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Characterization of the Major DNA Repair Methyltransferase Activity in Unadapted *Escherichia coli* and Identification of a Similar Activity in *Salmonella typhimurium*

G. WILLIAM REBECK, C. MARK SMITH, DALE L. GOAD, AND LEONA SAMSON*

Charles A. Dana Laboratory of Toxicology, Harvard School of Public Health, 665 Huntington Avenue, Boston, Massachusetts 02115

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***Escherichia coli* has two DNA repair methyltransferases (MTases): the 39-kilodalton (kDa) Ada protein, which can undergo proteolysis to an active 19-kDa fragment, and the 19-kDa DNA MTase II. We characterized DNA MTase II in cell extracts of an *ada* deletion mutant and compared it with the purified 19-kDa Ada fragment. Like Ada, DNA MTase II repaired O^6-methylguanine (O^6MeG) lesions via transfer of the methyl group from DNA to a cysteine residue in the MTase. Substrate competition experiments indicated that DNA MTase II repaired O^4-methylthymine lesions by transfer of the methyl group to the same active site within the DNA MTase II molecule. The repair kinetics of DNA MTase II were similar to those of Ada; both repaired O^6MeG in double-stranded DNA much more efficiently than O^6MeG in single-stranded DNA. Chronic pretreatment of *ada* deletion mutants with sublethal (adapting) levels of two alkylating agents resulted in the depletion of DNA MTase II. Thus, unlike Ada, DNA MTase II did not appear to be induced in response to chronic DNA alkylation at least in this *ada* deletion strain. DNA MTase II was much more heat labile than Ada. Heat lability studies indicated that more than 95% of the MTase in unadapted *E. coli* was DNA MTase II. We discuss the possible implications of these results for the mechanism of induction of the adaptive response. A similarly active 19-kDa O^6MeG–O^4-methylthymine DNA MTase was identified in *Salmonella typhimurium*.**

Methyl groups can be either added to or removed from DNA, and the enzymes responsible for these reactions are termed DNA methylases and methyltransferases (MTases). In *Escherichia coli* at least three known gene products transfer methyl groups to DNA: the *dam* and *dcm* DNA methylases are involved in mismatch repair systems, while the *hsd* methylase is involved in DNA restriction (18). These enzymes transfer methyl groups from *S*-adenosylmethionine to specific sites in DNA to form 5-methylcytosine and 6-methyladenine residues. The reactive methyl donor *S*-adenosylmethionine can also methylate DNA nonenzymatically at various other sites (1, 33). DNA repair MTases and other DNA repair enzymes can repair the methylated lesions introduced into the genome by *S*-adenosylmethionine and other alkylating agents (15). The first DNA repair MTase identified in *E. coli* was the product of the *ada* gene (26, 45), whose levels can be induced dramatically upon adaptation of cells with low doses of alkylating agents (11, 39). The 39-kilodalton (kDa) Ada protein removes methyl groups from O^6-methylguanine (O^6MeG), O^4-methylthymine (O^4MeT), and methylphosphotriester (MePT) DNA lesions (19, 20, 45). By repairing the premutagenic O^6MeG and O^4MeT DNA lesions, the Ada MTase provides the cell with resistance to mutation by methylating agents (11, 32, 39).

The exact mechanism of action of the Ada MTase is unclear, although it is known that the Ada protein has two separate active sites for the removal of distinct methyl lesions. Methyl groups from MePT lesions are transferred from damaged DNA to the cysteine-69 residue of the Ada MTase (42), and methyl groups from O^6MeG and O^4MeT lesions are transferred to the cysteine-321 residue (6). Transfer of methyl groups to each of these cysteine residues is irreversible. The *S*-methylcysteine residues formed at the two active sites within Ada prevent further DNA repair reactions, and thus Ada is termed a suicide enzyme (14). Mechanistically, Ada may share some features with other methylases and MTases of *E. coli*, with the activated cysteines acting as nucleophilic residues (38). Indeed, the two active sites of Ada are similar to the active site of the *E. coli* thymidylate synthase, the MTase responsible for the transfer of methyl groups from 5,10-methylenetetrahydrofolate to dUMP to form dTMP (6, 37).

