed in wild type cells. After infection with herpes simplex virus there were marked changes in the levels of all deoxyribonucleoside triphosphate pools; the most predominant being a 25- to 50-fold expansion of the dTTP pool. The pools of dCTP and dGTP also increased while the pool of dATP was very much reduced. These effects could be observed in both wild type and mutant cells.

INTRODUCTION

Infection of cells by herpes simplex virus type 1 (HSV-1) results in alterations in a large number of the host cell's metabolic processes (Kaplan, 1969). In particular, there is an induction of many of the enzymes involved in DNA synthesis and DNA precursor metabolism. Among those induced are: a new polymerase (Keir et al. 1966), a new exonuclease (Keir & Gold, 1963), a thymidine kinase (Kit et al. 1963) and a deoxycytidine kinase (Hay et al. 1971). More recently the latter two activities were said to reside in a new enzyme deoxypyrimidine kinase which acts to phosphorylate both thymidine and deoxycytidine (Jamieson, Gentry & Subak-Sharpe, 1974). There is also evidence for the induction of a new ribonucleotide reductase activity having different properties from that of the host cell (Cohen, 1972). In addition, there is an increase in activity of some of the pre-existing cell enzymes such as dCMP deaminase (Keir, 1968) and thymidylate kinase (Newton 1964).

The activity of many of the cell enzymes involved in DNA precursor metabolism appear to be controlled by deoxyribonucleoside triphosphates (Koerner, 1979). At least some of the enzymes in herpes virus infected cells are similarly affected by feedback inhibition; dCMP deaminase (Keir, 1968), deoxypyrimidine kinase (Jamieson & Subak-Sharpe, 1974),

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and ribonucleotide reductase (Cohen, 1972), although the effects exerted by the deoxyribo-
nucleotides in some cases may differ from those exerted on the host cell enzyme activities,
in either magnitude or type. It is of interest, therefore, to determine the size of the pools
of these nucleotides in relation to both enzyme induction and DNA synthesis in the infected
cells.

By using different cell systems, we might also obtain some information about the impor-
tance of the different host functions on deoxyribonucleotide metabolism in infected cells
and of the effect of selectively removing some of these functions.

Studies with polyoma virus have shown that during virus infection there is considerable
fluctuation of deoxyribonucleoside triphosphate pool levels which appear to be correlated
with the rate of viral DNA synthesis (Nordenskjold et al. 1970). However, it is not clear
whether the levels of the deoxyribonucleoside triphosphate pools are governed by the rate
of DNA synthesis, and/or by the activity of the enzymes involved in their synthesis, or whether
they themselves are part of a homeostatic control system which regulates their production
and utilization. Results from mammalian cell studies indicate that this form of homeostatic
regulation does not occur (Bray & Brent, 1972). In fact, expansion of certain pools does
not occur until after maximum DNA synthesis has taken place. However, it must be
remembered that these results convey static measurements of a dynamic situation and that
the regulation may involve control of supply from the pools, and may not solely depend
on the pool concentrations.

It was thought that by infecting cells with herpes simplex virus, the ensuing increase in
the rate of DNA synthesis, plus the enhanced rate of production of deoxyribonucleoside
triphosphates caused by the induction of viral enzymes, might accentuate the changes of the
deoxyribonucleoside triphosphate pools and give more information on deoxyribonucleotide
metabolism in general, as well as any specific effects due to herpes simplex virus.

METHODS

Cells. The cells used in the following experiments were either BHK C13 cells (Maepherson
& Stoker, 1962) or an analogue resistant cell line PyY/TG/CAR/BUdR cells, selected
from a polyoma virus transformed BHK cell line (Jamieson et al. 1974). The cells were
normally grown in Eagle’s medium supplemented with 10% calf serum.

Virus. Herpes simplex type 1 (HSV-1) strain 17 was isolated by Dr C. Ross and after
purification was isolated in a syncytial plaque morphology form (HSV 17 syn) by J. Subak-
Sharpe. All cell lines and virus stocks were checked for freedom from mycoplasma and
bacterial contamination by staining and were found to be free of both (Fogh & Fogh, 1964).

