Nucleic Acids Research

A system in mouse liver for the repair of O^6-methylguanine lesions in methylated DNA

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ABSTRACT

    An activity from mouse liver which catalyzes the disappearance of O^6-methylguanine from DNA methylated with methylnitrosourea has been partially purified by ammonium sulfate fractionation and DNA-cellulose chromatography. The activity does not require divalent metal ions and is not affected by EDTA. It is specific for the repair of O^6-methylguanine lesions and does not affect the removal of 7-methylguanine, 7-methyladenine or 3-methyladenine. The disappearance of O^6-methylguanine is linear with respect to the concentration of protein and is dependent on incubation temperature. The kinetics and substrate dependence experiments suggest that the protein factor is product-inactivated. Amino acid analysis of hydrolysates of protein obtained after incubation of methylated DNA with the protein factor indicates the presence of radiolabeled S-methyl-L-cysteine, suggesting that during the repair of O^6-methylguanine from methylated DNA, the methyl group is transferred to a sulfhydryl of a cysteine residue of a protein. This represents the first such demonstration in a mammalian system.

INTRODUCTION

    The alkylation of DNA after administration of simple alkylating agents such as N-ethyl-N-nitrosourea and dimethylnitrosamine has been implicated in the production of mutations and cancer (1,2). In particular, alkylation of the oxygens in nucleic acids has been considered as a crucial lesion (3,4). Perhaps the most extensively studied nucleic acid alkylation product is O^6-alkylguanine. This area of research was stimulated by the suggestion that O^6-alkylation was premutagenic due to anomalous base pairing (5). This view is supported by the observations that templates containing O^6-methylguanine direct RNA polymerase (6) and DNA polymerase I (7) to incorporate inappropriate ribonucleoside 5'-triphosphates and deoxyribonucleoside 5'-triphosphates, respectively. Recently, DNA damaged by dimethylnitrosamine has been demonstrated to permit replication in vivo (8).

    In other studies, O^6-alkylguanine has been causatively related to the production of mutations and to the formation of tumors. The presence of O^6-

methylguanine in the DNA of E. coli correlated with the appearance of muta-
tions (9,10), while the production of tissue-specific tumors by simple alky-
lating agents has been correlated with the formation and persistence of O^6-
alkylguanine in the DNA of the susceptible tissues (11-14).

In light of the above observations, the repair of O^6-alkylguanine
lesions in DNA has been considered of great importance. The excision of
O^6-methylguanine from DNA under in vivo conditions has been demonstrated
in rats (15,16), mice (12) and in E. coli (9,17). O^6-Ethylguanine excision
from DNA has also been shown in mice (12), rats (18,19) and in E. coli (20).

The in vitro repair of O^6-alkylguanine, however, has been less exten-
sively studied. Crude extracts obtained from rat liver catalyze the re-
moval of O^6-methylguanine (21) and O^6-ethylguanine (19) from DNA. The isola-
tion of a rat chromatin protein capable of removing O^6-ethylguanine from
DNA has also been reported (22). Crude E. coli extracts have also been
isolated which are capable of removing O^6-methylguanine from DNA (23).
Until recently, the product of this repair process had eluded a number of
investigators. However, recently, Olsson and Lindahl (24) have shown that
during its repair, the methyl group of O^6-methylguanine is transferred to a
cysteine residue of a protein, resulting in the formation of S-methyl-L-
cysteine.

We report here the partial purification and preliminary characteriza-
tion of an activity from mouse liver which also catalyzes the removal of
O^6-methylguanine residues from DNA. As in E. coli, the methyl group of
O^6-methylguanine is transferred to a cysteine residue of protein during
the repair process.

