S-Adenosylmethionine metabolism in herpes simplex virus type 2-infected cells

Carol Seivwright, Joan C. M. Macnab and Roger L. P. Adams

Infection of primary rat embryo cells with herpes simplex virus type 2 has previously been reported to produce a dramatic and rapid inhibition of cellular DNA methylation. However, it has neither an immediate effect on S-adenosylmethionine breakdown nor on the relative pool sizes of S-adenosylmethionine and S-adenosylhomocysteine.

Infection of primary rat embryo (RE) cells with herpes simplex virus type 2 (HSV-2) inhibits the methylation of newly synthesized cellular DNA, and various HSV-transformed cell lines also show reduced levels of methylation in comparison with the parent RE cells (Macnab et al., 1988). It has been postulated that this widespread hypomethylation may be involved in the expression of a set of tumour-specific cellular proteins that accumulate on HSV infection. Although the functions of DNA methylation are beginning to be understood, little is known about how DNA methylation patterns are altered. This communication investigates one mechanism whereby HSV infection might cause the hypomethylation of newly synthesized cellular DNA in RE cells namely a change in the availability of the methylating agent S-adenosylmethionine (AdoMet).

T3 bacteriophage encodes the enzyme AdoMet hydro-lase (EC 3.3.1.2) which catalyses the breakdown of AdoMet to 5'-deoxy-5'-(methylthio)adenosine (MTA) and homoserine. The expression of this protein results in an inhibition of the methylation of newly synthesized cellular DNA (Gefter et al., 1966). In a similar manner, HSV-2 infection may alter the metabolism of AdoMet, causing it to be a limiting factor in the methyl transfer.

Alternatively, HSV-2 infection could alter the metabolism of one of the products of the DNA methylase reaction, S-adenosylhomocysteine (AdoHcy), a potent inhibitor of methyltransferase-catalysed reactions (Ueland, 1982). AdoHcy can rise and lead to an inhibition of methylation of DNA and other molecules such as proteins, lipids or polysaccharides. For example, Kredich & Martin (1977) showed that the treatment of cultured S49 mouse lymphoma cells with a specific inhibitor of adenosine deaminase resulted in high levels of adenosine, and consequently high levels of AdoHcy, which resulted in an inhibition of DNA methylation. It is possible, therefore, that HSV-2 infection could lead to metabolic alterations in the cell which could result in the accumulation of AdoHcy and cause an inhibition in DNA methylation.

A modification of the assay developed by Gefter et al. (1966) was used to monitor AdoMet breakdown. In this assay the formation of [3H]MTA from [3H]AdoMet was determined. The former does not bind to an Amberlite CG50 column (equilibrated with 0.02 M-potassium phosphate pH 7.0) whereas AdoMet does bind under these conditions. Dishes (50 mm) of subconfluent RE cells were mock-infected or infected at 20 p.f.u./cell for 4 h with HSV-2 strain HG52 (Timbury, 1971). After 4 h almost complete inhibition of cellular DNA methylation is observed (Macnab et al., 1988). Cell lysates were prepared, 250 μl was incubated at 37 °C for 30 min with 10 μCi [3H]AdoMet (15 Ci/mmol) and the formation of tritiated MTA was determined.

AdoMet breakdown (pmol Adomet cleaved/30 min/ mg protein) in mock-infected and infected RE cells was 83.0±17.4 and 83.6±22.4, respectively. These results show that HSV-2 infection does not significantly alter the rate of breakdown of AdoMet. The figures are the mean values of five observations±s.d. About 7% of the added AdoMet was degraded in the incubation.

Intracellular pool sizes of AdoMet and AdoHcy in RE
cells were measured according to Arnaud et al. (1985). Briefly, L-[³⁵S]methionine (800 mCi/mmole) was added to the culture medium of the cells at a final concentration of 20 μCi/ml. After the appropriate incubation period at 37 °C the cells were washed three times with PBS containing 600 mg/l methionine and harvested. The precipitate was washed twice with 7.5% TCA, dissolved in 0.3 M NaOH and used for protein determination. A 10 ml ion-exchange column of P-11 phosphocellulose was equilibrated with 10 mM HCl at 4 °C. The sample was applied and eluted with a stepwise gradient of HCl (10 mM, 100 mM, 500 mM) at a rate of 70 ml/h. The protocol used is a slight modification of that used by Eloranta et al. (1976) and in our hands gave an improved separation of methionine, AdoHcy and AdoMet. Samples (10 ml) were collected, 5 ml of each was removed to scintillation vials containing 5 ml of Ecoscint scintillator and the radioactivity was measured using a liquid scintillation counter. The remainder of each of the samples was used to determine the absorbance at 260 nm (A₂₆₀).

Fig. 1 shows a typical separation profile of a [³⁵S]methionine-labelled cell extract obtained from RE cells labelled for 2 h with [³⁵S]methionine. The cell extract alone gave no detectable absorbance at 260 nm and the A₂₆₀ values correspond only to the non-radioactive AdoHcy added as a marker. The position of elution of methionine and AdoMet was established by applying unlabelled cell extracts to which [³²S]methionine or [³H]AdoMet had been added.

