Volume 14 Number 14 1986 Nucleic Acids Research

Mutant *Escherichia coli* Ada proteins simultaneously defective in the repair of O^6-methylguanine and in gene activation

Bruce Demple

Department of Biochemistry and Molecular Biology, Harvard University, 7 Divinity Avenue, Cambridge, MA 02138, USA

Received 10 June 1986; Accepted 23 June 1986

Abstract

The activated Ada protein triggers expression of DNA repair genes in Escherichia coli in response to alkylation damage. Ada also posesses two distinct suicide alkyltransferase activities, for O^6-alkylguanines and for alkyl phosphotriesters in DNA. The mutant Ada3 and Ada5 transferases repair O^6-methylguanine in DNA 20 and 3000 times more slowly, respectively, than the wild-type Ada protein, but both exhibit normal DNA phosphotriester repair. These same proteins also exhibit delayed and sluggish induction of the ada and alkA genes. Since the C-terminal O^6-methylguanine methyltransferase domain of Ada is not implicated in the direct binding of specific DNA sequences, this part of the Ada protein is likely to play an alternative mechanistic role in gene activation, either by promoting Ada dimerization, or via direct contacts with RNA polymerase.

INTRODUCTION

Exposure of growing Escherichia coli bacteria to low concentrations of alkylating agents induces high-level resistance to both the toxic and the mutagenic effects of much higher alkylation doses (1). This adaptive response to alkylation results from the elevated levels of two DNA repair activities and the induction of at least two genes of unknown function (2). Lethal damages in alkylated DNA are repaired by an inducible DNA glycosylase, the product of the alkA gene (3), which removes N3-alkylated purines (4) and O^2-alkylated pyrimidines (5).

Alkylation-induced mutagenesis is averted by the intervention of an unusual DNA repair system. An inducible protein, the product of the E.coli ada gene (2,6) scavenges the methyl (alkyl) groups from the O^6 position of O^6-methylguanine (O^6MeG) and the O^4 position of O^4-methylthymine, transferring these groups irreversibly to one of the Ada protein's own cysteine residues (7,8) near the polypeptide C-terminus (2). The O^6MeG methyl-

© IRL Press Limited, Oxford, England.

transferase activity can be liberated from the 39-kilodalton (kDa) Ada protein as a 19-kDa polypeptide (9) by proteolytic cleavage in cell-free extracts (6). The protease responsible for this specific cleavage has not been identified. Each O^6MeG-DNA methyltransferase appears to be used only once *in vivo* (10) and reactions with the purified protein exhibit suicide kinetics *in vitro* (11). An analogous suicide methyltransferase repairs O^6MeG in the DNA of mammalian cells, with the concomitant production of S-methylcysteine in the protein (12, 13, 14). The *E.coli* Ada protein also removes the methyl (alkyl) groups from methyl (alkyl) phosphotriesters (MeP) in DNA, using a separate suicide alkyl-transferase site on the protein (15, 16).

The induction of the adaptive response in *E.coli* is controlled in a positive fashion by the *ada* gene (17, 18, 19, 20). Self-alkylation at the (N-terminal) MeP-active site appears to be the signal that triggers Ada protein to bind specific DNA sequences, transcriptionally activating the *ada* and *alkA* genes (21). Thus, Ada functions in the repair of alkylated DNA bases, in the repair of DNA phosphotriesters and in gene control. Given these multiple functions, *ada* mutants are expected to fall into several categories depending on the activity affected. Some *ada* mutants induce substantial Ada protein in response to MNNG (N-methyl-N'-nitro-N-nitrosoguanidine) without inducing resistance to alkylation mutagenesis (6, 17). These strains were examined for altered O^6MeG methyltransferase activity. Two such repair mutants were identified in this way and, surprisingly, both are also defective in the regulation of the adaptive response. The basis of this defect in transcriptional control is discussed.

EXPERIMENTAL PROCEDURES

Chemicals and enzymes. MNNG, $Na_2S_2O_3$, O-nitrophenyl-β-galactoside, *Micrococcus luteus* DNA, and lysozyme were purchased from Sigma Chemical Company. [^{3}H-Methyl]-N-methyl-N-nitrosourea (declared specific activity 1.5 to 3 Ci/mmol) was supplied by Amersham International. O^6MeG-DNA methyltransferase was purified as described (9, 22). The rabbit anti-bacterial O^6MeG methyltransferase antiserum was a generous gift of I. Teo and T. Lindahl.