MATERIALS AND METHODS
    Materials: [^{14}C]-D,L-serine (10.3 mCi/mmole) and N-[^{3}H-methyl]-N-nitro-
sourea (1.1-1.6 Ci/mmole) were obtained from Cal Atomic and New England
Nuclear Corp., respectively. The sources of the following substances
were as indicated: proteinase K, from Beckman; Cellex 410 cellulose pow-
der, from Bio-Rad Laboratories; aminopeptidase M, calf thymus DNA, 7-
methylguanine (7-MeG) and S-methyl-L-cysteine (S-Me-L-Cys), from Sigma
Chemical Co.; other unlabeled amino acids, from Pierce Chemical Co.;
7-methyladenine (7-MeA) and 3-methyladenine (3-MeA), from Chemical Pro-
curement Laboratories, Inc. O^6-Methylguanine (O^6-MeG) was a generous
gift from Dr. A.E. Pegg of the Milton S. Hershey Medical Center.
    Preparation of Liver Fractions: The livers obtained from male 20-25g

C57BL/6J mice were used as the source of the protein. All operations in the purification were performed at 0-4°C. The purification was modified from that which was reported by Pegg and Hui (21). The liver was homogenized in three volumes of buffer A (50 mM Tris-HCl/1.0 mM dithiothreitol/0.1 mM disodium EDTA, pH 7.8), the homogenate was centrifuged at 10,000 x g for 5 min and the supernatant was removed. The pellet was suspended in 3 additional volumes of buffer A and the suspension was sonicated for 6 periods of 15 sec each separated by 4 min intervals. The sonicated extract was added to the supernatant from the centrifugation. This procedure was used because both the 10,000 x g supernatant and pellet contained significant activity, and sonication increased the activity obtained from the 10,000 x g pellet. The combined preparations represent the sonicated homogenate.

The sonicated homogenate was centrifuged at 105,000 x g in a Beckman Type 50 Ti rotor for 60 min, the supernatant was removed and ammonium sulfate was added to 35% saturation. After stirring for 30 min, the precipitate was removed by centrifugation. The resulting supernatant was adjusted to 50% saturation with ammonium sulfate, the suspension was stirred for 30 min, and the resulting 35-50% precipitate was removed by centrifugation. This precipitate was redissolved in a small volume of buffer A, dialyzed overnight against 2 liters of buffer A and centrifuged to remove any aggregated material. The resultant supernatant represented the 35-50% ammonium sulfate fraction.

The next step in the purification was DNA-cellulose chromatography. DNA-cellulose was prepared from calf thymus DNA by the method of Alberts and Herrick (25). The 35-50% ammonium sulfate fraction was loaded onto a 0.9 x 6 cm column of DNA-cellulose that had been equilibrated with buffer A; the ammonium sulfate fraction was allowed to incubate with the DNA-cellulose for 90 min. The column was then eluted with buffer A at a flow rate of 8 ml/hr, followed sequentially with buffer A containing 0.25 M, 0.5 M, 0.75 M and 1.0 M sodium chloride. Fractions of 2 ml were collected and the absorbance at 280 nm (A_{280}) was determined. The fractions were dialysed overnight against 2 liters of buffer A and assayed for activity. In subsequent enzyme isolations, 4 ml of the 35-50% ammonium sulfate fraction was split into two portions which were each chromatographed on separate DNA-cellulose columns. Protein in the 0.25 M NaCl fractions were combined and immediately stored at -70°C. The 0.25 M NaCl fraction was dialyzed overnight against 2 liters of buffer A before use.

Preparation of Substrate: DNA was alkylated with N-[methyl-^{3}H]-N-

nitrosourea (MNU) by a modification of the method of Lawley and Shah (26).
To a solution containing 3.1 mg of calf thymus DNA in 1.0 ml of 0.2 M
Tris-HCl, pH 8.0 was added 144 µl of 1.0 mCi/ml [^{3}H]-MNU in ethanol. This
solution was incubated for 60 min at 37°C, and the DNA was precipitated by
the addition of 0.1 vol of 2.5 M sodium acetate, and two volumes of cold
ethanol. After centrifugation, the DNA pellet was washed several times
with cold 95% ethanol, dried under nitrogen and redissolved overnight at 4°C
in 0.15 N sodium chloride/0.015 N sodium citrate, pH 7.0. The DNA was repre-
cipitated, washed, dried as above and stored at -20°C. The DNA alkylated in
this manner contained 0.082 to 0.131 mCi/mmole DNA-P.