In *E. coli*, chronic low-level DNA alkylation damage induces the adaptive response repair system (34); when Ada has transferred a methyl group from a MePT lesion to its cysteine-69 residue, it becomes a positive regulator of transcription of the *ada-alkB* operon (46), the *alkA* gene (46, 47), and the *aidB* gene (47). The *alkA* gene product, a DNA glycosylase, repairs the potentially lethal N^3-methylpurine and O^2-methylpyrimidine lesions (7, 10, 19). Thus, Ada provides cells with resistance to alkylation-induced mutations directly through the repair of O^6MeG and O^4MeT and with resistance to cell killing through the induction of *alkA*. The functions of the *alkB* and *aidB* gene products are not yet known. In addition to these inducible repair activities, *E. coli* also possess a second DNA glycosylase (Tag I) which removes N^3-methyladenine (but evidently no other lesions) from alkylated DNA but which is constitutively expressed (31).

We recently identified a second DNA repair MTase in *E. coli* that repairs O^6MeG and O^4MeT but not MePT lesions (29). This 19-kDa DNA MTase has been detected in two strains of *E. coli* bearing deletions of the *ada* gene (29, 43). Here we further characterize the MTase with respect to its mechanism of action, inducibility by DNA damage, repair kinetics, and heat stability. We found it to be the predominant DNA repair MTase in unadapted *E. coli* and identified a similar DNA repair MTase in *Salmonella typhimurium*.

* Corresponding author.

Potter et al. (28), using a functional assay for O^6MeG DNA repair MTases (17), recently cloned an *E. coli* gene (*ogt*) that encodes a 19-kDa O^6MeG DNA MTase; it seems likely that *ogt* encodes the DNA MTase described here.

MATERIALS AND METHODS

Bacterial strains. BS21 and BS23 are derivatives of F26, a *his thy* mutant strain of *E. coli* B/r. BS21 cells constitutively express high levels of Ada (41); BS23 is an *ada-alkB* deletion mutant derived from BS21 (29, 40). PM155 is a *leu* mutant strain of *S. typhimurium* LT2 (27).

Methylated DNA substrate preparation. *Micrococcus luteus* DNA (ICN Biomedicals, Inc., Costa Mesa, Calif.) containing O^6-[^{3}H]MeG as the predominant base lesion (refered to here as O^6MeG DNA substrate) was prepared as described previously (11) by using [^{3}H]methylnitrosourea (2.9 Ci/mmol; Dupont, NEN Research Products, Boston, Mass.); the specific activity of the alkylated DNA was 104 cpm/μg of DNA. Single-stranded O^6MeG DNA substrate was prepared by boiling double-stranded substrate in 10 mM Tris–1 mM EDTA (pH 7.5) for 10 min and then chilling the substrate on ice. Methylated poly(dT) was prepared as described previously (29), producing O^4MeT-MePT DNA substrate with a specific activity of 2,500 cpm/μg. Nonradioactive O^4MeT-MePT DNA substrate was prepared similarly by using 1 mM nontritiated methylnitrosourea. The presence of approximately 2% O^4MeT lesions in the radioactive substrate was confirmed by the transfer of radioactivity to the purified 19-kDa Ada fragment (data not shown). A 25-mer double-stranded DNA (dsDNA) oligonucleotide synthesized to contain a single O^4MeT lesion was obtained from Manjit Dosanjh and John Essigmann.

Purification of the 19-kDa Ada protein fragment. Approximately 3 mg of the 19-kDa form of the Ada protein was purified to apparent homogeneity from 190 g of *E. coli* BS21 cells by the method of Demple et al. (5).

Gel analysis of DNA MTase activity. Bacterial extracts were prepared from log-phase cultures in 50 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid–KOH (pH 7.8)–10 mM dithiothreitol–1 mM EDTA–5% glycerol (MTase buffer) as described previously (29). Extract proteins (100 to 200 μg) were incubated with 19 μg of O^6MeG DNA substrate (2,000 cpm) or 4 μg of O^4MeT-MePT DNA substrate (10,000 cpm) at 37°C for 30 min, and the extract proteins were separated by electrophoresis in a 12% polyacrylamide gel. The gel was cut into 2-mm-wide slices and counted for tritium (29).

