Deoxyribonucleoside Triphosphate Pools in Cells Infected with Deoxypyrimidine Kinaseless Herpes Simplex Virus

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SUMMARY

Deoxyribonucleoside triphosphate pools were analysed after infection of cells with mutant herpes simplex virus which lacks the ability to induce the enzyme deoxypyrimidine kinase. After infection of exponentially growing BHK C13 cells, an increase in all four dNTP pools was observed. However, after infection of cells which themselves cannot incorporate exogenous pyrimidine deoxynucleosides only the purine deoxynucleoside triphosphate pools increased in size.

In a system which is non-permissive for virus infection, i.e. resting BHK C13 cells which have been infected with dPyK− HSV-1, there is an increase in all dNTP pool sizes except for dTTP.

A comparison of the changes in dNTP pool sizes after infection with either wild type or dPyK− mutant HSV suggests an important role for dTTP in the control of both the production of the other DNA precursors and of viral DNA synthesis.

INTRODUCTION

In the preceding paper it has been shown (Jamieson & Bjursell, 1975) that after herpes simplex virus infection there are dramatic changes in the sizes of all four deoxynucleoside triphosphate pools. These changes are both complicated and diverse. It is likely that the observed changes reflect both the increased rate of DNA synthesis, and the increased availability of enzymes involved in the synthesis of these pools in the infected cells.

It seems possible that using virus which is deficient in the production of enzymes involved in DNA precursor metabolism, one might be able to determine the roles played in the expansion of the deoxynucleoside triphosphate pools by the various influencing factors.

Whereas the thymidine phosphorylating ability of the herpes simplex virus-induced deoxypyrimidine kinase activity can be detected directly by the incorporation of labelled thymidine into acid precipitable material, the deoxycytidine phosphorylating function cannot be detected in this manner. This has been shown to be due to some blockage in the metabolism of the dCMP formed by the herpes deoxypyrimidine kinase such that it cannot be phosphorylated to dCTP and thus used for DNA synthesis (Jamieson & Subak-Sharpe, 1976).

The induction of deoxypyrimidine kinase by herpes simplex virus seems under normal conditions to be an unnecessary viral function except when the infected cells have a low rate of basal metabolism (Jamieson, Gentry & Subak-Sharpe, 1974). That is, mutant virus selected for loss of the ability to induce this enzyme grows as well as wild-type virus except in resting cells.

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Polyoma virus, although not coding for enzymes involved in DNA precursor metabolism, does induce a number of the cellular enzyme activities (Green, 1970). Among those activities induced are thymidine kinase (Kit et al. 1965), deoxycytidine kinase (Kara & Weil, 1967), TMP synthetase and dihydrofolate reductase (Green, 1970).

After infection with polyoma virus both the dATP and dTTP pools are expanded (Skoog, Nordenskjöld & Lindberg, 1970). These results indicate that the main factor in establishing deoxynucleoside triphosphate pools in polyoma-infected cells is more likely to be the onset of viral DNA synthesis than the induction of enzymes involved in the metabolism of the precursors of the deoxynucleoside triphosphates. Utilizing mutant virus lacking the ability to induce the deoxynucleoside kinase, the relative contributions of virus induced and cell-coded de novo and salvage pathways in the establishment of the pools of DNA precursors can be established.

This report plus the findings of the preceding paper (Jamieson & Bjursell, 1975), together give some indication as to the interdependence of DNA synthesis, precursor pool size and the activity of enzymes of DNA metabolism, and perhaps as to the mechanism involved in the control of DNA synthesis in virus-infected cells.

METHODS

Cells. The cells used in the following experiments were either BHK 21/C13 cells (Macpherson & Stoker, 1962) or an analogue resistant cell line PyY/TG/CAR/BUdR cells, selected from a polyoma-transformed BHK cell line (Jamieson et al. 1974). The cells were normally grown in Eagle's medium supplemented with 10% calf serum.

Virus. Deoxypyrimidine kinaseless (dPyK-) mutant herpes simplex virus (HSV) was isolated as described previously (Jamieson et al. 1974) by selecting progeny virus resistant to the base analogue cytosine arabinoside.

Production of resting cells. BHK C13 cells were seeded at a concentration of 10^6 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 (v/v) 1% calf serum (EC1) added and cells incubated for 5 to 6 days at 37°C (Burk, 1966).