Bacterial strains and plasmids. Strain AB1157 was a stock of this laboratory. The ada⁻ strains (17) were from the E.coli Genetic Stock Center (B. Bachmann, curator). Strain GW5352 (ada10::Tn10) and plasmid pGW2622 bearing an ada::lacZ fusion (19) were kindly provided by P. LeMotte. Strain MV1902 (alkA::lacZ (λpSG1; ref. 23) was used for transduction (24) of the alkA::lacZ fusion, selecting for chloramphenicol resistance.

Methyltransferase substrates. M.luteus DNA containing $O^6[^3H]$MeG (1800 to 2100 cpm/pmol) as the predominant methylated base was made as previously described (22) and calibrated using O^6MeG methyltransferase standardized by a non-radioactive assay (25). The MeP methyltransferase substrate was prepared by treating the O^6MeG substrate with a 2-fold excess of the homogeneous 19-kDa O^6MeG methyltransferase for 20 min at 37°C, followed by phenol extraction and ethanol precipitation, and redissolved with 10 mM Tris·HCl, pH 7.5, 1 mM EDTA. This treatment removes all the O^6MeG and O^4-methylthymine methyl groups (5, 11), but not those linked to DNA phosphates (16).

Induction of Ada protein and preparation of cell extracts. Strains to be induced were grown to mid-log phase at 37°C in M9 medium (24) supplemented with 0.2% glucose, 0.1% casamino acids, 0.1 mM $CaCl_2$ and 1 µg/ml thiamine-HCl (supplemented M9). For small-scale extracts, MNNG was added to 0.25 µg/ml, incubation at 37°C continued, and 50-ml aliquots were removed to chilled tubes containing 50 ml M9 with 100 µg/ml chloramphenicol. The cells were collected by centrifugation, washed with M9 containing chloramphenicol, and the pellets frozen overnight at -80°C. Lysates were made according to Wickner et al. (26) using for the lysis buffer 50 mM Tris·HCl, pH 7.5, 0.25 M NaCl, 10 mM dithiothreitol, 1 mM EDTA, 10% sucrose.

Induction by MNNG on a large scale (2 l of culture) was performed in a similar manner, except that MNNG was added to 0.1 µg/ml for 45 min, followed by an additional 0.5 µg/ml for 45 min. $Na_2S_2O_3$ was then added to 25 µg/ml, the cells were harvested and washed with M9 salts, then frozen at -80°C. The resuspended cells (50 ml) were extracted either by the method described above, or by passage through a French pressure cell (2 passes at 10,000 psi). Extracts prepared by either procedure

yielded identical results.

Isolation of 19-kDa and 39-kDa Ada proteins. Large-scale extracts were treated with polymin P as described previously (9, 22) followed by precipitation with 450 g/l $(NH_4)_2SO_4$. The supernatant was removed, and the precipitate redissolved in approximately 1/10 the original sample volume using AcA54 buffer (50 mM Tris·HCl, pH 7.9, 10 mM dithiothreitol, 1 mM EDTA, 0.5 M NaCl, 5% glycerol). This material (3.5 ml to 7 ml) was chromatographed at 30 ml/h on a precalibrated column of Ultrogel AcA54 (2.4 cm x 205 cm) equilibrated with AcA54 buffer. Fractions (4.5 ml) were collected and assayed for O^6MeG methyltransferase. The active fractions corresponding to the 39-kDa and 19-kDa Ada proteins were pooled and concentrated by $(NH_4)_2SO_4$ precipitation (450 g/l), then stored frozen at -50°C. The Ada$^+$, Ada3 and Ada5 methyltransferases all retained full activity after multiple freezing/thawing cycles.

Methyltransferase assays. The extract samples in the O^6MeG-DNA methyltransferase assay (22) always comprised $\leq$0.1 of the total reaction volume. For kinetic experiments, reactions were initiated by the addition of enzyme extract to prewarmed (37°C) mixtures, and samples removed at the indicated times to chilled tubes containing an equal volume of 0.8 M trichloroacetic acid, then processed as described (9, 22).

MeP methyltransferase was assayed as described by McCarthy and Lindahl (16). The assay measures the transfer of labelled methyl groups from DNA to protein by their susceptibility to proteolysis. As observed by others (16), a plateau value of 50% repair was consistently reached for MePs.