Detection of *S*-methylcysteine. Human cell extracts were prepared as described previously (35). Cell extracts (containing about 3 mg of protein) that were reacted with O^6-[^{3}H]MeG DNA substrate were proteolytically digested to completion by incubation with protease (0.5 mg/ml at 37°C for 4 h) and then leucine aminopeptidase (0.1 mg/ml at 37°C overnight). Each hydrolysate was lyophilized and then suspended in 50 μl of 5 mM Tris (pH 7.5) and spotted onto Whatman 3MM chromatography paper (40 by 5 cm), which was developed in 1-propanol–H_2O (7:3) for about 18 h. The authentic *S*-methylcysteine marker was visualized in an adjacent lane after it was stained with a ninhydrin spray (0.2% in acetone). The chromatogram was dried and cut into 1-cm-wide strips which were eluted for 2 h at room temperature into 2 ml of H_2O and counted for tritium after the addition of 16 ml of Aquasol (Dupont, NEN Research Products).

Kinetics of O^6MeG DNA repair. The rate of DNA MTase-mediated O^6MeG repair from dsDNA and single-stranded DNA (ssDNA) was determined as described previously (14). BS23 extract (2 mg) was incubated at 37°C with 190 μg of O^6MeG DNA substrate in a total volume of 1 ml of DNA MTase buffer; 100-μl fractions were removed at various times. The kinetics of O^6MeG repair by the purified Ada protein fragment were determined in the presence of BS23 extract that was made from cells depleted of DNA MTase activity by exposure to 0.5 μg of *N*-methyl-*N'*-nitro-*N'*-nitrosoguanidine (MNNG) per ml for 1 h prior to extract preparation (see Results and Table 1).

Heat inactivation of DNA MTase. Cell extract protein (4 mg) in 1 ml of MTase buffer was heated to 55°C, and 100-μl fractions were transferred to ice-cold tubes at various times. The O^6MeG DNA substrate (2,000 cpm) was added to each fraction, which was then incubated at 37°C for 30 min. MTase activity was determined as described previously (16). The heat stability of the 19-kDa Ada protein was determined in BS23 extracts from cells depleted of DNA MTase activity as described above.

RESULTS

The 39-kDa Ada DNA MTase can undergo a specific proteolytic cleavage (44), and the 19-kDa carboxy-terminal peptide generated can repair O^6MeG and O^4MeT DNA lesions (5, 19, 45). A second 19-kDa O^6MeG-O^4MeT DNA repair MTase in *E. coli* was identified in cell extracts of *ada* deletion mutants (29, 43). Here, using cell extracts of *E. coli* BS23, a spontaneously arising *ada* deletion mutant, we characterized this *ada*-independent DNA MTase and compared it with the purified 19-kDa form of Ada.

Repair of O^4MeT prevents repair of O^6MeG by DNA MTase in BS23 cell extracts. Since DNA repair MTases act by covalently binding methyl groups from alkylated DNA, one can identify and assay these activities by reacting cell extracts with DNA containing the appropriate tritium-labeled methyl groups, separating the reacted extract proteins by polyacrylamide gel electrophoresis, and counting gel slices for tritium. Thus, the purified Ada fragment was detected as a 19-kDa protein that accepted methyl groups from O^6MeG (Fig. 1A). The O^6MeG DNA MTase activity identified in cell extracts from the *ada* deletion mutant *E. coli* BS23 was also 19 kDa, and a similar O^6MeG DNA MTase was found in cell extracts of *S. typhimurium* (Fig. 1B and C). All three 19-kDa MTases also accepted methyl groups from the O^4MeT-MePT DNA substrate (20, 29) (Fig. 1A to C). However, none of the MTases could accept methyl groups from this substrate if O^4MeT was removed by mild acid hydrolysis; i.e., these MTases could repair O^4MeT but not MePT lesions (20, 29) (data not shown).

We wished to determine whether methyl groups from O^6MeG and O^4MeT competed for the same DNA MTase-active sites in BS23 cell extracts, or whether the transfer of methyl groups from O^6MeG and O^4MeT lesions was to separate active sites, perhaps on different 19-kDa DNA MTases. If the same active sites could accept methyl groups from either O^6MeG or O^4MeT lesions, then the preincubation of BS23 cell extracts with a DNA substrate containing O^4MeT should deplete the capacity of this extract to subsequently repair O^6MeG. The preincubation of BS23 extracts with poly(dT) · poly(dA) DNA containing nonradioactive O^4MeT lesions (see above) abolished the ability of BS23 extracts to accept labeled methyl groups from O^6MeG (Fig. 2A). As expected, a similar depletion of MTase activity was observed when the Ada fragment was preincubated with DNA containing O^4MeT (Fig. 2B). MTase-depleted extracts