Incubations: The standard assay, performed for 1.0 hr at 37°C, con-
tained 50 mM Tris-HCl (pH 7.8), 1.0 mM dithiothreitol, 5.0 mM disodium EDTA,
100 µg of DNA methylated with [^{3}H]-MNU and protein in a total volume of
200 µl. The reaction was stopped by precipitation of the DNA and protein
with 0.1 vol of 2.5 M sodium acetate and two volumes of cold ethanol. In
control incubations, the protein preparation was added after the incubation
period and immediately before ethanol precipitation. The reactions were
allowed to stand for 1 hr at -20°C, centrifuged, the supernatant was
removed and the precipitated DNA was depurinated by heating in 300 µl of
0.1 N HCl for 30 min at 70°C (20,21). This solution was cooled, neutralized,
recentrifuged and the acid hydrolysate was removed and stored in a capped
vial at 4°C. For routine assays, the acid hydrolysates were filtered
through a 0.45 µm Millipore filter, unlabeled marker 0^6-methylguanine was
added and high pressure liquid chromatography was performed as described
below. One unit is defined as that amount of activity which catalyzes the
release of 1 pmole of 0^6-methylguanine under the standard assay conditions.

High-Pressure Liquid Chromatography (HPLC): All HPLC analysis of acid
hydrolysates containing [methyl-^{3}H]-purine bases was performed using a
Varian Model 5000 liquid chromatography apparatus. For routine assays, a
modification of the procedure of Hartwick and Brown (28) was used. A
Waters C$_{18}$ µBondapak column (0.39 x 30 cm) was eluted at 1.0 ml/min using
an isocratic solvent system consisting of 0.01 M potassium dihydrogen
phosphate, pH 5.5-methanol (4:1 v/v). In this system, 0^6-methylguanine
eluted at 10 min, well after all the other radioactive material, pre-
dominantly 7-methylguanine.

To achieve a better separation of [methyl-^{3}H]-purines, a Whatman
Partisil 10-SCX column (0.46 x 25 cm) was used (12,29). The column was
eluted at 1 ml/min using a 25 min gradient from 0.02 M ammonium formate,

pH 4.0 containing 6% methanol (v/v) to 0.2 M ammonium formate, pH 4.0 con-
taining 8% methanol (v/v). Gradient 9 adapted from a Waters model 660
solvent programmer to the Varian Model 5000 liquid chromatograph was
employed. For both HPLC procedures, 0.5 ml fractions were collected and the
radioactivity was determined by liquid scintillation counting.

Partial Depurination of [Methyl-^{3}H]-DNA Substrate: To aid in product
identification, [methyl-^{3}H]-DNA substrate was heat-treated to remove N-
alkylated purines (23). The DNA in 100 mM NaCl/10 mM sodium citrate/10 mM
potassium phosphate, pH 7.4 was incubated at 80°C for 16 hr in a sealed
glass ampoule followed by dialysis at 4°C against 2 liters of buffer A for
24 hr and 2 additional liters of buffer A for 4 hr. The DNA, which con-
tained 0.016 to 0.020 mCi/mmole DNA-P, was stored frozen in aliquots at
-20°C. About 50% of the total radioactivity in the DNA was contained in
0^6-methylguanine residues.

Amino Acid Analysis: The standard reaction mixture, using the DNA-cellu-
lose fraction and the partially depurinated [methyl-^{3}H]-DNA substrate, was
scaled-up 15 times for analysis of the methyl acceptor. After the incubation
period, proteinase K (200 µg/ml) was added to the reaction mixture and incuba-
tion was continued at 37°C for 4 hr. Aminopeptidase M (380 µg/ml) was then
added to the reaction mixture and incubated for 4 additional hr at 37°C (24).
After the digestion period, residual DNA was removed by ethanol precipitation.
The supernatant was evaporated under nitrogen to remove ethanol and the re-
maining aqueous solution was lyophilized. The residue was dissolved in sample
buffer at pH 2.2, adjusted to pH 4.0 with 2.0 M citric acid and supplemented
with a small amount of [^{14}C]-D,L-serine as an internal standard (900 µl final
volume). 750 µl of this solution was employed in a Beckman Model 121
Automatic Amino Acid Analyser. 1 min (1 ml) fractions were collected and
their radioactivity was determined by liquid scintillation counting.