Immunoblotting. Na-dodecyl sulfate-polyacrylamide (12.5%) gels were electroblotted onto nitrocellulose (Schleicher and Schuell BA85) and probed with rabbit anti-E.coli methyltransferase (19-kDa) antiserum as described (6). Goat anti-rabbit IgG linked to horseradish peroxidase (Bio-Rad) was used at 1/2000 dilution, and the bound antibody detected as recommended by the supplier.

Other methods. Protein was assayed by the method of Bradford (27). β-Galactosidase activity in bacterial cultures was determined according to Miller (24).

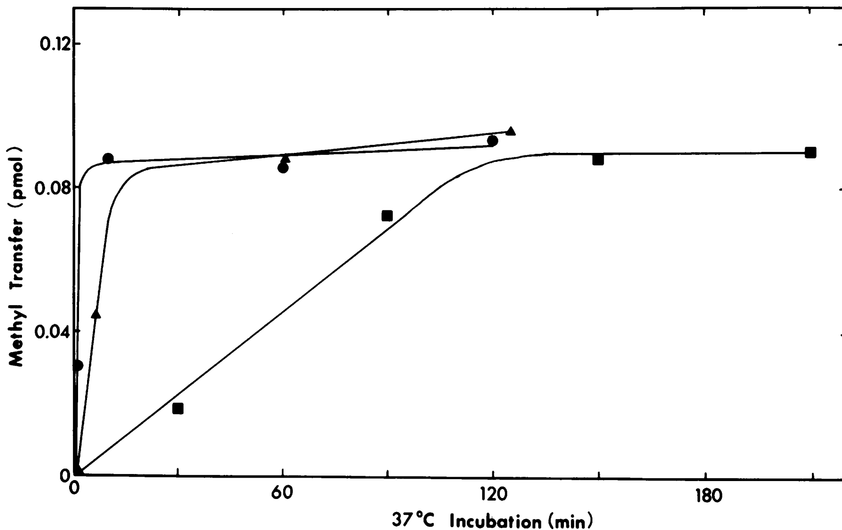

Figure 1. Kinetics of O^6MeG-DNA methyltransferase in crude extracts. Bacteria were induced with MNNG and extracts prepared and assayed as described in Methods. ●, AB1157 (ada+), 8.3 μg/sample; ▲, PJ3 (ada3), 51 μg/sample; ■, PJ5 (ada5), 31 μg/sample.

RESULTS

Defective O^6MeG-DNA methyltransferase in ada mutants.

The Ada protein is induced by MNNG in the ada3 and ada5 mutants (strains PJ3 and PJ5, respectively; ref. 17) as seen using antibodies directed against the homogeneous 19-kDa O^6MeG methyltransferase (6). Since both of these strains are sensitive to alkylation mutagenesis even after an inducing treatment (17), ada3 and ada5 are candidates for alleles that encode proteins defective in the repair of O^6MeG. This possiblity was tested by examining crude extracts of MNNG-treated PJ3 and PJ5 for the presence of O^6MeG methyltransferase activity. Cell-free extracts of the adapted mutants did contain active O^6MeG-DNA methyltransferase, but the mutant activities clearly differed from the wild-type enzyme (Fig. 1). The Ada+ O^6MeG-DNA methyltransferase in crude extracts had a half-time for methyl transfer of < 2 min, while the Ada3 activity required 15 to 20 min for repair of 1/2 of the O^6MeG methyl groups present. The Ada5 methyltransferase was even more impaired, achieving 50% transfer only after about 60 min (Fig. 1).