Production of resting cells. BHK C13 cells were seeded at a concentration of 10⁶ cells/
50 mm Petri dish in 5 ml Eagle’s medium supplemented (v/v) with 10% calf serum (EC10).
After incubation for 24 h at 37 °C the medium was removed, the cell sheet washed, 5 ml
Eagle’s medium containing 1% (v/v) calf serum (EC1) added and cells incubated for 5 to
6 days at 37 °C (Burk, 1966).

In comparison to cultures of exponentially growing BHK C13 cells in which 98% of
the nuclei synthesize DNA during a 24 hour period, the resting cell population only
contains ~ 0.5% of the cells exhibiting any DNA synthesis in the same time period as
determined autoradiographically.

Virus growth curves. 50 mm Petri dishes (Nuncelon) containing confluent monolayers of
cells were infected with virus at an input multiplicity of 10 p.f.u./cell in a total volume of
0.2 ml. After adsorption for 1 h at 37 °C, the cell sheet was washed to remove unadsorbed
Triphosphate pools in herpes infected cells

virus and 5 ml EC10 added. (In the case of resting cells the original EC1 was replaced.) At subsequent times after infection the cell sheets were suspended in the medium and virus released by ultrasonic vibration. This liberated virus was then titrated for infectivity in BHK C13 cells (Russell, 1962).

Thymidine incorporation. Cells seeded on glass coverslips in 50 mm Petri dishes were infected at a multiplicity of 10 p.f.u./cell with HSV-1 and the virus allowed to adsorb for 1 h at 37 °C. After replacement of the medium, [H]-dThd was added (2 μCi/ml) and the cultures incubated at 37 °C. At various times after infection the medium was removed and the cells washed twice with phosphate buffered saline (PBS), three times with ice cold trichloroacetic acid (10 %), three times in distilled water and finally rinsed in alcohol before drying. The coverslips were then placed in vials containing a toluene-based scintillant and the acid precipitable radioactivity measured by liquid scintillation spectroscopy. The rate of DNA synthesis at various times in the virus growth cycle was measured by pulsing the infected cells for 1 h with [H]-dThd (2 μCi/ml) at the required times. The samples were then treated as above.

Enzyme determination. Revolving Winchester bottles containing a cell sheet which had just reached confluence, i.e. containing approx. 3 × 10^8 (BHK C13), or 10^8 (PyY/TG/CAR/BUdR) cells were infected at an input multiplicity of 10 p.f.u. cell in 20 ml of EC10 and the virus allowed to adsorb at 37 °C for 1 h; 100 ml EC10 was then added and incubation continued. Eight hours after infection the cells were removed in 10 ml trypsin/versene. After all the cells had detached from the glass 1 ml of calf serum was added to neutralize the trypsin. The cells were then pelleted, washed twice in 10 ml of ice cold saline and once in 10 mM-tris HCl, pH 7.5, containing 5 mM-2-mercaptoethanol, 5 μM-thymidine and 5 μM-deoxycytidine. The cells were then resuspended in 5 ml of the same buffer and disrupted by sonicating for 4 × 10 s in a Dawe bath sonicator. The resulting solution was then centrifuged at 100000g for 1 h. The supernatant fluid was removed and used as the crude enzyme fraction. The enzyme could be stored for long periods of time at −70 °C.

The viral deoxypyrimidine kinase was measured by determining the thymidine kinase activity of the enzyme. The enzyme assay mixture contained in a final volume of 100 μl; 10 μM-[H]-thymidine (10 μCi/μmol), 10 mM-ATP, 10 mM-MgCl2, 0.02 M-Na phosphate buffer, pH 6, and 50 μl enzyme fraction. After addition of the enzyme fraction, the tubes were shaken and incubated at 37 °C for 15 min. The reaction was stopped by immersing the mixtures in a boiling water bath, followed by cooling in ice.