RESULTS

The majority of the activity that caused the disappearance of 0^6-methyl-
guanine from methylated DNA was associated with the 35-50% ammonium sulfate
fraction of the homogenized and sonicated mouse liver. This fraction was
then subjected to DNA-cellulose chromatography. These experiments are
demonstrated in Figure 1a. Essentially all of the activity eluted in the
0.25 M NaCl fraction. On the other hand, when cellulose alone was employed
all of the activity was associated with the breakthrough peak (Figure 1b).
The results of a typical protein purification are summarized in Table 1. In
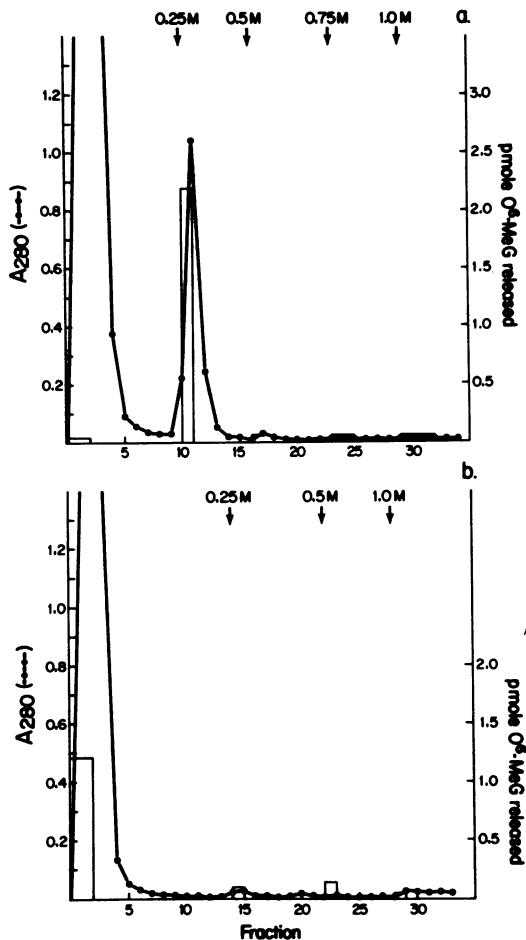
Figure 1.   DNA-Cellulose Chromatography of the 35-50% Ammonium Sulfate
Fraction.   The ammonium sulfate fraction was chromatographed on (a) a DNA-
cellulose column or (b) a cellulose column. 150 μl aliquots of the column frac-
tions were assayed for activity using the standard procedure described in
Methods.   The absorbance at 280 nm (A_{280}) and the pmol O^6-methylguanine
(O^6-MeG) released during the assay are presented on the left and right
ordinates, respectively.

this case, the overall purification of the factor by the three step procedure
was 86-fold with a 20% yield of activity. We attempted to chromatograph the
35-50% ammonium sulfate fraction on a DNA-cellulose column that had been pre-
pared using DNA alkylated with unlabeled MNU. We observed a protein peak with
the 0.25 M NaCl step, but no activity was associated with this fraction.   The

TABLE 1

Partial Purification of Activity from Mouse Liver

Fraction	Total Protein (mg)	Total Units	Units/mg Protein	Yield (%)	Purification (-fold)
Sonicated homogenate	3000	252	0.084	100	-
35-50% $(NH_4)_2SO_4$	317	62.1	0.196	24.7	2.33
0.25 M NaCl DNA-Cellulose	6.85	49.6	7.24	19.7	86.2

Legend: These results are representative of many experiments performed with different mouse liver preparations.

alkylated DNA-cellulose column was eluted with up to 4.0 M NaCl without any recovery of activity.

We next contrasted the specificity of release of the methylated bases. These data are illustrated in Figure 2. It is apparent that an almost complete disappearance of 0^6-methylguanine was observed in the incubation as compared to the control. In this case, 93% of the original 0^6-methylguanine present in the alkylated DNA was removed. On the other hand, no significant disappearance of the other major methylated purines, that is, 7-methylguanine, 7-methyladenine and 3-methyladenine, occurred.

The disappearance of 0^6-methylguanine as a function of protein concentration was investigated (Figure 3). It was essentially linear up to at least 185 μg of protein added to the reaction mixture. At this protein concentration, approximately 74% of the original 0^6-methylguanine had been released from the alkylated DNA.