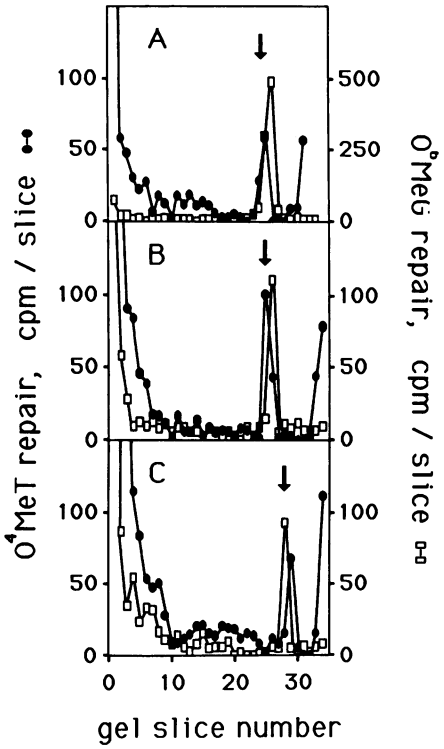

FIG. 1. Repair of O⁶MeG and O⁴MeT by 19-kDa bacterial DNA MTases. A total of 0.1 pmol of 19-kDa Ada (A), 100 μg of BS23 cell extract protein (B), or 100 μg of *S. typhimurium* LT2 cell extract protein (C) was incubated with 2,000 cpm of O⁶MeG DNA substrate (□) or 10,000 cpm of O⁴MeT-MePT DNA substrate (●) and separated by polyacrylamide gel electrophoresis. Radioactivity was recorded as counts above background. Slice 1 is the top of the gel. The counts at the bottom of the gels with O⁴MeT-MePT DNA substrate were not due to the transfer to protein (29). Arrows indicate the locations of the 19-kDa proteins.

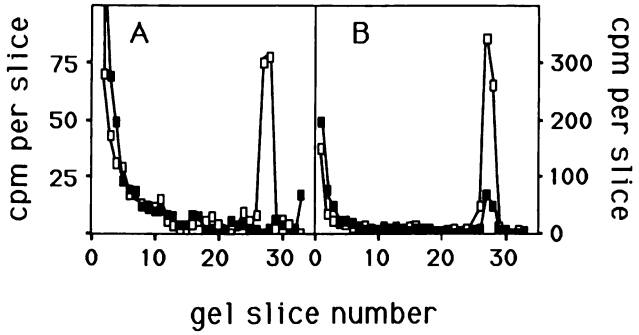

FIG. 2. Depletion of O⁶MeG DNA repair activity by O⁴MeT repair. A total of 200 μg of BS23 cell extract protein (A) or 0.4 pmol of 19-kDa Ada (B) was incubated for 30 min at 37°C with nonradioactive O⁴MeT-MePT DNA substrate (■) or with unalkylated poly (dT) · poly(dA) (□). After the addition of 2,000 cpm of O⁶MeG DNA substrate, each sample was incubated at 37°C for an additional 30 min. Proteins were separated and counted for tritium as described in the text. Residual Ada O⁶MeG DNA repair activity was presumably due to incomplete competition by the O⁴MeT-MePT DNA substrate.

were compared with extracts preincubated with unalkylated poly(dT) · poly(dA) DNA, which retained full MTase activity (Fig. 2A and B). A similar depletion of MTase activity was observed when BS23 cell extracts or purified 19-kDa Ada fragments were preincubated with a dsDNA oligonucleotide synthesized to contain a single O⁴MeT lesion (data not shown). These results are consistent with the existence of a single 19-kDa DNA MTase in BS23 cell extracts with a single active site for the repair of either O⁶MeG or O⁴MeT lesions; we refer to this activity as DNA MTase II. We cannot, however, exclude several more complicated models, including the existence of two 19-kDa DNA MTases in BS23 cells, both of which repair O⁶MeG and O⁴MeT, or the existence of one DNA MTase in BS23 cells with two active sites which interact in some manner to inhibit DNA repair.

