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## 3-Methyladenine DNA Glycosylase Activity in a Glial Cell Line Sensitive to the Haloethylnitrosoureas in Comparison with a Resistant Cell Line<sup>1</sup>

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### Abstract

Extracts of a glial cell line (SF-126) which is sensitive to the cytotoxic effect of the haloethylnitrosoureas and of a cell line (SF-188) which is resistant to these agents have been tested for their ability to release methylated bases from a DNA substrate which has been modified with [<sup>3</sup>H]dimethyl sulfate. In comparison with the sensitive cell line, extracts from the resistant cell line have 2-3-fold higher enzymatic activity. High performance liquid chromatography profiles of the bases which are released by these extracts show that the activity is specific for 3-methyladenine, suggesting that the resistant cells contain elevated levels of 3-methyladenine DNA glycosylase. Previous studies have shown that these cells also contain elevated levels of O<sup>6</sup>-alkylguanine-DNA alkyltransferase, suggesting that both enzyme activities may be involved in the resistance of this cell line to the haloethylnitrosoureas.

### Introduction

The haloethylnitrosoureas evidently produce their cytotoxic effect by modifying DNA, and repair of these modifications plays an important role in determining the sensitivity of various cell lines to these agents (1, 2). Cells with the methyl excision repair positive trait are more resistant to the action of the haloethylnitrosoureas than methyl excision repair negative cells (2, 3), and O<sup>6</sup>-alkylguanine-DNA alkyltransferase has been shown to prevent DNA interstrand cross-linking by these agents (4-6). Recent studies by Bodell *et al.* (7) have shown that a resistant glial cell line, SF-188, with elevated levels of O<sup>6</sup>-alkylguanine-DNA alkyltransferase, has fewer G-C cross-links than a sensitive cell line, SF-126.

The finding that the resistant cell line is also depleted in other DNA modifications has suggested that other repair activities besides O<sup>6</sup>-alkylguanine-DNA alkyltransferase may be acting in these cells. Recent studies have shown that several 7-alkylguanines including the cross-linked 1,2-bis(7-guanyl)ethane are released from haloethylnitrosourea-modified DNA by bacterial 3-methyladenine DNA glycosylase II (8, 9). Several of these 7-alkyl-guanines fall in the region of the HPLC profile which is depleted in the resistant glial cell line, suggesting that this line might contain more glycosylase activity than the sensitive cell line.

In this paper, we report evidence that the resistant SF-188 glial cells do, in fact, contain more glycosylase activity than the sensitive SF-126 cells.

### Materials and Methods

**Materials.** [<sup>3</sup>H]Dimethyl sulfate (specific activity, 1 Ci/mmol) was purchased from New England Nuclear. 7-Methylguanine and 3-meth-

yladenine were purchased from Cyclo; calf thymus DNA and bovine albumin were from Sigma.

**Modification of DNA.** [<sup>3</sup>H]DMS<sup>3</sup>-DNA was prepared by alkylating 0.8 ml of calf thymus DNA (5 mg/ml) in 50 mM sodium cacodylate buffer, pH 7.5, with 2 mCi [<sup>3</sup>H]DMS for 120 min at 37°C. Then, the solution was made 0.3 M in NaCl, DNA was precipitated with 2.5 volumes of ethanol, redissolved in water, and reprecipitated until a constant activity (4.1 pmol/μg) was obtained. The distribution of alkylation products was determined by HPLC analysis following depurination in 0.1 N HCl at 37°C for 16 h. Essentially all of the alkylation was at position 7 of guanine and position 3 of adenine: m<sup>7</sup>Gua, 76.8%; and m<sup>3</sup>Ade, 23.2%.

**Preparation of Cell Extracts.** Human glioma cell lines SF-126 (sensitive to the haloethylnitrosoureas) and SF-188 (5-fold more resistant to chloroethylnitrosourea than SF-126 cells) have been maintained in tissue culture as described previously (10, 11). Cells were grown to confluence in eight 850-cm<sup>2</sup> roller bottles, harvested mechanically in the absence of trypsin, and kept frozen. All steps in the preparation of cell extracts were carried out on ice; 0.5-1 g of cells was resuspended in 50 mM Tris buffer, pH 8, containing 1 mM EDTA, 100 mM NaCl, 0.1 mM phenylmethylsulfonyl fluoride and 0.03 unit/ml of aprotinin. The NaCl concentration was increased to 700 mM by the addition of 5 M NaCl and the cells were sonicated; NaCl concentration was then adjusted to 300 mM by the addition of 50 mM Tris buffer, pH 8, containing 1 mM EDTA, 2 mM 2-mercaptoethanol, and 0.1 mM phenylmethylsulfonyl fluoride. After being stirred for 2 h at 4°C, the solution was centrifuged for 2.5 h at 17,200 × g in a Sorvall SS-34 rotor. The supernatant was distributed in 100-μl aliquots and assayed for enzyme activity either immediately or after storage at -70°C; the activity of frozen aliquots was found to be unchanged over 6 months.