The 0^6-methylguanine release as a function of incubation time at 2 different protein concentrations is illustrated in Figure 4a. In both cases, the reaction was essentially complete by 8 to 10 min, the amount of 0^6-methylguanine lost reaching a constant value by this time. It is apparent that a reduction by one-half of the protein in the reaction mixture resulted in a 50% decrease in 0^6-methylguanine release. In neither case, however, did 0^6-methylguanine release appraoch completion (i.e., 100%).

We believed the inability to achieve 100% release of the 0^6-methylguanine under the above conditions might have been due to heat inactivation
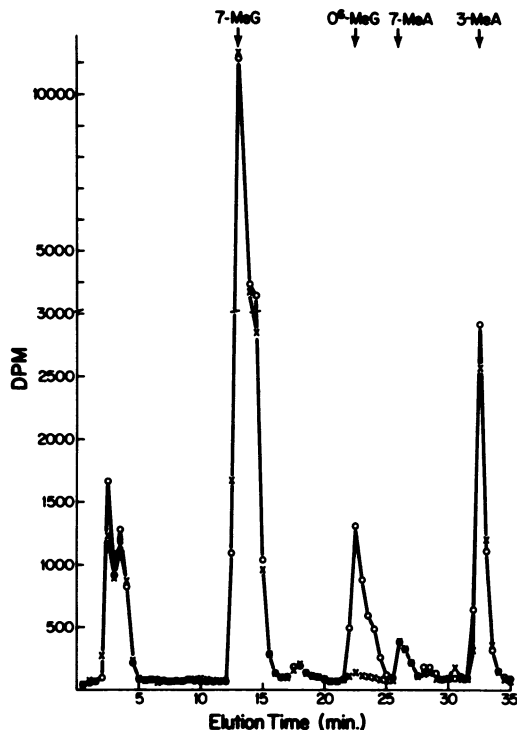
Figure 2. HPLC Analysis of Acid Hydrolysates of DNA on a Whatman Partisil
10-SCX Column. The standard assay procedure was performed using 174 μg of
the 0.25 M NaCl DNA-cellulose fraction as described in Methods. Acid
hydrolysates from sample (— ✕ —) and control (— O —) incubation were
chromatographed by HPLC. The arrows show the elution postions of unlabeled
marker compounds.

of the protein. For this reason, the protein preparation was preincubated
for various time periods at 37°C in the absence of substrate before being
assayed for activity (Figure 4b). No loss of activity occurred during the
first 6 min of preincubation. After 10 min of preincubation, 88% of the
original activity remained and even after 60 min of preincubation about 30%
of the original activity was still present. Thus, the effect shown above
cannot be explained by heat inactivation of the factor.

    The factor is 3-fold less active against denatured DNA alkylated with
[^{3}H]-MNU than against native DNA alkylated with [^{3}H]-MNU. The activity is
sensitive to NaCl with 50% inhibition occurring at 0.5 M, while no effect
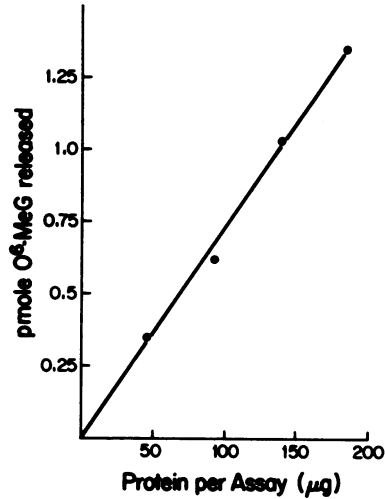was observed at less than 0.1 M. The reaction does not require divalent