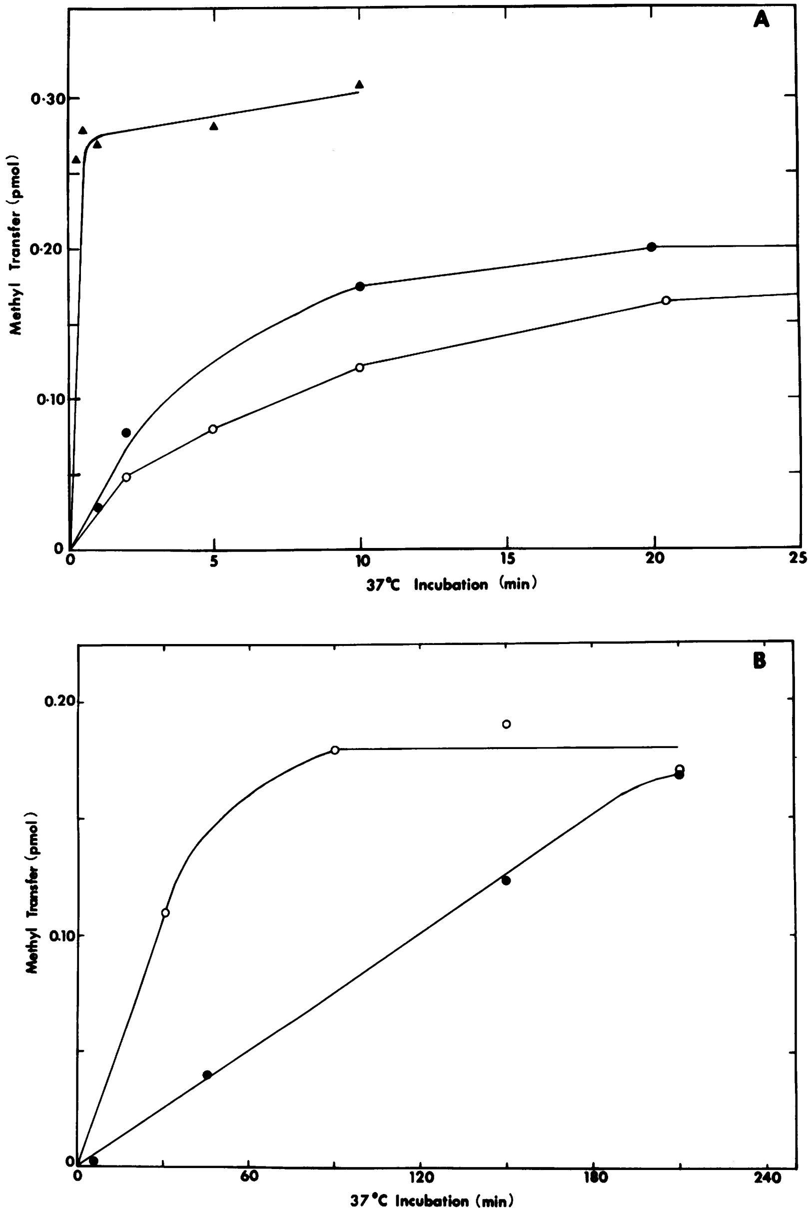

Figure 2. Kinetics of the 19-kDa and 39-kDa O^6MeG methyltransferases. Kinetic assays were conducted as described in Methods. A. Ada3 proteins. B. Ada5 proteins. ●, 19-kDa fraction; ○, 39-kDa fraction; ▲, purified 19-kDa Ada^+ methyltransferase (9).

The induced levels of active protein in extracts of the mutants (2 to 6 pmol/mg-protein, measured upon completion of methyl transfer) were only somewhat lower than wild type E.coli K12 (approximately 10 pmol/mg-protein). Comparable amounts of Ada protein were detected previously in immunoblotting experiments following adaptation of all three strains (6). The induced levels of Ada protein observed in the present experiments were only somewhat lower in the mutant than in the wild-type strains (see below). Thus, the Ada^{+}, Ada3 and Ada5 proteins each appear to take up 1 methyl group from O^6MeG per molecule. Thus, the Ada3 and Ada5 methyltransferases are notably defective in the rate of methyl group transfer, but not in its ultimate extent.

Location of the defect in the Ada3 and Ada5 proteins.

The kinetic defects in the mutant transferases could be intrinsic to the 19-kDa O^6MeG methyltransferase domain formed by the C-terminal half of the Ada polypeptide (2) or might result from mutations that affect other regions of the protein. To distinguish between these alternatives, the 19-kDa fragment generated in cell-free extracts (6) and 39-kDa form (intact) of each of the three methyltransferases were separated by gel filtration chromatography (9). Both the 39-kDa and the 19-kDa Ada3 proteins exhibited a 10- to 20-fold slower repair rate than the purified 19-kDa Ada^{+} methyltransferase (Fig. 2A). Analogous results were obtained with the induced Ada5 activity following gel filtration, except that both the 39-kDa and 19-kDa fractions were 3000- to 4000-fold slower than the wild type methyltransferase (Fig. 2B). The significance of the apparent kinetic difference between the 39-kDa and the 19-kDa Ada5 methyltransferases (Fig. 2B) is not known, but it is clear that both carry out repair much more slowly than the wild type protein. Similarly fractionated Ada^{+} methyltransferase exhibited kinetics indistinguishable from the purified 19-kDa Ada^{+} protein. The slow rates of methyl group transfer seen with Ada3 and Ada5 are therefore an intrinsic property of the mutant O^6MeG methyltransferase domains, and not the result of mutational changes elsewhere in the respective proteins.