Normally the phosphorylated compounds were separated from the nucleoside by spotting 10 μl of the reaction mixture on Whatman DE81 paper discs, which were then washed once in 4 mM-ammonium formate + 10 μM-dThd + 10 μM-CdR at 37 °C for 7 min, once in 4 mM-ammonium formate at 37 °C for 7 min and once for 5 min in distilled water at room temperature. The discs were finally rinsed in absolute alcohol, dried and counted in a scintillation spectrometer using a toluene based scintillant (2:4 l toluene, 12:5 g PPO and 0:125 g POPOP).

Protein estimation. The protein content of the enzyme fractions was determined by the method of Lowry et al. (1951).

Isolation of deoxyribonucleoside triphosphate pools. At various times after infection of 50 mm Petri dishes of cells at a multiplicity of 10 p.f.u./cell, the medium was removed and the nucleotide pools extracted in 60 % methanol, which allows quantitative recovery of the nucleotides (Lindberg & Skoog, 1970). After 24 h at −20 °C the methanol was removed by evaporation and the residue dissolved in 200 μl 100 mM-tris/HCl, pH 8.2. The fractions were then stored at −20 °C until assayed.
DNA determination. The precipitate formed on treating the cells with 60% methanol was stored and used for DNA determination by the colorimetric method described by Burton (1956).

Pool determination. The estimation of the deoxynucleoside triphosphate pools was carried out as described by Lindberg & Skoog (1970) and Skoog (1970). The data shown were drawn from two separate sets of experiments.

RESULTS

Thymidine incorporation and enzyme induction

The time course of DNA synthesis in herpes simplex type 1 (HSV-1) infected exponentially growing BHK C13 cells, PyY/Tg/CAR/BUdR cells and resting BHK C13 cells is essentially the same, commencing 2 h after infection with total production reaching a maximum 10 h p.i. The pattern of DNA synthesis is parallel to the growth cycle of the virus but precedes it by approx. 2 h (Fig. 1). In all three systems the maximum rate of DNA synthesis occurs between 4 and 6 h p.i. (Fig. 2) and the rates of DNA synthesis are essentially the same in each case.

The induction of deoxypyrimidine kinase by HSV-1 commences about the same time as DNA synthesis and continues linearly until 8 h p.i., after which time the activity remains constant. Because of background enzyme activity in exponentially growing BHK C13 cells, the relative increase of deoxypyrimidine kinase induced in these cells appears to be less than in PyY/Tg/CAR/BUdR cells, or resting BHK 13 cells; however the total amount of enzyme activity is the same in all three cases, having a maximum value of 0.3 nmol TMP formed/min/mg protein (Fig. 2).

Deoxynucleoside triphosphate pools

After infection by HSV-1, the total deoxyribonucleoside triphosphate (dNTP) content of the cells is increased to 500% of that found before infection. Uninfected BHK C13 or
Triphosphate pools in herpes infected cells

Fig. 2. Rate of thymidine incorporation (○ ○) and deoxypyrimidine kinase activity (□ □□□) expressed as nmol thymidine phosphorylated/min/mg protein in (a) Exponentially growing BHK C13 cells, (b) PyY/TG/CAR/BUdR cells, (c) Resting BHK C13 cells after infection with wild type HSV-1 at a multiplicity of 10 p.f.u./cell.

Table 1. Deoxynucleoside triphosphate pool sizes in uninfected cells and in cells six hours after infection with herpes simplex virus type 1

<table>
<thead>
<tr>
<th>Cells</th>
<th>Virus infected at a m.o.i. of 10</th>
<th>Deoxynucleoside triphosphate pmol/μg DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>dTTP</td>
</tr>
<tr>
<td>Exp BHK*</td>
<td>—</td>
<td>6</td>
</tr>
<tr>
<td>Exp BHK</td>
<td>17 syn†</td>
<td>146</td>
</tr>
<tr>
<td>PyY/TG/CAR/BUdR</td>
<td>17 syn†</td>
<td>5</td>
</tr>
<tr>
<td>PyY/TG/CAR/BUdR</td>
<td>17 syn</td>
<td>62</td>
</tr>
<tr>
<td>Res BHK§</td>
<td>—</td>
<td>1-25</td>
</tr>
<tr>
<td>Res BHK</td>
<td>17 syn</td>
<td>22-5</td>
</tr>
</tbody>
</table>