Figure 3. O^6-Methylguanine Release as a Function of Protein Concentration.
The standard assay procedure was performed using various amounts of the
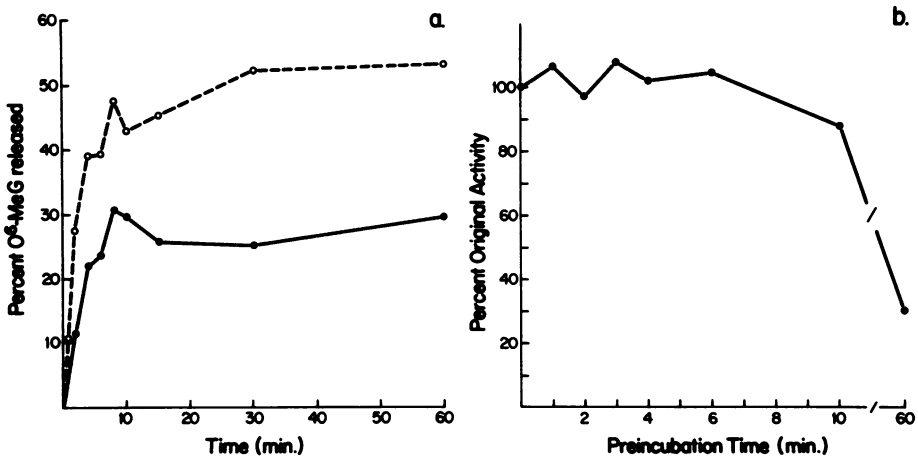0.25 M NaCl DNA-cellulose fraction as a protein source.

Figure 4. A. O^6-Methylgunaine Release as a Function of Incubation Time.
126 µg (—O—) and 63 µg (—●—) aliquots of the DNA-cellulose fraction
were assayed by the standard assay procedure for the incubation times
shown. B. Effect of Preincubation Time on Activity. 117 µg aliquots of
the DNA-cellulose fraction were preincubated for various time periods at
37°C in the absence of substrate before being assayed by the standard
assay procedure.

metal ions, and in fact, the standard assay procedure is performed in the presence of 5 mM disodium EDTA.

O^6-Methylguanine release as a function of incubation time at different temperatures is illustrated in Figure 5. At 4°C, essentially no release of O^6-methylguanine occurred by 60 min while at 37°C, the reaction was fairly rapid. At an intermediate temperature of 20°C, O^6-methylguanine dissappeared at a much slower rate than at 37°C. It is noteworthy that after 60 min of incubation time, the amount of O^6-methylguanine released at 20°C approached the value achieved at 37°C.

The effect of initial substrate concentration, i.e., methylated DNA, on the amount of O^6-methylguanine released is summarized in Table 2. It can be seen that increasing the substrate concentration did not result in any increase in O^6-methylguanine release. This suggested that the release was dependent only upon the amount of protein available in the assay.

Throughout these studies, product identification had proven a substantial problem. Using the partially depurinated [methyl-^{3}H]-DNA substrate, under conditions in which 84% of the O^6-methylguanine was removed, only 4% of the initial radioactivity could be found in ethanol soluble form. Initially, we entertained the possibility that the methyl group would be released either as formaldehyde or methanol but were unable to
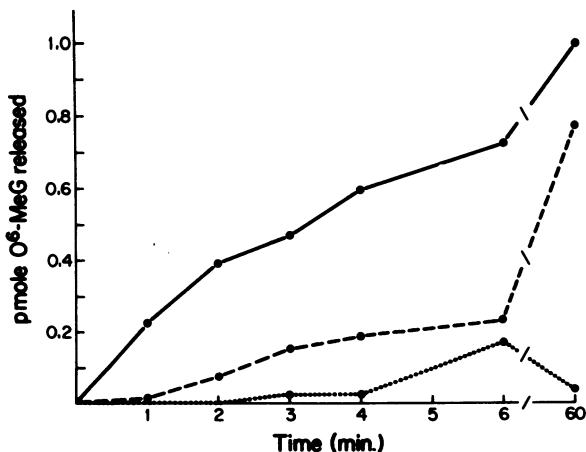

Figure 5.   The Kinetics of O^6-Methylguanine Release at Different Temperatures. 129 μg aliquots of the DNA-cellulose fraction were assayed for the indicated incubation times at 4°C (·· ● ·), 20°C (- - ● - -) and 37°C (— ● —) by the standard assay procedure.