Formation of *S*-methylcysteine in DNA MTase II. The transfer of a methyl group from methylated DNA to the active site of Ada results in the formation of an *S*-methylcysteine residue within the Ada protein (26). Since DNA MTase II also appears to transfer methyl groups to itself, we determined whether *S*-methylcysteine was also formed in DNA MTase II. After reaction with a radioactive O⁶MeG DNA substrate, BS23 extract proteins were enzymatically hydrolyzed to amino acids and chromatographed to identify *S*-methylcysteine. A radioactive peak that comigrated with *S*-methylcysteine was seen in reacted BS23 extracts when separated by paper chromatography (Fig. 3C) or by high-pressure liquid chromatography (data not shown) (21). The formation of *S*-methylcysteine is most likely associated with the 19-kDa DNA MTase II activity, because this is the only component of BS23 cell extracts which accepts radioactive methyl groups from alkylated DNA. Thus, like Ada, the repair of O⁶MeG by DNA MTase II probably involves the formation of *S*-methylcysteine residues. Since no bacteria have been isolated that completely lack DNA MTase activity, we used extracts from DNA MTase-deficient S3-7 HeLa cells (35) as a negative control for the formation of *S*-methylcysteine; as a positive control, we used extracts from S3-9 HeLa cells, which express high levels of the *E. coli* Ada protein (35). *S*-Methylcysteine is not formed in MTase-deficient extracts (Fig. 3A) and is formed, as expected, in Ada MTase-rich extracts (Fig. 3B).

Lack of induction of DNA MTase II in BS23 cells. When wild-type *E. coli* cells are treated with nontoxic levels of methylating agents, the level of the Ada protein is induced dramatically (11, 39). We tested whether DNA MTase II is induced in BS23 cells exposed to various doses of MNNG or methyl methanesulfonate (MMS) for 1 h. (Both MNNG and

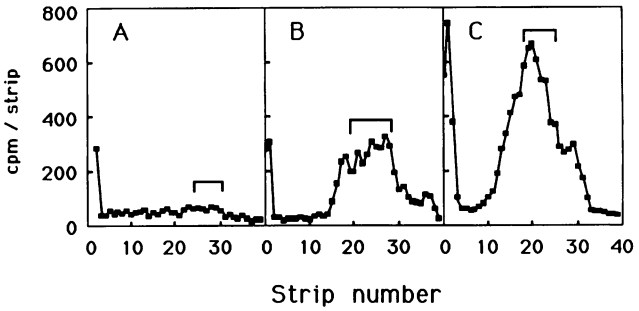

FIG. 3. Formation of *S*-methylcysteine in DNA MTase II. A total of 3 mg of cell extract protein from DNA MTase-deficient S3-7 cells (A), S3-9 cells expressing the Ada MTase (B), or *E. coli* BS23 cells (C) was incubated with 10,000 cpm of O⁶MeG DNA substrate. Proteins were enzymatically hydrolyzed to amino acids, which were separated by paper chromatography, eluted from 1-cm-wide strips, and counted for tritium. The brackets mark the position of authentic *S*-methylcysteine.

TABLE 1. Depletion of DNA MTase II by MNNG or MMS

Pretreatment (μg/ml) at 1 h	fmol of DNA MTase/μg of BS23 extract[a]
None	0.54 ± 0.13
MNNG	
0.005	0.58 ± 0.10
0.01	0.68 ± 0.07
0.1	0.66 ± 0.21
0.5	0.01 ± 0.01
MMS	
26	0.28 ± 0.06
129	0.19 ± 0.03
625	0.04 ± 0.02

[a] Values represent the averages of two to five DNA MTase activity determinations.

MMS can induce the genes of the adaptive response in *E. coli* [12, 47].) At the lowest doses tested, there was no significant induction of DNA MTase II by MNNG or MMS (Table 1). At higher doses of MNNG and MMS, the level of active DNA MTase II decreased (Table 1), presumably because of a depletion of the suicide MTase. Thus, unlike Ada, DNA MTase II is not induced in response to chronic low-dose exposure of BS23 cells to methylating agents under the conditions tested.

