when DNA synthesis is low, or cells treated with hydroxyurea to block DNA replication have lesser amounts of DNA methylase in this 'nuclear matrix' fraction. In addition, studies with cycloheximide indicate the association with the nuclear matrix also requires continuing protein synthesis.

Solubilization of the matrix-bound DNA methylase activity has been achieved by using large amounts of micrococcal nuclease, and present investigations are aimed at comparing the enzymic properties of this DNA methylase activity with that which is normally readily solubilized from mouse cell nuclei.


### Increased uptake of polyamines by baby-hamster kidney cells (BHK-21/C13) treated with α-methylornithine

HEATHER M. WALLACE, MAUREEN A. L. MELVIN and HAMISH M. KEIR

Department of Biochemistry, University of Aberdeen, Marischal College, Aberdeen AB9 1AS, Scotland, U.K.

α-Methylornithine is a reversible, competitive inhibitor of the first enzyme in the polyamine biosynthetic pathway, ornithine decarboxylase (EC 4.1.1.17). As such, it has been shown to decrease the intracellular concentrations of both putrescine and spermidine in a number of cell lines (Mamont et al., 1976; Seidenfeld & Marton, 1980; Pegg et al., 1981), including BHK-21/C13 cells (Wallace, 1979). In contrast, treatment of cells with α-methylornithine does not appreciably alter the intracellular spermine content. In addition to its effects on polyamine biosynthesis, α-methylornithine has also been shown to affect the excretion of polyamines from cells. Confluent cultures of BHK-21/C13 cells treated with this drug showed a marked decrease in the total amount of polyamines released from the cells into the extracellular medium (Wallace et al., 1979). In the present study we have examined the effect of α-methylornithine on the uptake and metabolism of exogenous polyamines by BHK-21/C13 cells.

BHK-21/C13 cells were seeded at a density of 2.6 × 10⁴ cells/cm² and were grown for 17 h in Dulbecco's modification of Eagle's medium supplemented with 10% (v/v) dialysed horse serum and 0.1 mM-hypoxanthine. The medium was then changed to Dulbecco's medium containing 0.1 mM-hypoxanthine and either 0.9% (w/v) NaCl or 5 mM-α-methylornithine in 0.9% NaCl. Then, 10 min later, dialysed horse serum was added to a final concentration of 10% (v/v). After a further 2h, ⁹⁹⁸H₈₂putrescine dihydrochloride (0.75 μCi/ml) or ¹⁴C₂spermidine trihydrochloride (0.5 μCi/ml) or ¹⁴C₂spermine tetrahydrochloride (0.5 μCi/ml) was added to the cultures. Samples were taken 24h later to determine the total intracellular acid-soluble radioactivity and the distribution of this radioactivity in the cells in the presence and absence of α-methylornithine.

In the presence of α-methylornithine, there was an increase in the incorporation of both putrescine and spermidine in the cells

(25.0 ± 1.07) (46.9 ± 1.7) (5.2 ± 0.6)

<table>
<thead>
<tr>
<th>¹^H-labelled polyamine</th>
<th>α-Methylornithine (5 milim)</th>
<th>¹^H labelled incorporated (cp.m.)</th>
<th>Distribution of radioactivity (% of total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Putrescine</td>
<td>+</td>
<td>12.41 ± 0.19</td>
<td>Spermine: 52.6 ± 11.7, Spermidine: 67.3 ± 6.1, Putrescine: 35.0 ± 7.8</td>
</tr>
<tr>
<td>+</td>
<td>3.03 ± 0.21</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spermidine</td>
<td>+</td>
<td>9.09 ± 0.54</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spermine</td>
<td>+</td>
<td>6.54 ± 0.41</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Effect of α-methylornithine on the uptake and metabolism of the polyamines

Values are means ± s.d. (n = 3). The radioactivity present as conjugated polyamines has not been included in this table. Conjugated polyamines account for up to 25% of the total radioactivity (H. M. Wallace, unpublished work).

1982
and therefore it seems likely that the cells increased the uptake of these two polyamines from the medium in order to compensate from the intracellular deficiency.

We thank the Medical Research Council for financial support (grant no. G979/267).


The effect of polyamines on the synthesis of poly(ADP-ribose) in permeabilized mammalian cells

AMANDA M. GORDON, HEATHER M. WALLACE, HAMISH M. KEIR and COLIN K. PEARSON

Department of Biochemistry, University of Aberdeen, Marischal College, Aberdeen AB9 1AS, Scotland, U.K.