TABLE 2

Disappearance of O^6-Methylguanine as a Function of Substrate

Methylated DNA in Reaction Mixture (µg)	O^6-Methylguanine Originally Present (pmol)	O^6-Methylguanine Released (pmol)	% O^6-MeG Released
100	1.34	0.95; 1.00	72.8
150	2.01	0.93; 1.21	53.3
200	2.68	0/94; 1.26	41.3

Legend: [^{3}H]- MNU-methylated DNA which was prepared as described in Methods was incubated with 110 µg of the DNA-cellulose fraction for 60 min. The disappearence of O^6-methylguanine was determined as described in the text. The results of 2 experiments are presented in the Table.

detect any volatile radioactivity during the course of the reaction. To test if the reaction product might be bound to macromolecules, reaction mixtures were treated with proteinase K, RNase A or DNase I. Treatment with proteinase K resulted in a significant release of ethanol-soluble counts, suggesting that the reaction product might be bound to protein. To further characterize the putative protein methyl acceptor, the standard assay procedure was performed and the protein contained in the mixture was then digested enzymatically to amino acids as described in Methods. All of the O^6-methylguanine radioactivity which disappeared from the partially depurinated DNA could be accounted for in the ethanol soluble fraction. This digest was chromatographed on an automatic amino acid analyzer (Figure 6). It can be seen that only one major tritium peak which accounted for 88% of the total radioactivity was found, and that this co-chromatographed with authentic S-methyl-L-cysteine. [^{14}C]-D,L-serine, added as an internal marker to the protein digest, co-chromatographed with authentic D,L-serine. It is therefore most likely that the methyl group of O^6-methylguanine was transferred to a L-cysteine residue contained in a protein during the course of the incubation.

DISCUSSION

The mouse protein fraction described in this paper appears to have an affinity for DNA, since it binds tightly to native DNA-cellulose but does not bind to cellulose alone. We cannot state at this time whether this fraction will bind to alkylated DNA-cellulose so tightly as to resist
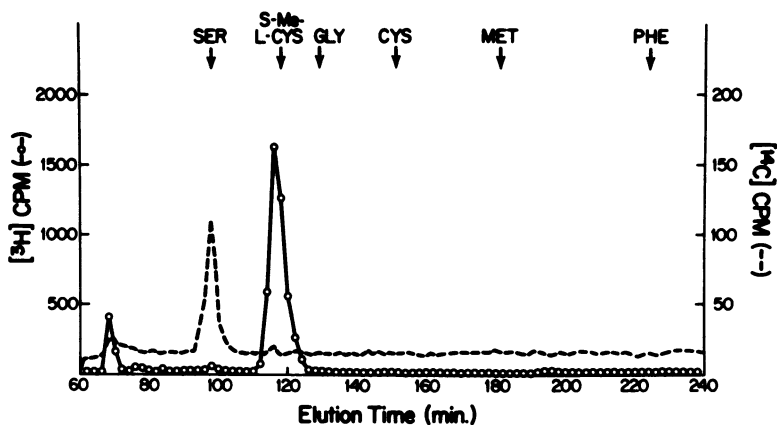
Figure 6. Amino Acid Analysis of an Enzymatically Hydrolyzed Protein Digest. The radioactivity profile of the protein hydrolysate is shown. Arrows show the ninhydrin maxima of several unlabeled marker amino acids. The [^{3}H] (left ordinate) represents tritium labeled material, i.e., from the methylation assay; the ^{14}C (right ordinate) represents the D,L-serine (Ser) used as an internal marker.

elution with 4.0 M NaCl or loses its activity once this binding is effected. The protein fraction causes the specific disappearance of 0^6-methylguanine from alkylated DNA but does not affect 7-methylguanine, 7-methyladenine or 3-methyladenine levels. This agrees with the observation of Pegg and Hui (21) that a crude rat liver extract was able to remove 0^6-methylguanine but not 7-methylguanine from methylated DNA. The mouse activity does not require divalent metal ions, and is active in the presence of 5 mM disodium EDTA. This characteristic is also exhibited by a rat liver chromatin protein which removes 0^6-ethylguanine (22) and by a crude E. coli factor which removes 0^6-methylguanine (23) from alkylated DNA. On the other hand, the assay of the activity of the crude rat liver extract was performed in the presence of Mg^{+2}, although an absolute requirement of divalent metal ions for activity was not demonstrated (21,30). Our mouse preparation is inactive at 4°C, in agreement with results obtained for the rat chromatin protein (22), although the E. coli factor was active in vivo at 0°C (31).