* Exp BHK – exponentially growing BHK C13 cells.
† 17 syn – herpes simplex virus type 1 strain 17 having a syncytial plaque morphology.
‡ PyY/TG/CAR/BUdR – a polyoma transformed line of BHK cells which are resistant to thioguanine araC and BUdR.
§ Res BHK – serum starved BHK C13 cells.

BHK C13-derived cells, whether they possess thymidine kinase and deoxycytidine kinase activities or not, contain the four deoxynucleoside triphosphates in unequal amounts, but have a characteristic order of C > T > A > G. In cells which have a low rate of basal metabolism, i.e. resting cells, a similar order is observed, although the total amount of the deoxynucleoside triphosphates is much smaller (Table 1). After infection by HSV-1, all four deoxynucleoside triphosphate pool sizes are altered and a new order of T > C > G > A is established in all three cell systems.

A more detailed picture of the changes in the deoxynucleoside triphosphate pool sizes caused by HSV-1 infection can be obtained by studying the changes in each pool during the virus growth cycle.

By 2 h p.i. of exponentially growing BHK C13 cells the dTTP pool has already been expanded sixfold; it continues to increase until it reaches a maximum value of 146 pmol/μg
DNA, which is a 30-fold increase over the uninfected cell level at 6 h p.i. Thereafter the amount of dTTP diminishes until at 12 h p.i., it is reduced to 36 pmol/µg DNA, the same value as is found 2 h p.i. (Fig. 3). The dCTP pool is expanded during HSV infection, but the increase does not begin until 4 h p.i.; it continues until 8 h p.i. when a level of 40 pmol/µg DNA is reached—a fourfold increase over the level in the uninfected cells. Thereafter the amount of dCTP decreases to a value of 22 pmol/µg DNA 12 h p.i. (Fig. 3).

Unlike the other three deoxynucleoside triphosphate pools, the dATP pool size is rapidly reduced after HSV-1 infection. By 2 h p.i. the amount of dATP present is 13 pmol/µg DNA which is 50% of the level found in uninfected cells. The pool of dATP continues to be reduced in size, although at a slower rate, having a value of 0.7 pmol/µg DNA 12 h p.i. (Fig. 3).

Although the overall effect on the deoxynucleoside triphosphate pools in PyY/TG/CAR/BUdR cells caused by the HSV-1 infection is similar to that found in exponentially growing BHK C13 cells, some interesting differences are found in both the timing and magnitude of the changes (Fig. 4).

While the initial effect in exponentially growing BHK C13 cells infected with HSV-1 is
Triphosphate pools in herpes infected cells

Fig. 4. Deoxynucleoside triphosphate pool sizes expressed as pmol dNTP/μg DNA in uninfected
PYY/Tg/CAR/BUDR cells (O-----O) and in PYY/TG/CAR/BUDR cells after infection with wild
type HSV-1 at a multiplicity of 10 p.f.u./cell (● - ●). (a) dTTP, (b) dCTP, (c) dATP, (d) dGTP.

the immediate expansion of the dTTP pool and reduction in the dATP pool, the first apparent change in HSV-1 infected
PYY/TG/CAR/BUDR cells is the expansion of the
dTTP and dCTP pools.

By 4 h p.i. the dCTP pool has a value of 24 pmol/μg DNA, and continues to increase reaching a maximum of 33 pmol/μg DNA 6 h p.i. – a twofold increase over the uninfected cell level. Thereafter the amount of dCTP decreases to a level of 14 pmol/μg DNA 12 h p.i. (Fig. 4).