**Enzyme Assay.** Incubation mixtures (total volume, 100 μl) contained: 20 mM Tris, pH 8; 2 mM 2-mercaptoethanol; 60 mM NaCl; approximately 3 μg (12,500 cpm) of DNA substrate; and an amount of cell extract containing 5-108 μg of protein. Mixtures were incubated at 37°C for 30 min and reactions were stopped by chilling on ice. DNA was precipitated with NaCl-ethanol, and the radioactivity in the supernatant was determined by counting an aliquot in a Beckman LS 1800 scintillation counter. Time-dependent release of radioactivity from the DNA substrate was determined by incubating [<sup>3</sup>H]DMS-DNA for different periods of time with 35 μg of protein under the same conditions. Heat-inactivated extract from SF-188 cells and bovine albumin were used as controls in all experiments.

Modified bases released by cell extracts were identified in the supernatant from an incubation mixture which had been scaled up 20-fold. The supernatant was passed through a DEAE-Sephadex A-25 column to remove oligonucleotides, lyophilized to dryness, redissolved in HPLC buffer, and separated on an Alltech Spherisorb 5-μm C<sub>18</sub> column eluted at 1 ml/min with increasing concentrations of KH<sub>2</sub>PO<sub>4</sub>, pH 4.5, and methanol as follows: 2.5 mM KH<sub>2</sub>PO<sub>4</sub>, with 15% methanol for 15 min; 25 mM KH<sub>2</sub>PO<sub>4</sub>, with 15-50% methanol for 10 min; and with 50% methanol for an additional 15 min. Under these conditions, the retention times for marker r-o m<sup>7</sup>Gua, m<sup>7</sup>Gua, m<sup>6</sup>Gua, and m<sup>3</sup>Ade were 5.0, 7.7, 15.8, and 28.3 min, respectively.

In order to compare the release from DNA by different cell extracts,

<sup>3</sup> The abbreviations used are: DMS, dimethyl sulfate; HPLC, high performance liquid chromatography; m<sup>3</sup>Ade, 3-methyladenine; m<sup>6</sup>Gua, O<sup>6</sup>-methylguanine; m<sup>7</sup>Gua, 7-methylguanine; r-o m<sup>7</sup>Gua, ring-opened 7-methylguanine.

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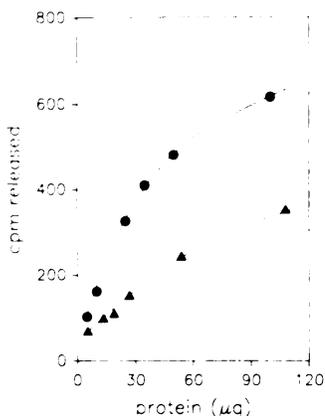


Fig. 1. Protein-dependent release of radioactivity from [<sup>3</sup>H]DMS-DNA by extracts from resistant (●) or sensitive (▲) glial cells. Background release by boiled extract has been subtracted.

the results from the HPLC profiles were normalized to the same volume analyzed, specifically to the volume of boiled extract.

**Results and Discussion**

The protein-dependent release of radioactivity from [<sup>3</sup>H]DMS-DNA is shown in Fig. 1. Extracts from both the sensitive (SF-126) and resistant cells (SF-188) release radiolabeled material from the substrate in a protein-dependent manner. At any given protein concentration, however, the resistant cell extract has approximately twice the activity of sensitive cell extract when it is assayed in this way.

Fig. 2 shows the time-dependent release of radioactivity from the substrate under the conditions described above. Under these conditions, the greater activity in the resistant cell line is even more apparent, approaching 3-fold.

A difficulty in assaying crude cell extracts is, of course, the possibility that release is nonspecific. However, the 3-methyladenine DNA glycosylases which have been described all show a significantly greater activity towards m<sup>3</sup>Ade in preference to m<sup>7</sup>Gua in the substrate. Since our [<sup>3</sup>H]DMS-DNA contains approximately 4 times as much m<sup>7</sup>Gua as m<sup>3</sup>Ade, evidence that the release of radioactivity shown in Figs. 1 and 2 is the result of glycosylase action comes from a comparison of the distribution of bases released by the extract in comparison with

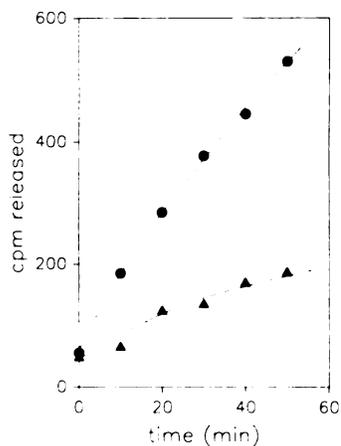


Fig. 2. Time-dependent release of radioactivity from [<sup>3</sup>H]DMS-DNA by extracts containing 35 μg protein from resistant (●) or sensitive (▲) glial cells. Background release by boiled extract has been subtracted.