To obtain reliable results for the pool sizes it was necessary to ensure equilibration of the extracellular [³⁵S]methionine with the intracellular pool of AdoMet. For this [³⁵S]methionine (80 μCi; 800 mCi/mmole) was added to the medium of duplicate plates of RE cells to give a final concentration of 20 μCi/ml. This does not affect the methionine concentration in the medium which remains at 30 mg/l. Cells were harvested at 15 min, 30 min, 1, 2 and 4 h after the addition of the [³⁵S]-methionine. In addition, at 4 h the medium from one set of plates was replaced with unlabelled medium and the cells were harvested after a further 2 h incubation at 37 °C. Extracts were prepared and fractionated as described for Fig. 1 to determine the amount of [³⁵S]-labelled AdoMet present in the cells. Equilibration of extracellular methionine with intracellular AdoMet occurred by around 2 h (Fig. 2) and hence this was the labelling time used in the subsequent analysis. This result is in agreement with that obtained by German et al. (1983) using WI-L2 lymphoblasts. The graph also shows that nearly all of the labelled AdoMet is metabolized within 2 h of the removal of [³⁵S]methionine from the extracellular medium, demonstrating the rapid turnover of AdoMet within the cell.

Table 1. AdoMet: AdoHcy ratio in mock-infected and infected RE cells

<table>
<thead>
<tr>
<th>Amount in cells (pmol/mg protein)</th>
<th>AdoMet</th>
<th>AdoHcy</th>
<th>AdoMet: AdoHcy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mock-infected</td>
<td>216:3</td>
<td>30:6</td>
<td>7:1</td>
</tr>
<tr>
<td>Infected</td>
<td>185:8</td>
<td>20:8</td>
<td>8:9</td>
</tr>
</tbody>
</table>

Table 1 shows the amounts of AdoMet and AdoHcy and the ratios of AdoMet: AdoHcy in HSV-2-mock-infected and-infected RE cells. The values shown are those obtained in two separate experiments. Sub-confluent RE cells in 90 mm plates were infected at 20 p.f.u./cell and, 2 h post-infection (p.i.), 80 μCi of [³⁵S]methionine (800 mCi/mmole) was added to the
extracellular medium at a final concentration of 20 μCi/ml. Four hours p.i., acid-soluble material was analysed as described. One-hundred μl of AdoHcy (10 mM in 5 mM-HCl) was added along with the extract as an internal standard. The results demonstrate that infection of RE cells with HSV-2 does not lower the AdoMet:AdoHcy ratio.

Measurement of the rate of AdoMet breakdown shows no differences between mock-infected cells and cells 4 h p.i., eliminating the possibility that infection with HSV-2 rapidly induces an AdoMet hydrolase or similar enzyme. The amount of AdoMet degraded in this assay corresponds to a turnover in 30 min of approximately 40% of the total amount of AdoMet present in cells. This is in accordance with the results shown in Fig. 2 where > 90% of 35S-labelled AdoMet is metabolized within 2 h of removing the source of the label, i.e. [35S]methionine. This also correlates with the results of Guilidori et al. (1984) who demonstrated that the half-life of AdoMet in vivo was approximately 30 min.

AdoHcy is a potent inhibitor of the DNA methylase reaction and alterations in its metabolism and therefore in the ratio of AdoMet:AdoHcy have been shown to be sufficient to cause a decrease in the 5-methylcytosine content of DNA in rats (Wilson et al., 1984). Adams & Burdon (1985) have shown that when AdoMet and AdoHcy are present at equimolar concentrations in vitro, DNA methylase activity is inhibited by 84%. In vivo, the ratio of AdoMet:AdoHcy has been estimated to be between 3 and 25 (Wilson et al., 1984; De Sanchez et al., 1991) which is in accordance with the results obtained in this study. However, no significant difference in this ratio was seen in HSV-2-infected and mock-infected RE cells. Similarly, Arnaud et al. (1985) found that the DNA hypomethylation of rat kidney cells transformed by avian sarcoma virus was not related to alterations in AdoMet:AdoHcy ratios which they calculated as being 10-94 and 12-32 in transformed and control cells, respectively. Therefore, HSV infection does not appear to cause the hypomethylation of newly synthesized DNA by altering the metabolism of AdoMet or AdoHcy.

We have recently found (C. Seivwright et al., unpublished) that on infection with HSV-2, the cellular DNA methylase is relocated to the perinuclear region, distant from the sites of residual cellular DNA replication, and this could explain the hypomethylation of cellular DNA found following HSV-2 infection of cells.

We would like to thank Professor M. D. Houslay and the University of Glasgow for the provision of facilities, Professor J. H. Subak-Sharpe for support that made this work possible and the MRC for funding.

References


(Received 23 November 1992; Accepted 15 February 1993)