Significant expansion of the dTTP pool in this system does not occur until 4 h p.i. and unlike the other changes, continues throughout the infectious cycle, the dTTP pool reaching a maximal size of 100 pmol/μg DNA 12 h p.i. (Fig. 4), which is an increase of 20-fold over the uninfected cell level.

The dGTP pool is only slightly affected in this system and increases up to 6 h p.i. when it has a size of 1.6 pmol/μg DNA (Fig. 4).

As is found in HSV-1 infected exponentially growing BHK C13 cells, the dATP pool is decreased, although not so rapidly (Fig. 4). The most rapid reduction occurring between 4 and 6 h p.i. in HSV-1 infected PYY/TG/CAR/BUDR cells compared to between 0 and 2 h p.i. in HSV-1 infected BHK C13 cells. The reduction of the dATP pool again continues throughout infection, the pool being reduced to 0.8 pmol/μg DNA 12 h p.i. which is 25% of the level found in the uninfected PYY/TG/CAR/BUDR cells (Fig. 4).
Fig. 5. Deoxynucleoside triphosphate pool sexpressed as pmol NTP/µg DNA in uninfected resting BHK C13 cells (○——○) and resting BHK C13 cells after infection with wild type HSV-1 at a multiplicity of 10 p.f.u./cells (●—●). (a) dTTP, (b) dCTP, (c) dATP, (d) dGTP.

Resting BHK C13 cells which themseves have a low rate of basal metabolism and which exhibit practically no DNA synthesis have much lower levels of all four deoxynucleoside triphosphate pools than are found in either exponentially growing BHK C13 or PyY/TG/ CAR/BUDR cells. After infection with wild type HSV-1 the initial effect is an increase in the dTTP pool which continues until 6 h p.i. when it has reached a level of 25 pmol/µg DNA—a 20-fold increase over the uninfected cell level. Thereafter the size of the dTTP pool decreases until at 12 h p.i. it has a value of 5 pmol/µg DNA (Fig. 5). Thus, like the dTTP pool in HSV-1 infected exponentially there is an induction then depletion.

The dCTP and the dGTP pools are not expanded over the same time scale as the dTTP pool, i.e. between 0 and 6 h p.i., but both reach a maximum at 8 h p.i. although the final sizes of these pools are vastly different (Fig. 5). The dCTP pool reaches a maximum size of 3.7 pmol/µg DNA while the maximum size of the dGTP pool is much smaller, 0.6 pmol/µg DNA. Although the dATP pool of resting BHK C13 cells is only about one sixth of the size found in exponentially growing BHK Cells, HSV-1 infection reduces this pool still further to a size of 0.2 pmol/µg DNA, between 0 and 6 h p.i. (Fig. 5).

The deoxypyrimidine nucleoside triphosphates make up approx. 80% of the total deoxynucleoside triphosphate content of the uninfected cells. During HSV-1 infection there is a steady increase in the proportion of deoxypyrimidine triphosphates until they constitute between 96% and 98% of the total deoxynucleoside triphosphate complement (Table 2) regardless of the type or state of the cell infected. As infection progresses there is a change of
Triphosphate pools in herpes infected cells

Table 2. Deoxypyrimidine nucleoside triphosphate content of HSV-1 infected cells as percent of total deoxyribonucleoside triphosphate