The O^6MeG methyltransferase suicide kinetics result directly from the irreversible formation of S-methylcysteine at a unique

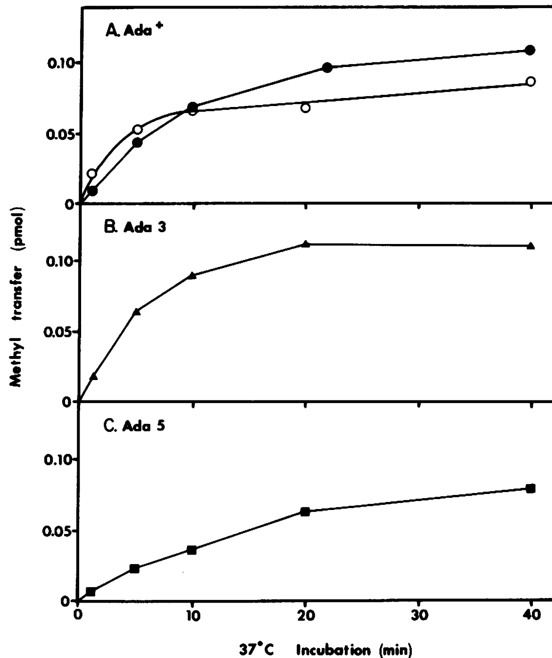

Figure 3. Methyl phosphotriester methyltransferase kinetics. Crude extracts of the MNNG-adapted strains were prepared as described in Methods. Each point represents an aliquot containing 0.22 pmol total MeP A. Wild-type Ada. ●, crude extract (100 μg protein per sample); ○, isolated 39-kDa fraction (1.6 μg per sample). B. Ada3 crude extract (80 μg per sample), ▲. C. Ada5 extract (40 μg per sample), ■.

active site in wild-type Ada protein (2,7,11). The 19-kDa forms of both the Ada3 and the Ada5 methyltransferases also appear to employ cysteine as the sole methyl group acceptor in O^6MeG repair (data not shown).

The DNA alkyl phosphotriester methyltransferase activity of Ada protein resides in the N-terminal portion of the polypeptide (16, 21). In contrast to O^6MeG repair, the rates of methyl group transfer from MeP in DNA are rapid and indistinguishable for the Ada^+, Ada3 and Ada5 proteins, both in crude extracts (Fig. 3) and in the isolated 39-kDa fractions (Fig. 3 and data not shown). The effects of the mutations in Ada3 and Ada5 thus seem to be confined to Ada's C-terminal 19-kDa O^6MeG repair domain.

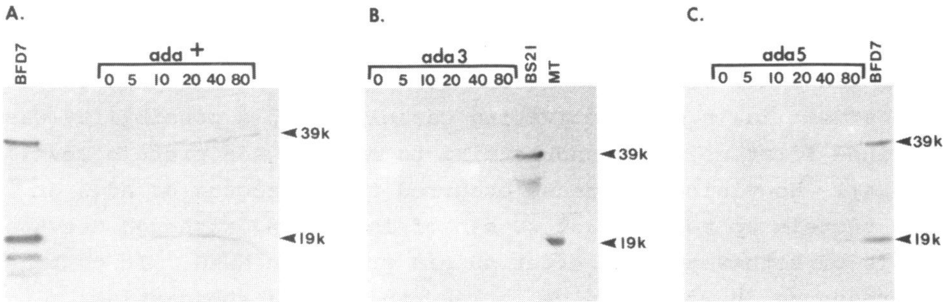

Figure 4. Induction of Ada protein by MNNG. Extracts were prepared at the indicated times, and samples containing 100 μg of protein were electrophoresed and immunoblotted as described in Methods. The induction time (min) is given above each lane. BS21 constitutively produces Ada protein (9); strain BFD7 harbors a plasmid with the cloned <u>ada</u>$^+$ gene (2); MT is the purified 19-kDa methyltransferase (0.1 μg). A. AB1157 (<u>ada</u>$^+$). B. PJ3 (<u>ada3</u>). C. PJ5 (<u>ada5</u>).