Kinetics of O⁶MeG repair by DNA MTase II. The purified 19-kDa fragment of Ada has been shown to transfer methyl groups in vitro at a very much faster rate from dsDNA than from ssDNA (14). We measured the reaction rates for DNA MTase II acting on dsDNA and ssDNA and compared these repair kinetics with those of the 19-kDa Ada fragment. DNA MTase II transferred methyl groups from dsDNA about 20-fold faster than it did from ssDNA (Fig. 4A). The Ada fragment also showed the expected strong preference for dsDNA over ssDNA (Fig. 4B). In order to compare the O⁶MeG repair kinetics of the purified Ada fragment with those of DNA MTase II in BS23 extracts, reactions with the Ada protein were carried out in extracts from BS23 cells

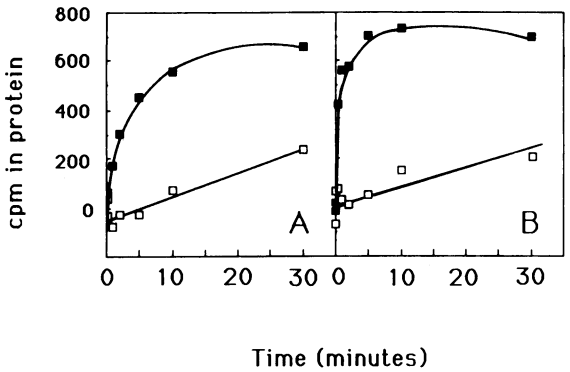

FIG. 4. Kinetics of repair of O⁶MeG in dsDNA and ssDNA. A total of 4 mg of BS23 cell extract protein (A) or 3 pmol of 19-kDa Ada in BS23 cell extracts depleted of DNA MTase II (B) was incubated at 37°C with 20,000 cpm of dsDNA (■) or ssDNA (□) O⁶MeG DNA substrate. Samples of 400 μg were removed at the indicated times and transferred to 0.8 M trichloroacetic acid to precipitate DNA and proteins. Unreacted O⁶MeG bases were hydrolyzed for 30 min at 70°C, and the hydrolysate was counted for tritium. MTase activity was recorded as a loss of hydrolyzable counts from the precipitate.

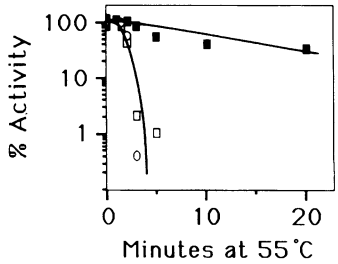

FIG. 5. Inactivation of DNA MTase II and 19-kDa Ada at 55°C. A total of 800 μg of BS23 cell extract protein (□), 800 μg of F26 cell extract protein (○), or 0.3 pmol of 19-kDa Ada in 800 μg of MTase-depleted BS23 cell extract protein (■) was diluted to 400 μl in DNA MTase buffer (see text). Samples were incubated at 55°C for the indicated times and then transferred to ice-cold tubes. After the addition of 2,000 cpm of O⁶MeG DNA substrate, each sample was incubated at 37°C for 30 min, and MTase activity was measured as described in the text. Background activity, which was measured by using samples of 100 μg of bovine serum albumin, was typically 40 to 60 cpm. Activity of 100% at time zero represented the transfer of 150 to 200 cpm above background.

depleted of DNA MTase II activity by a pretreatment with 0.5 μg of MNNG per ml (see Table 1 and Materials and Methods). This type of comparison was important because the 19-kDa Ada fragment reacted more slowly in crude cell extracts than in buffer alone (data not shown). On average, in cell extract at 37°C, DNA MTase II and Ada both removed O⁶MeG lesions from dsDNA with half-lives of 1.5 to 3 min (averaged data not shown).

Heat stability of DNA MTase II. Since the purified 19-kDa Ada protein is relatively heat stable (5), we determined whether one could differentiate between the Ada fragment and DNA MTase II by their heat labilities. The inactivation of Ada and DNA MTase II in BS23 cell extracts at 55°C is shown in Fig. 5; relative to Ada, DNA MTase II was extremely heat labile. Indeed, after 3 min at 55°C, the activity of DNA MTase II was nearly undetectable, while the activity of the Ada fragment remained unaffected (Fig. 5).

Unadapted *E. coli* F26 contains 30 to 40 molecules of 19-kDa MTase per cell, as judged from the activity in cell extracts (22, 29). We used heat inactivation to determine how many of these molecules were DNA MTase II and how many were derived from the 39-kDa Ada protein. All the MTase activity in extracts from nonadapted wild-type F26 cells appeared to be heat labile (Fig. 5). Taking into account the sensitivity of our assay, we estimated that at least 95% of the MTase activity in unadapted *E. coli* was DNA MTase II.