<table>
<thead>
<tr>
<th>Time p.i. (h)</th>
<th>Exp BHK*</th>
<th>Exp BHK + 17 syn†</th>
<th>PyY/TG/CAR/BUdR‡</th>
<th>PyY/TG/CAR/BUdR + 17 syn</th>
<th>Res BHK§</th>
<th>Res BHK + 17 syn</th>
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<tbody>
<tr>
<td>Exp BHK</td>
<td>84.0</td>
<td>83.3</td>
<td>82.0</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Exp BHK + 17 syn†</td>
<td>95.0</td>
<td>98.1</td>
<td>98.0</td>
<td>98.4</td>
<td>96.0</td>
<td>--</td>
</tr>
<tr>
<td>PyY/TG/CAR/BUdR‡</td>
<td>84.8</td>
<td>77.5</td>
<td>79.4</td>
<td>83.9</td>
<td>90.7</td>
<td>--</td>
</tr>
<tr>
<td>PyY/TG/CAR/BUdR + 17 syn</td>
<td>77.8</td>
<td>90.0</td>
<td>96.5</td>
<td>96.8</td>
<td>98.2</td>
<td>--</td>
</tr>
<tr>
<td>Res BHK§</td>
<td>85.5</td>
<td>84.9</td>
<td>80.7</td>
<td>85.5</td>
<td>79.9</td>
<td>--</td>
</tr>
<tr>
<td>Res BHK + 17 syn</td>
<td>87.9</td>
<td>96.0</td>
<td>96.7</td>
<td>95.6</td>
<td>95.4</td>
<td>--</td>
</tr>
</tbody>
</table>

* Exp BHK – exponentially growing BHK C13 cells.
† 17 syn – herpes simplex virus type 1 strain 17 having a syncytial plaque morphology.
‡ PyY/TG/CAR/BUdR – a polyoma transformed line of BHK cells which are resistant to thioguanine araC and BUdR.
§ Res BHK – serum starved BHK C13 cells.

Table 3. Distribution of individual deoxynucleoside triphosphates as percent of total dNTP content before and after infection with HSV-1

<table>
<thead>
<tr>
<th>Time p.i. (h)</th>
<th>dTTP</th>
<th>dATP</th>
<th>dCTP</th>
<th>dGTP</th>
</tr>
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<tr>
<td>Exp BHK</td>
<td>24.0</td>
<td>23.8</td>
<td>24.27</td>
<td>--</td>
</tr>
<tr>
<td>PyY/TG/CAR/BUdR</td>
<td>15.4</td>
<td>24.8</td>
<td>18.9</td>
<td>21.9</td>
</tr>
<tr>
<td>Res BHK</td>
<td>31.0</td>
<td>23.6</td>
<td>31.8</td>
<td>41.9</td>
</tr>
<tr>
<td>Exp BHK + 17 syn†</td>
<td>73.9</td>
<td>87.6</td>
<td>86.7</td>
<td>75.0</td>
</tr>
<tr>
<td>PyY/TG/CAR/BUdR + 17 syn</td>
<td>23.7</td>
<td>52.1</td>
<td>63.0</td>
<td>81.0</td>
</tr>
<tr>
<td>Res BHK + 17 syn</td>
<td>70.6</td>
<td>80.0</td>
<td>83.7</td>
<td>76.7</td>
</tr>
</tbody>
</table>

* Time of maximal rate of viral DNA synthesis.

the dominant triphosphate form from dCTP to dTTP. This changeover occurs extremely rapidly in HSV-1 infected exponentially growing and resting BHK cells, being completed by 2 h p.i., while in HSV-1 infected PyY/TG/CAR/BUdR cells it does not occur until 4 h p.i. Thereafter any increase in the dTTP pool is synonymous with an increase in the dCTP pool and vice versa. This correlation results from changes in the sizes of both the deoxypyrimidine pools.

Table 3 shows that although there are minor differences in the changes of the deoxynucleoside triphosphates in relation to one another, between the different cell types after infection, the general trends are the same in each case. At the time of maximal viral DNA synthesis, 6 h p.i., the dominant pool (dTTP) makes up 83 to 86% of the total deoxynucleoside triphosphate in BHK cells and 63% in PyY/TG/CAR/BUdR cells while the smallest pool is dATP, only comprising between 0.4 and 1.6% of the total.