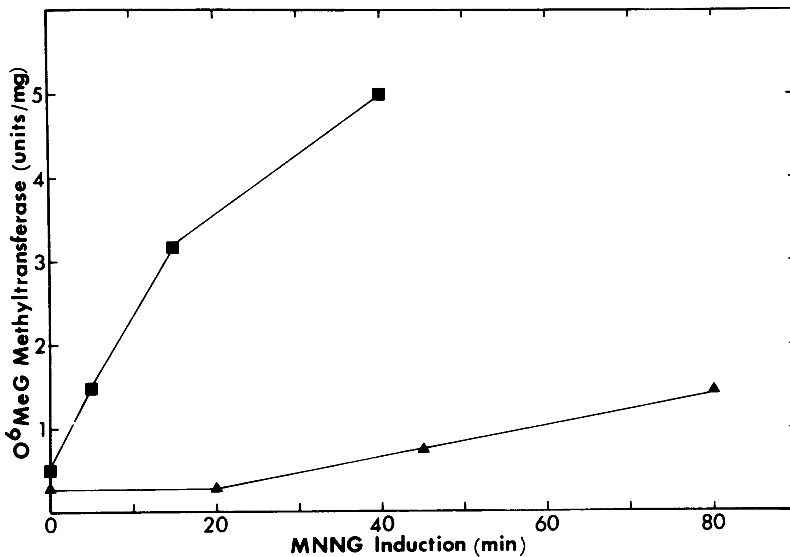

Figure 5. Induction of O^6MeG methyltransferase in <u>ada</u>$^+$ and <u>ada3</u>. Crude extracts were prepared from cells adapted with MNNG for the indicated times, and assayed as described in Materials and Methods. ■, AB1157 (<u>ada</u>$^+$); ▲, PJ3 (<u>ada3</u>).

Induction of Ada3 and Ada5.

It was of interest to determine whether the mutations that alter the O^6MeG methyltransferase function of the Ada protein also affect Ada's gene activating capacity. This possibility was examined first using immunoblotting to monitor Ada protein levels (Fig.4). No visible increase occurred in the amount of Ada3 or Ada5 protein up to at least 20 min of induction, although elevated levels of both were seen after 40 min growth in MNNG. In contrast, the response in wild type cells was rapid, with substantial induction of Ada^+ protein detectable after only 20 min (Fig.4). The induction of O^6MeG methyltransferase activity was measured directly in extracts of the ada^+ and ada3 strains, and a similar picture emerged (Fig.5). No increase in Ada3 methyltransferase activity was seen following 20 min of induction, a period sufficient to increase the Ada^+ activity 8-fold. Thus, gene regulation in both of the mutants appeared to be defective.

Gene regulation in ada3 and ada5.

The ada3 and ada5 mutations exhibited diminished trans activation of gene fusions. Expression of a plasmid-borne ada::lacZ fusion (19) increased within 10 min of exposure of ada^+ bacteria to MNNG, and β-galactosidase was elevated to very high levels in these cells over the next 60 min (Fig. 6A). In contrast, no induction of ada::lacZ was seen in the ada3 or ada5 strains during the first 20 min of MNNG exposure, after which time the mutants activated expression at about 1/10 the rate measured in ada^+ E.coli (Fig. 6A). A similar difference (10-fold) between ada^+ and ada3 or ada5 was observed at various inducing concentrations of MNNG (data not shown). These results have been confirmed with several independent isolates of PJ3 and PJ5 transformed with the ada::lacZ plasmid.

The mutant Ada proteins were also clearly deficient in activating an alkA::lacZ fusion present in the chromosome (23). Both the ada3 and ada5 alleles conferred sluggish induction of the fusion, again by a factor of about 10 compared to wild type E.coli (Fig. 6B). Impaired induction of an alkA::lacZ fusion in ada5 was reported previously (28). The exact degree of deficiency varied depending on the concentration of MNNG used for the induction, probably because these $alkA^-$ strains are exceptionally sensitive to alkyation killing (3). At every concentration,