In comparison with exponentially growing BHK C13 cells which have 98% of the nuclei synthesizing DNA during a 24 h period, the resting cell population only contains <0.5% of the cells exhibiting any DNA synthesis over a similar time period, as determined autoradiographically.

Isolation of deoxyribonucleoside triphosphate pools. At various times after infection of 50 mm Petri dishes of cells at an m.o.i. of 10 p.f.u./cell, the medium was removed and the nucleotide pools extracted in 60% methanol. 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 till 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 earlier (Lindberg & Skoog, 1970; Skoog, 1970).
Changes in dNTP pools caused by dPyK⁻ HSV

RESULTS

After infection of exponentially growing BHK cells with dPyK⁻ HSV there is a threefold increase in the total amount of deoxynucleoside triphosphate between 2 and 8 h post infection (p.i.). A similar increase is observed in the total dNTP content after infection of resting BHK C13 cells between 2 and 8 h p.i. However, after infection of PyY/TG/CAR/BUdR cells with dPyK⁻ HSV the total deoxynucleoside triphosphate content decreases (Fig. 1). The data for wild-type HSV-infected cells is included for comparison.

Unlike wild-type HSV, which after infection causes a rearrangement of the relationship between the four triphosphates, in dPyK⁻ cells, at the time of maximal viral DNA synthesis, the relative amounts of the four deoxynucleoside triphosphates are the same as are found in the uninfected cells, C > T > A > G (Table 1).

The initial effect of dPyK⁻ HSV on the deoxynucleoside triphosphate pools of exponentially growing BHK C13 cells is an increase in the dATP pool, which occurs between 0 and 6 h p.i. having a maximum size of 7-8 pmol/μg DNA – a threefold increase over the uninfected cell level. Accompanying this is a stimulation in the size of the dCTP and dGTP pools, the dCTP pool reaching a maximum size of 27-5 pmol/μg DNA 6 h p.i.; the dGTP pool, on the other hand, continues to increase throughout infection, reaching a maximum size of 3-3 pmol/μg DNA (Fig. 2). Although the virus does not possess the ability to induce a thymidine kinase activity, the dTTP pool is expanded after infection between 2 and 8 h, reaching a maximum size of 40 pmol/μg DNA. Thereafter, like the dATP and dCTP pools, the size of the dTTP pool decreases (Fig. 2).

A different pattern of changes in pool size is observed after infection of PyY/TG/CAR/BUdR cells with dPyK⁻ HSV. There is no increase in the dCTP pool, in fact the size of this pool decreases progressively during infection (Fig. 3). There is only a very small expansion of the dTTP pool, this having a maximum size of 10 pmol/μg DNA 6 h p.i. – twice the level found in uninfected cells. The dGTP pool size is essentially unaffected, being similar to that of the uninfected cell throughout infection (Fig. 3). The dATP pool on the other hand is expanded to a similar extent as is found in dPyK⁻ HSV-infected exponentially growing BHK21 C13 cells, although in this case it reaches a maximum size of 8.8 pmol/μg DNA.
Table 1. Deoxyribonucleoside triphosphate content of cells infected with dPyK− HSV 6 h p.i.

<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>Exp BHK*</td>
<td>—</td>
<td>dTTP 12.5 dCTP 7.8 dATP 1.85 dGTP 0.9</td>
</tr>
<tr>
<td>Exp BHK 17 dPyK†</td>
<td>26</td>
<td>dTTP 27.5 dCTP 7.8 dATP 1.85 dGTP 0.9</td>
</tr>
<tr>
<td>PyY/TG/CAR/BUdR‡</td>
<td>7</td>
<td>dTTP 15 dCTP 4.4 dATP 0.95 dGTP 1.1</td>
</tr>
<tr>
<td>PyY/TG/CAR/BUdR</td>
<td>10</td>
<td>dTTP 10 dCTP 6 dATP 1.06 dGTP 0.24</td>
</tr>
<tr>
<td>Res BHK§</td>
<td>1.3</td>
<td>dTTP 2 dCTP 0.4 dATP 2.6 dGTP 1.06</td>
</tr>
<tr>
<td>Res BHK 17 dPyK−</td>
<td>2.5</td>
<td>dTTP 4.1 dCTP 2.6 dATP 1.06 dGTP 0.24</td>
</tr>
</tbody>
</table>

* Exp BHK – exponentially growing BHK C13 cells.
† 17 dPyK− – mutant HSV type 1 strain 17, lacking the ability to induce deoxypyrimidine kinase activity.
‡ PyY/TG/CAR/BUdR – a polyoma-transformed BHK cell line which is resistant to the base analogues, thioguanine, araC and BUdR.
§ Res BHK – resting BHK C13 cells.