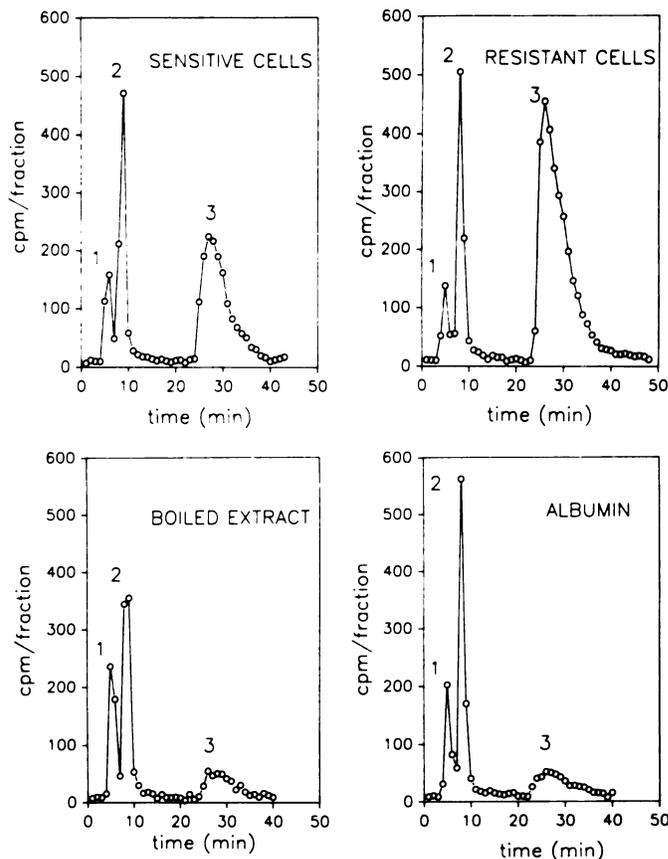


Fig. 3. HPLC profiles of bases released from [<sup>3</sup>H]DMS-DNA. Peak 1, r-o m<sup>7</sup>Gua; Peak 2, m<sup>7</sup>Gua; and Peak 3, m<sup>3</sup>Ade.

spontaneous release, or release in the presence of inactivated extract.

Fig. 3 shows the distribution of radioactivity released by the cellular extracts in comparison with the distribution released in control experiments. In these HPLC profiles, the first two peaks represent r-o m<sup>7</sup>Gua and m<sup>7</sup>Gua, respectively, while m<sup>3</sup>Ade is the late-appearing peak 3. It is apparent that release of m<sup>3</sup>Ade constitutes a relatively small fraction of the radioactivity in the supernatant from control experiments. In fact, this distribution approximates that in the original substrate.

At the top of Fig. 3, releases by extracts from sensitive and resistant cells are compared. It is apparent that the increase in radioactivity released by extracts from both cell lines is in the m<sup>3</sup>Ade peak. Furthermore, as expected from the results shown in Figs. 1 and 2, release of m<sup>3</sup>Ade by extracts from the resistant cell line greatly exceeds the release by extracts from the sensitive cell line.

These results are quantitated in Table 1. It is apparent that the sum of r-o m<sup>7</sup>Gua and m<sup>7</sup>Gua released by both sensitive and resistant cell extracts is approximately the same as in the control experiments and that the increase in radioactivity re-

Table 1 Release of m<sup>3</sup>Ade by cell extracts<sup>a</sup>

Protein	cpm	
	r-o m <sup>7</sup> Gua + m <sup>7</sup> Gua	m <sup>3</sup> Ade
Albumen	1106	341
Boiled extract	1199	303
Sensitive cell extract	1035	1411
Resistant cell extract	1150	3048

<sup>a</sup> Values normalized to the volume of boiled extract analyzed as described in "Materials and Methods."

leased by extracts of both cell lines can be attributed entirely to m<sup>3</sup>Ade. Moreover, the increase in m<sup>3</sup>Ade release over control levels is more than 2-fold higher for the resistant than for the sensitive cells which confirms the conclusion that the resistant cells have higher glycosylase activity. If this glycosylase has a range of specificity similar to that of bacterial 3-methyladenine-DNA glycosylase II (8, 9), its presence would explain the observed depletion of other haloethylnitrosoarea-induced DNA modifications in the resistant cell line (7).

Thus, resistant SF-188 cells have higher levels of 3-methyladenine DNA glycosylase than do the sensitive SF-126 cells, as well as higher levels of O<sup>6</sup>-alkylguanine-DNA alkyltransferase as found previously (11). Since levels of some repair enzymes increase in a coordinated way in response to DNA alkylation in bacteria (12), it is possible that the increased level of two enzymatic activities seen here is also coordinated in some way. Further experimentation with other cell lines will be necessary to determine the generality of this result as well as to explore possible control mechanisms.

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