To date, studies of the effects of polyamines on the synthesis of poly(ADP-ribose) have been performed with isolated nuclei as the experimental system (Purnell et al., 1980). However, Halldorsson et al. (1978) have emphasized that the activity of poly(ADP-ribose) polymerase is artificially high in isolated nuclei as a consequence of the damage to the DNA which occurs during nuclear isolation. The same authors have suggested that cells rendered permeable to NAD⁺, by incubation in hypo-osmotic buffer, provide a better system for estimating the activity of the polymerase in vivo. In this study we have examined the effect of incubating permeabilized cells in the presence of polyamines on the synthesis of poly(ADP-ribose).

BHK 21/C13 cells were grown routinely in Dulbecco's modification of Eagle's medium supplemented with 10% (v/v) horse serum. Cells were permeabilized by the method of Berger & Johnson (1976). The poly(ADP-ribose) polymerase assay contained (final concns.) 50 mM-Tris/HCl, pH 8.0, 10 mM-MgCl₂, 1 mM-dithiothreitol, 0.5 mM-[¹⁴C]NAD⁺ (28 µCi/mmol; 5 µCi/assay) and polyamines at the concentrations shown in the experiment. The reaction was started by the addition of approx. 2 × 10⁶ permeabilized cells and was incubated at 26°C for 30 min. Polymerase activity was measured by the incorporation of radioactivity into the trichloroacetic acid-insoluble material (Furneaux & Pearson, 1980).

Spermidine and spermine stimulated the synthesis of poly(ADP-ribose) in a dose-dependent manner (Fig. 1). Stimulation by spermidine was observed with increasing concentration of the polyamine up to 5 mM, the highest concentration tested. Stimulation by spermine was maximal (2.5 fold) when this polyamine was present at 1 mM. At higher concentrations the polymerase activity decreased, but even at 5 mM spermine, the highest concentration tested, activity was 2-fold higher than in the control assay. In contrast, putrescine had little effect on the synthesis of poly(ADP-ribose) in the concentration range tested (0.1–5 mM; Fig. 1).

This enhancement of poly(ADP-ribose) synthesis could be due to an increase in the number of polymer chains synthesized, or in their extent of elongation, or both. The average chain lengths of the poly(ADP-ribose) moieties were determined by hydroxyapatite chromatography. Poly(ADP-ribose) chains were synthesized in the presence of [¹⁴C]NAD⁺, the radiolabelled chains were then cleaved from the acceptor proteins by alkali and loaded onto a column of hydroxyapatite. The various lengths of chains were then eluted by a step-gradient of potassium phosphate (1–500 mM; Farzaneh & Pearson, 1978). No significant difference was detected in the overall distribution of chain lengths synthesized in the presence or absence of the polyamines. This implies that polyamines increased the synthesis of poly(ADP-ribose) by increasing the number of chain-initiation sites, since they had no effect on the lengths of the chains.

An alternative explanation for the increased synthesis of poly(ADP-ribose) in the presence of polyamines is that these molecules inhibit the glycohydrolase enzyme which degrades the polymer chains. However, a number of workers have shown that polyamines have no inhibitory effect on the activity of poly(ADP-ribose) glycohydrolase in isolated nuclei (Whitby et al., 1979; Tanigawa et al., 1980).

Polyamines also stimulate poly(ADP-ribose) synthesis in isolated nuclei. This effect has been shown to be the result of both increased initiation of poly(ADP-ribose) synthesis and increased elongation of polymer chains (Tanigawa et al., 1980). In addition to these effects, polyamines have been shown to alter the pattern of ADP-ribosylation of nuclear proteins (Perrella & Lea, 1978). The addition of spermine, and to a lesser extent spermidine, to isolated nuclei resulted in the preferential ADP-ribosylation of histone H1 compared with histones H2a, H2b and H3. Similarly, in the presence of spermine there was increased modification of non-histone proteins. Perrella & Lea (1978) have suggested that polyamines may regulate the activity of some nuclear proteins by altering the patterns of ADP-ribosylation of these proteins. The permeabilized cell system can be used to study this.

![Fig. 1. Effect of polyamines on the synthesis of poly(ADP-ribose) in permeabilized cells](image-url)