Although BHK C13 cells and PyY/TG/CAR/BUdR cells contain DNA with a G+C con-
Table 4. % G+C content of deoxynucleoside triphosphate pools in HSV-I infected cells

<table>
<thead>
<tr>
<th>Time (p.i.)</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>12</th>
</tr>
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<tbody>
<tr>
<td>Exp BHK</td>
<td>64</td>
<td>63</td>
<td>63</td>
<td></td>
<td></td>
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<tr>
<td>Exp BHK + 17 syn</td>
<td>23</td>
<td>11</td>
<td>13</td>
<td>25</td>
<td>39</td>
</tr>
<tr>
<td>PyY/TG/CAR/BUdR</td>
<td>73</td>
<td>56</td>
<td>64</td>
<td>63</td>
<td>79</td>
</tr>
<tr>
<td>PyY/TG/CAR/BUdR + 17 syn</td>
<td>58</td>
<td>40</td>
<td>35</td>
<td>18</td>
<td>14</td>
</tr>
<tr>
<td>Res BHK</td>
<td>60</td>
<td>67</td>
<td>55</td>
<td>49</td>
<td>48</td>
</tr>
<tr>
<td>Res BHK + 17 syn</td>
<td>22</td>
<td>19</td>
<td>15</td>
<td>22</td>
<td>23</td>
</tr>
</tbody>
</table>

content of 41%, the dGTP+dCTP content of these cells is in the order of 60%. After HSV-I infection the dominant species of DNA being synthesized has a G+C ratio of 67% and there is a reduction of the dGTP+dCTP ratio in the infected cells to approx. 20% (Table 4). The reduction of dGTP+dCTP content of the cells is extremely rapid, and may reflect both the changes in the balance of the enzymes available for deoxynucleoside triphosphate synthesis, and in the withdrawal rates from the pools in order to cope with synthesizing DNA having a different base composition, after virus infection.

DISCUSSION

Cells which lack the enzymes thymidine kinase and deoxycytidine kinase (PyY/TG/CAR/BUdR cells) contain the same level of deoxypyrimidine triphosphates as those which possess these activities. This indicates that either these ‘salvage’ enzymes are not normally used to supply the triphosphate pools or that the cells which lack them compensate for the loss by having an increased de novo production of deoxyribonucleoside triphosphate. It may be inferred that PyY/TG/CAR/BUdR cells compensate for their loss of the deoxypyrimidine kinases by having an increased supply of deoxypyrimidine nucleotides via the enzyme ribonucleotide reductase.

In cells which do possess the ‘salvage’ enzymes but which have a low rate of basal metabolism, the activities of these enzymes plus that of enzymes involved in de novo deoxynucleotide synthesis are extremely low. Resting BHK C13 cells have much lower levels of all four deoxyribonucleoside triphosphates than do normally-growing cells. The reasons for this are probably twofold. Firstly, the low level of metabolizing enzymes and secondly, the minimal demand made for deoxyribonucleoside triphosphates because of the very low rate of DNA synthesis.

Our experiments with herpes simplex virus clearly show that virus infection causes major changes in the levels of all four deoxyribonucleoside triphosphate pools. In all three cell systems the major change is the vast increase in the dTTP pool. This appears to be mainly due to induction of the HSV deoxypyrimidine kinase, but not solely as there are definite differences between the three different host cell systems studied. The increase in the dTTP content follows the same time course as the increase in rate of DNA synthesis. In the case of the BHK C13 cells this reaches a maximum at the same time as the rate of DNA synthesis then falls off. In HSV infected PyY/TG/CAR/BUdR cells there is no depletion of the TTP pool late in infection.

In HSV infected PyY/TG/CAR/BUdR, the increase in the dTTP pool is somewhat delayed compared to the HSV infected BHK C13 cell systems. This may well be a consequence of the fact that there are no detectable cell pyrimidine deoxynucleoside kinases in
the mutant cells even after infection (Jamieson et al. 1974). Thus, the initial increase in the
dTTP pool size after infection may be the result of an induction of the host cell activities
which in HSV infected PyY/TG/CAR/BuD cells does not occur because the de novo
pathway is already working maximally. An additional contribution is that cells lacking
salvage enzymes excrete more nucleotides into the extracellular medium than do wild type
cells (Chan, Meuth & Green 1974).