Fig. 2. Deoxynucleoside triphosphate pool sizes expressed as pmol dNTP/μg DNA in exponentially growing BHK C13 cells (○——○) and in exponentially growing BHK C13 cells after infection with dPyK− HSV at a multiplicity of 10 p.f.u./cell (●——●). (a) dTTP, (b) dCTP, (c) dATP, (d) dGTP.
Changes in dNTP pools caused by dPyK- HSV

Changes in dNTP pools caused by dPyK- HSV

2 h earlier, i.e. at 4 h p.i. Thereafter, the dATP pool size decreases to a level which is less than is found in uninfected cells (Fig. 3).

dPyK- HSV infection of resting BHK C13 cells produces no progeny virus (Jamieson et al. 1974). All of the deoxynucleoside triphosphate pools except dTTP are markedly stimulated over the uninfected cell levels, which are small because of the low rate of basal metabolism (Fig. 4). The initial effect of dPyK- HSV infection is an expansion of the dATP pool which continues throughout infection, reaching a maximum size of 3.4 pmol/µg DNA. The dGTP pool is expanded between 2 and 8 h p.i., when it has obtained the maximum value of 1.24 pmol/µg DNA. The dCTP pool is expanded over a similar time scale, although the final size at 8 h p.i. is much larger, being 6.4 pmol/µg DNA. Between 8 and 12 h p.i. the total dCTP content decreases to a level similar to that found in uninfected resting cells (Fig. 4). The dTTP pool is only slightly stimulated, having a value of 4.5 pmol/µg DNA 8 h p.i., which is a threefold increase over the uninfected cell level (Fig. 4a).

The deoxypyrimidine triphosphates comprise 80 % of the total dNTP content of uninfected cells, and on infection with wild-type HSV the proportion of deoxypyrimidines increases from 96 to 98 % (Jamieson & Bjursell, 1975). Such a change does not occur after dPyK- HSV infection of any of the cell types used (Table 2), the deoxypyrimidine content...
Fig. 4. Deoxynucleoside triphosphate pool sizes expressed as pmol dNTP/μg DNA in uninfected resting BHK C13 cells (○) and after infection with dPyK- HSV at a multiplicity of 10 p.f.u./cell (●). (a) dTTP, (b) dCTP, (c) dATP, (d) dGTP.

Table 2. Deoxypyrimidine triphosphate content of HSV dPyK- infected cells as percentage of total deoxyribonucleoside triphosphate

<table>
<thead>
<tr>
<th>Time p.i. (h)</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exp BHK*</td>
<td>84</td>
<td>83</td>
<td>82</td>
<td>83</td>
<td>85</td>
</tr>
<tr>
<td>Exp BHK+17dPyK†</td>
<td>81.4</td>
<td>80</td>
<td>84.7</td>
<td>89.3</td>
<td>85.1</td>
</tr>
<tr>
<td>PyY/TG/CAR/BUdR‡</td>
<td>85</td>
<td>77</td>
<td>80</td>
<td>82.9</td>
<td>90</td>
</tr>
<tr>
<td>PyY/TG/CAR/BUdR+17dPyK-</td>
<td>78.3</td>
<td>65</td>
<td>73.8</td>
<td>85</td>
<td>81.7</td>
</tr>
<tr>
<td>Res BHK§</td>
<td>85.5</td>
<td>84.9</td>
<td>81</td>
<td>86.4</td>
<td>80</td>
</tr>
<tr>
<td>Res BHK+17dPyK-</td>
<td>86.4</td>
<td>69</td>
<td>64</td>
<td>73</td>
<td>57</td>
</tr>
</tbody>
</table>

* Exp BHK – exponentially growing BHK C13 cells.
† 17dPyK- – mutant HSV type 1 strain 17, lacking the ability to induce deoxypyrimidine kinase activity.
‡ PyY/TG/CAR/BUdR – a polyoma-transformed BHK cell line which is resistant to the base analogues, thioguanine, araC and BUdR.
§ Res BHK – resting BHK C13 cells.
Changes in dNTP pools caused by dPyK− HSV