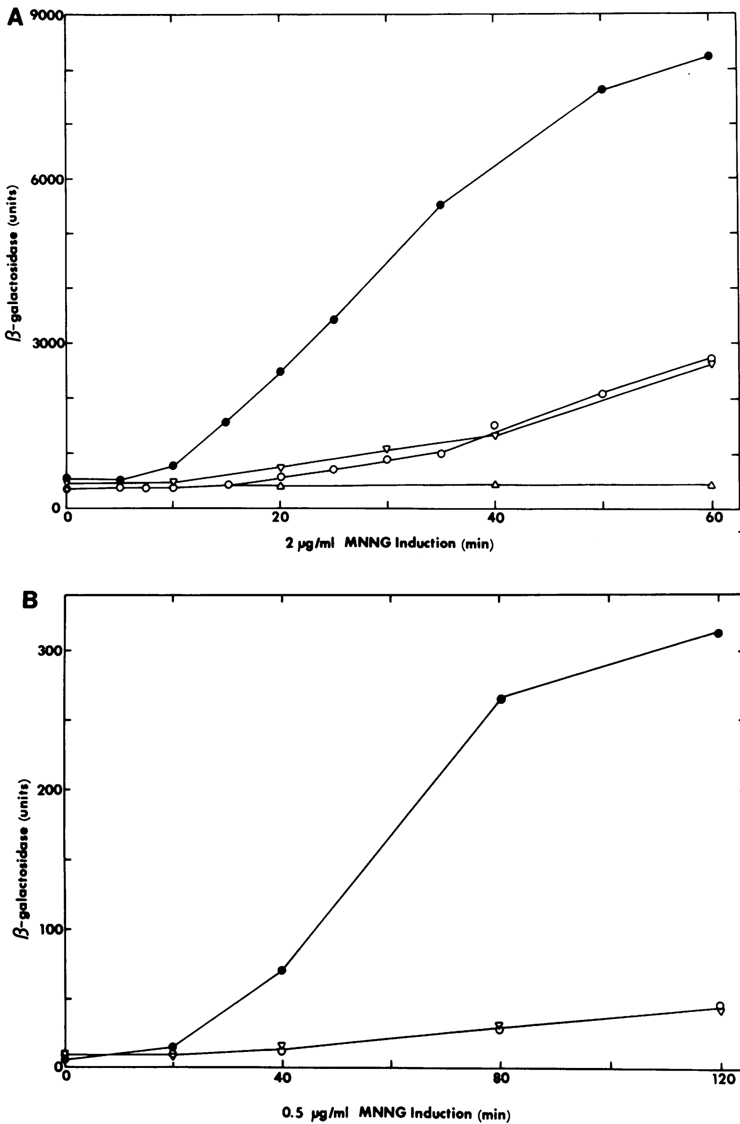

Figure 6. MNNG-induced expression of gene fusions. A. Strains bearing the plasmid pGW2622 (ada::lacZ) were grown in supplemented M9 medium containing 25 μg/ml kanamycin to 10^8 cells per ml and induced by the addition of 2 g/ml MNNG. B. Transductants with an alkA::lacZ fusion were grown in supplemented M9 medium containing 25 μg/ml chloramphenicol to 10^8 cells per ml and treated with 0.5 μg/ml MNNG. ●, ada^+; ○, ada3; ▽, ada5; △, ada::Tn10 (10 μg/ml tetracycline was added to the medium for this strain).

however, the induction was notably slower in the mutant strains. The ada3 and ada5 mutations therefore affect not only O^6MeG methyltransferase function, but also the regulatory ability of the Ada protein.

DISCUSSION

The Ada3 and Ada5 proteins each exhibit markedly reduced repair rates for O^6MeG in DNA, and this accounts in part for the sensitivity of the ada3 and ada5 mutants to the mutagenic effects of methylation damage. The two proteins are also defective in another Ada function, the transcriptional activation of the ada and alkA genes. A third activity of the Ada protein, the suicide repair of MeP in DNA, appears to be normal in these proteins.

The effectiveness of the adaptive response in averting alkylation mutagenesis and the known rate of replication fork movement in E.coli indicate that each O-alkylated guanine is repaired in <0.5 sec in adapted bacteria (29). Consistent with this expection, the homogeneous 19-kDa methyltransferase scavenges methyl groups in <2 sec at nanomolar concentrations of the protein and of O^6MeG (11). The sluggish Ada3 and Ada5 proteins are thus likely to constitute poor defences against mutagenic mispairing by O^6MeG during DNA replication. Indeed, attempted MNNG adaptation of an ada5 mutant confers no protection against MNNG mutagenesis (17), even though the level of Ada5 protein is increased (Fig.5; ref.6). The mammalian O^6MeG methyltransferases described so far (12,13,14) all repair O^6MeG in vitro significantly more slowly than the E.coli activity. The speed of repair required in animal cells may be slower, owing to the slower rate of DNA replication fork movement compared to bacteria.