The increase of dCTP pool either precedes (PyY/TG/CAR/BuD cells) or follows
(exponentially growing and resting BHK C13 cells) the increase in dTTP. We think the
induction of a new ribonucleotide reductase by HSV, having less sensitivity to feedback
inhibition by TTP, as described by Cohen (1972) to be the primary cause of the dCTP pool
increase. That this occurs earlier in PyY/TG/CAR/BuD cells than in the BHK C13 cell
system may be a result of a lower demand on dCDP for the synthesis of thymidine nucleo-
tides allowing the majority of dCDP to be available for dCTP synthesis.

The dGTP pool of HSV infected cells is increased along with the dTTP pool, this is similar
to the situation in synchronized cells moving into S phase (Bjursell & Reichard, 1973), i.e.
dTTP stimulates GDP reduction.

In all cases there is a depletion of the dATP pool coincident with the increase in dTTP.
The situation concerning the dATP pool in HSV infected cells is difficult to correlate with
the changes in the other three deoxyribonucleoside triphosphate pools. This is the only
pool which is depleted throughout HSV infection. The reduction in the size of the dATP
pool occurs rapidly during the initial stages of infection. There are a number of reasons
why this may occur. It may be that this pool has a much higher turnover rate than the other
three dNTP pools and that early in infection the rate of withdrawal from the pool exceeds
the rate of dATP synthesis, while the rates of production of dTTP, dCPT and dGTP exceed
the rates of utilization. Also the ribonucleotide reductase induced during HSV infection
although being less sensitive to dTTP inhibition for the reduction of CDP than the host
enzyme, might be sensitive to triphosphate inhibition for the reduction of dATP.

The amounts of dATP and dTTP required for DNA synthesis are equal as are the
amounts of dGTP and dCPT. The vast differences in the four dNTP pool sizes may reflect
the fact that the DNA polymerase might have different affinities for the four substrates
necessitating different concentrations to allow equal utilization. Also, the four pools may
have different turnover rates governed by the enzymes involved in production plus the
demand made for DNA synthesis. The fact that the sizes of the dTTP, dGTP and dCPT
pools reach a maximum and then are reduced is probably due to a change in turnover rate
of these pools as infection progresses, ending up with the situation where the rate of
utilization is greater than the rate of production.

The vast concentration of dTTP which accounts for 85% of the total deoxyribonucleoside
triphosphate complement of the infected cell may not solely be required for DNA synthesis,
but may have some role in controlling metabolic processes, such as switching off host ribo-
nucleotide reductase and dCMP deaminase activities which are sensitive to dTTP inhibition.
At later times in infection this inhibition would not be required, and therefore the size of the
dTTP pool could be allowed to diminish. This reduction of dTTP might then reflect itself
in the lowering of the dGTP and dCPT pools.

Herpes simplex virus type 1 has a G+C content of 67% and therefore requires more
dCPT and dGTP in relation to dATP and dTTP, than do the host cells. This is not apparent
from the relative sizes of the deoxyribonucleoside triphosphate pools. The G+C content
of the deoxynucleotide pool population after infection is in fact decreased from 63% to
12%. This is partly a consequence of the large amount of dTTP synthesis but may also be
controlled to some extent by the different demands made by the necessities of viral DNA synthesis.

The data presented here indicate that the normal balance of dNTPs in mammalian cells is drastically altered after infection with HSV. The changes in the dNTP pool sizes support the possibility of a new ribonucleotide reductase activity after infection such that high levels of dTTP reduces ADP reduction but stimulates CDP and GDP reduction. The results also suggest an important role for dTTP in the control of DNA precursor production.

It should be stressed that while the information given in this report does to a certain extent elucidate nucleotide metabolism in herpes infected cells, it simply measures fixed sizes of the pools at different times of infection and gives no information on the dynamics of the processing of these pools. It does, however, suggest a great deal of importance to precursor pools in DNA synthesis and regulating processes.

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REFERENCES


Triphosphate pools in herpes infected cells


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