DISCUSSION

E. coli possesses three separate pathways for the repair of the premutagenic O⁶MeG lesion: the *uvr* excision repair pathway (36), the inducible Ada protein, and DNA MTase II. These overlapping pathways of repair are apparently regulated separately and could provide the cell with mutation resistance under various conditions of exposure to DNA-alkylating agents. However, DNA MTase II does not appear to fulfill any DNA repair function that the 19-kDa Ada protein could not; heat lability and induction in response to chronic exposure to alkylating agents are the only features that distinguish the two proteins. It should be noted that our data for the lack of induction of DNA MTase II in BS23 cells could be explained by two models: (i) DNA MTase II is constitutively expressed in *E. coli* or (ii) DNA

MTase II is induced as part of the adaptive response and we did not observe the induction because BS23 cells were *ada* mutants. The following evidence indicates that none of the genes currently known to be regulated by Ada encode DNA MTase II: (i) DNA MTase II is expressed in *E. coli* BS23, which is deleted for both the *ada* gene and the *alkB* gene; (ii) DNA MTase II is expressed in an *ada aidB* double mutant strain of *E. coli* (data not shown); and (iii) the *alkA* gene product is a DNA glycosylase and does not repair O^6MeG (7). The cloned DNA MTase II gene can be used to determine directly whether it is regulated in response to DNA damage.

We estimate that *S. typhimurium* constitutively expressed about 30 molecules of O^6MeG-O^4MeT DNA MTase per cell. A saturable O^6MeG DNA repair function was first suggested by the mutation and in vivo alkylation repair studies of Guttenplan and Milstein (9); *S. typhimurium* exposed to low levels of MNNG did not accumulate O^6MeG DNA lesions or mutations, but above a certain dose the accumulation was linear, presumably because the O^6MeG DNA MTase was consumed.

Unadapted wild-type *E. coli* F26 also expressed about 30 to 40 molecules of DNA MTase per cell, predominantly in the form of DNA MTase II. Indeed, immunoblots of unadapted *E. coli* extracts with polyclonal antibodies to the Ada protein did not display a 39- or a 19-kDa protein (45) (data not shown). The existence of at most one or two molecules of Ada per cell raises the question of how the adaptive response is induced, since the methylated Ada protein is believed to be required for its induction (46). Adaptation is a relatively slow process, taking at least 60 min of chronic exposure to alkylation before the culture attains maximal resistance and the expression of the Ada regulon is at its highest level (4, 24, 34). The extremely low levels of Ada might be responsible for the slow induction of the regulon; alternatively, if the Ada protein is, in fact, absent from nonadapted cells, there may be some other signal which initially induces the adaptive response slightly and the methylated Ada protein may only act to amplify and maintain the induction. However, *ada*::*lacZ* fusions in *E. coli* strains deficient in the Ada protein show no induction of β-galactosidase after MNNG pretreatments (4, 13), suggesting that any *ada* induction is almost entirely dependent on the Ada protein. The use of *ada*::*lacZ* and *alkA*::*lacZ* fusions in strains altered in the expression of DNA MTase II could help to determine whether DNA MTase II is involved in the induction of the adaptive response.

The repair of O-methylated lesions in DNA by an irreversible transfer of the methyl group to suicide DNA MTases is emerging as a ubiquitous form of DNA repair, having been found in mammals (2, 3), fish (25), *Drosophila melanogaster* (8), yeasts (M. Sassanfar, S. Coyne, and L. Samson, manuscript in preparation), and bacteria (15, 26). Several different types of inducible and constitutive DNA MTases have been found in a number of bacterial species, namely, *E. coli* (29, 39), *Bacillus subtilis* (23), *M. luteus* (30), and *Rhizobium meliloti* (Allan Kaufman and Graham Walker, personal communication); and we report here the existence of a constitutive DNA MTase in *S. typhimurium*. The bacterial DNA MTases are capable of repairing O^6MeG, O^4MeT, and MePT lesions; the inducible *E. coli* Ada DNA MTase can repair all three lesions, while the other MTases can repair only one or two of the lesions. So far, it appears that MePT lesions are exclusively repaired by inducible MTases, while O^6MeG and O^4MeT can be repaired by both constitutive and inducible MTases. It remains to be determined whether the use of constitutive and inducible DNA alkylation repair enzymes with overlapping substrate specificities is common among different cell types.

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