Our data show that during the repair of 0^6-methylguanine in vitro the partially purified mouse preparation causes the transfer of the methyl group to the sulfhydryl group of a cysteine residue of a protein, resulting in S-methyl-L-cysteine formation. The rat chromatin protein appeared to convert 0^6-ethylguanine to some other product which remained in DNA

(22), while the crude rat liver extract apparently demethylated either the alkylated DNA or an oligonucleotide derived from it, resulting in the appearance of free methanol (30). We have been unable to detect the presence of methanol under the conditions of our assay.

The kinetic characteristics exhibited by the mouse protein are somewhat unusual. The reaction is complete by 10 min, with the amount of 0^6-methylguanine removed being proportional to the amount of protein present in the reaction mixture. No additional release of 0^6-methylguanine occurred after 10 min, even though excess substrate was present. This could not be attributed to lability of the activity during the incubation. Increasing the substrate concentration did not effectively enhance further disappearance of 0^6-methylguanine; the absolute amount of 0^6-methylguanine removed remained the same at all substrate concentrations. These results suggest that the factor is inactivated during the incubation period, possibly caused by product inactivation. In contrast to our results, the rat chromatin protein was still active after 2 hr in the presence of excess ethylated DNA substrate, and the reaction rate depended on the concentration of 0^6-ethylguanine in the incubation medium (22).

That the factor may be product inactivated even in vivo is supported by a significant body of circumstantial evidence. In C57BL mice, 0^6-methylguanine was actively removed from liver DNA in vivo after low (12) but not high (27) doses of methylating agent. In rats, the rate of 0^6-methylguanine removal from DNA in various tissues in vivo was greater after low doses of methylating agent than after high doses (15,16). Pretreatment of rats with high doses of unlabeled methylating agent resulted in a decreased rate of removal of subsequently formed radiolabelled 0^6-methylguanine from liver DNA in vivo (32,33), and a decreased capacity of crude liver extracts to remove 0^6-methylguanine from alkylated DNA in vitro (34). These results were translated as meaning that those mouse and rat tissues which can remove 0^6-methylguanine from their DNA possess a finite capacity to do so, that is, they have the ability to remove only a fixed amount of 0^6-methylguanine.

In E. coli, a similar situation exists. This organism possesses a highly-inducible repair system resulting in an effective although limited ability to repair 0^6-methylguanine lesions. The system functions much less efficiently if the amount of DNA methylation is too great (9,35). It was suggested that the molecules responsible for the repair of 0^6-methylguanine might be expended after their interaction with the methylated

base, and therefore could act only once (24,31). This proposal is more
attractive since the finding of Olsson and Lindahl (24) that the E. coli
factor transfers the methyl group of 0^6-methylguanine of DNA to a protein
cysteine residue. Methylation of this amino acid residue could account for
the subsequent inactivation.

Our kinetic data can also be explained in an analogous fashion, The
apparent inactivation of the factor in the presence of substrate could
result from methylation of a reactive sulfhydryl group. This would explain
our failure to recover activity from a DNA-cellulose column containing
DNA alkylated with unlabeled MNU, the DNA of course containing substantial
amounts of 0^6-methylguanine. In this case, the factor might become in-
activated on the column. Assuming that a limited number of molecules of
the protein exist in vivo, the limited capacity of mammalian tissues to
remove 0^6-methylguanine from their methylated DNA can also be explained in
this manner. In fact, recent in vivo experiments suggest that the activity
responsible for removal of 0^6-methylguanine from methylated rat liver DNA
is inactivated during the reaction with 0^6-methylguanine (36).

It is still unclear whether the methyl acceptor removes the methyl
group directly from 0^6-methylguanine contained in methylated DNA, or if an
intermediate methyltransferase is involved. In either case, the supply of
methyl acceptor proteins could be the rate-limiting factor in the removal
of 0^6-methylguanine from methylated DNA (37). Further purification of the
activity, now in progress, is needed to clarify this situation. Neverthe-
less, our experiments with mouse liver preparations indicate an interesting
repair system which is analogous to that reported in E. coli (24).

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