Table 3. % G+C content of deoxynucleoside triphosphate pools in HSV dPyK−-infected cells

<table>
<thead>
<tr>
<th>Time p.i. (h)</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exp BHK*</td>
<td>64</td>
<td>63</td>
<td>63</td>
<td>62</td>
<td>64</td>
</tr>
<tr>
<td>Exp BHK + 17dPyK†</td>
<td>55</td>
<td>45</td>
<td>46</td>
<td>32</td>
<td>43</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 + 17dPyK−</td>
<td>60</td>
<td>47</td>
<td>73</td>
<td>49</td>
<td>56</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 + 17dPyK−</td>
<td>72</td>
<td>59</td>
<td>60</td>
<td>51</td>
<td>34</td>
</tr>
</tbody>
</table>

* Exp BHK – exponentially growing BHK C13 cells.
† 17dPyK− – mutant HSV type I strain 17, lacking the ability to induce deoxypyrimidine kinase activity.
‡ PyY/TG/CAR/BUdR – a polyoma-transformed BHK cell line which is resistant to the base analogues, thioguanine, araC and BUdR.
§ Res BHK – resting BHK C13 cells.

Table 4. Changes in deoxynucleoside triphosphate pools after infection with wild-type and dPyK− mutant HSV, in relation to the enzymes available in the infected cell

<table>
<thead>
<tr>
<th>Enzymes available*</th>
<th>Cell red.</th>
<th>Cell TK</th>
<th>Cell dCK</th>
<th>Viral red.</th>
<th>Viral TK</th>
<th>Viral dCK</th>
<th>dTTP (pmol/μg DNA)</th>
<th>dCTP</th>
<th>dATP</th>
<th>dGTP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exponentially growing BHK</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>6</td>
<td>11</td>
<td>4.1</td>
<td>0.75</td>
</tr>
<tr>
<td>+ 17 syn HSV</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>6</td>
<td>11</td>
<td>4.1</td>
<td>0.75</td>
</tr>
<tr>
<td>+ dPyK− HSV</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>6</td>
<td>11</td>
<td>4.1</td>
<td>0.75</td>
</tr>
<tr>
<td>PyY/TG/CAR/BUdR</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>6</td>
<td>11</td>
<td>4.1</td>
<td>0.75</td>
</tr>
<tr>
<td>+ 17 syn HSV</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>6</td>
<td>11</td>
<td>4.1</td>
<td>0.75</td>
</tr>
<tr>
<td>+ dPyK− HSV</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>6</td>
<td>11</td>
<td>4.1</td>
<td>0.75</td>
</tr>
<tr>
<td>Resting BHK</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>6</td>
<td>11</td>
<td>4.1</td>
<td>0.75</td>
</tr>
<tr>
<td>+ 17 syn HSV</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>6</td>
<td>11</td>
<td>4.1</td>
<td>0.75</td>
</tr>
<tr>
<td>+ dPyK− HSV</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>6</td>
<td>11</td>
<td>4.1</td>
<td>0.75</td>
</tr>
</tbody>
</table>

* Cell red. – host cell ribonucleotide reductase; Viral red. – virus induced ribonucleotide reductase; TK – thymidine kinase activity; dCK – deoxycytidine kinase activity.
† , increase in pool size; =, no significant change in pool size, e.g. ×30, thirty-fold increase; †, decrease in pool size.

being similar to that found in the uninfected cells. In the case of dPyK−-infected resting BHK C13 cells the deoxypyrimidine content decreases to as little as 62% of the total.

As is found after wild-type HSV infection, there is a progressive reduction in the dCTP + dGTP pool content in the infected cells (Table 3). It should be noted however, that while wild-type virus infection results in a G+C triphosphate pool content of < 14%, the dPyK− virus only reduces the G+C pool content from 60% to 40%. Since both wild-type and mutant virus have DNA with the same G+C content, the differences in the dCTP + dGTP pool sizes found cannot be primarily due to withdrawal demands made by viral DNA synthesis, but rather reflect the differences in the rates of synthesis of the DNA precursors.
DISCUSSION

Wild-type and dPyK− mutant herpes simplex virus, while both growing equally well in cells—whether or not the cells possess the enzymes thymidine kinase—exert different influences on the establishment of deoxynucleotide pools. A summary of the changes in deoxynucleoside triphosphate pool sizes at the time of maximum viral DNA synthesis, caused by wild-type and dPyK− HSV in different host cell systems, is given in Table 4 where the changes in pool sizes are correlated with the different combinations of cell and virus enzyme activities available.

In general the changes that occur during dPyK− HSV infection in the deoxynucleoside triphosphate pools are less severe than those caused by the wild-type virus.

The dPyK− mutant virus, although lacking the ability to induce the enzyme deoxypyrimidine kinase, still causes an expansion of both the dCTP and dTTP pools in exponentially growing cells. However, the increase in dTTP is only 17% of that caused by wild-type virus at this point in infection. It should be noted, however, that in dPyK− virus-infected cells the maximum dTTP pool size occurs 2 h later, and at this point is 27% of the maximum size found in wild-type-infected cells. The increase caused by dPyK− virus in the dCTP pool at the time of maximum DNA synthesis is actually more than that found 6 h after wild-type infection.

dPyK− mutant HSV causes no significant increase in the sizes of dTTP and dCTP pools after infection of PyY/TG/CAR/BUdR cells (which lack both thymidine and deoxycytidine kinases). This indicates that the salvage enzymes play a large part in establishing the observed pool sizes. Cells which contain both the viral and cellular thymidine kinase activities have an enormous dTTP pool, while if the viral activity is not present this is decreased by 73%. The absence of both the viral and cellular activities results in the de novo pathways in infected cells only being able to supply dTTP at a rate equivalent to the rate of removal of triphosphates from the pool, i.e. no build-up of the triphosphate is observed.

The viral deoxycytidine kinase does not play an important part in the establishment of the dCTP pool in infected cells; the major contribution seems to come from the reduction of CDP. When cellular, but not viral, deoxycytidine kinase is present the amount of dCTP present 6 h p.i. is in fact greater than when both activities are present. This difference may be a result of the excess demands being made on the de novo dCDP produced for both dTTP and dCTP synthesis, thus preventing a build-up of the dCTP pool. These results are consistent with the finding that although HSV-infected cells incorporate exogenous deoxycytidine the dCMP formed is not available for further metabolism (Jamieson & Subak-Sharpe, 1976).

In exponentially growing BHK C13 cells, the dGTP pool is expanded after infection with dPyK− virus, while a similar expansion is not found after infection of PyY/TG/CAR/BUdR cells. The reason for this is not clear, but it may be that the sizes of all four deoxynucleoside triphosphate pools are interrelated, and thus change in one pool size merely reflects changes in others. Alternatively, the differences found may be a reflection of some intrinsic differences in the metabolism of the two cell types.

Unlike wild-type HSV, dPyK− mutant virus does not cause a reduction of the dATP pool but in fact initially increases this pool during infection of all cell types tested. Again there is no obvious reason for the different effects found between wild-type and mutant virus infection. One possibility is that the dTTP pool size controls the production of dATP, probably at the level of ADP reduction by the enzyme ribonucleotide reductase, and that in cases where there are high levels of dTTP dATP synthesis is inhibited, but when lower levels of dTTP are present, dATP synthesis is enhanced.
Changes in dNTP pools caused by dPyK- HSV

After infection of resting BHK C13 cells with dPyK- HSV, while there is no significant change in the dTTP pool size, the sizes of the dATP, dCTP and dGTP pools are increased. In this case, when the levels of the cell enzymes involved in the conversion of deoxycytidine nucleotides to thymidine nucleotides are at a low level and there are no enzymes available for the uptake of exogenous thymidine, there is no facility for synthesizing dTTP. Of all the deoxynucleoside triphosphates dTTP is the only one which is at a level which in other cases has not been found to be able to sustain DNA synthesis. It is possible that although this measurement does not reflect the dynamic processes in which the pools are involved, a certain minimal level of dTTP is required in order for DNA synthesis to occur.

This report, taken in conjunction with the preceding paper, indicates that after herpes simplex virus infection there are substantial changes in the control mechanisms involved in the establishment of DNA precursors. dTTP appears to play a central role in the control processes in that it can act as a positive effector for the production of dGTP and dATP, yet at high concentrations dTTP may inhibit dATP production. In addition, it appears as if there is a lower limit to the dTTP pool size below which DNA synthesis does not occur.

These results are indicative of the deoxynucleoside triphosphates being involved in a homeostatic control process whereby they not only control their own production, but also under certain conditions control their own utilization.

REFERENCES


(Received 15 July 1975)