Defects in methyltransferase activity.

All of the ada mutants examined to date either contain a normal O^6MeG-DNA methyltransferase activity that is not induced by MNNG (30) or an inducible methyltransferase that exhibits slow kinetics (Fig. 1). A strain bearing a Tn10 insertion in the N-terminal coding portion of the ada gene (19) also appears to retain O^6MeG repair activity (E. Loechler, personal communication). The absence of a methyltransferase-null mutant from this small collection could indicate a restricted target for mutagenic disruption of the activity. Alternatively, it seems possible

that the methyltransferase active center could perform some other (essential) function, with inactivating mutations rendering the cell inviable. Such a previously unsuspected role would also help to rationalize the unusual suicide mechanism of O^6MeG repair, although not the suicide activity for MeP. The construction of ada deletion mutants will help to answer this question.

Regulation of the adaptive response.

Recent evidence (21) indicates that activation of the adaptive response depends on the covalent self-methylation of the Ada protein resulting from the repair of MeP in DNA. Such methylated Ada protein activates *in vitro* transcription of the cloned *ada* and *alkA* genes and binds to specific sites near the 5' ends of those genes ("ada boxes"; ref. 21). Intact Ada protein or the isolated 19-kDa domain, methylated only at their O^6MeG repair sites, were ineffective in DNA binding and gene activation. This is consistent with the observation reported here, that the Ada3 and Ada5 proteins activate expression *in vivo* of *ada* and *alkA* equally (and severalfold more slowly than the Ada^+ protein), despite a 100-fold difference in their O^6MeG repair rates. There is thus no evidence for a quantitative linkage of the suicide repair of O-methylated bases and activation of the adaptive response.

Since the C-terminal 19-kDa domain of Ada does not bind the "ada box" sequences (21), an alternative role for it in positive control must be examined. By analogy to known examples (31), the C-terminal domain of Ada could mediate dimerization of the protein to increase DNA binding affinity, or directly contact RNA polymerase to mediate transcriptional activation. It is possible that self-methylation at the O^6MeG repair site in Ada enhances such functions. Indeed, the *in vitro* experiments (21) indicate that the doubly methylated protein (at both the N-terminal and the C-terminal active sites) is somewhat more effective in DNA binding and transcriptional activation than is Ada blocked only at the MeP repair site.

The Ada3 and Ada5 proteins offer attractive opportunities to study the mechanism by which O^6MeG-DNA methyltransferase recognizes and repairs O^6MeG and O^4-methylthymine in DNA. Additional information on the mechanism of positive control by activated Ada protein is also likely to be forthcoming from the

study of these mutant proteins. Towards these ends we are cloning the ada mutant alleles. The cloned genes will reveal the exact sequence alterations responsible for the mutant phenotypes, and provide us with sufficient amounts of the proteins for detailed mechanistic and structural studies.

ACKNOWLEDGEMENTS

I wish to thank P. Millirons for help in analyzing Ada5, and S. DePalma for help with some of the immunoblots. I am grateful to L. Samson, T. Maniatis and E. Loechler for discussions and critical reading of earlier drafts of the manuscript. This work was supported by start-up funds from the Department of Biochemistry and Molecular Biology, Harvard University.

REFERENCES

1. Samson, L., and Cairns, J. (1977) Nature (London) 267, 281-283.
2. Demple, B., Sedwick, B., Robins, P., Totty, N., Waterfield, M.D., and Lindahl, T. (1985) Proc. Natl. Acad. Sci. USA 82, 2688-2692.
3. Evensen, G., and Seeberg, E. (1982) Nature (London) 296, 773-775.
4. Karran, P., Hjelmgren, T., and Lindahl, T. (1982) Nature (London) 296, 770-773.
5. McCarthy, T.V., Karran, P., and Lindahl, T. (1984) EMBO J. 3, 545-550.
6. Teo, I., Sedgwick, B., Demple, B., Li, B., and Lindahl, T. (1984) EMBO J. 3, 2151-2157.
7. Olsson, M., and Lindahl, T. (1980) J. Biol. Chem. 255, 10569-10571.
8. Nakabeppu, Y., Kondo, H., Kawabata, S., Iwanaga, S., and Sekiguchi, M. (1985) J. Biol. Chem. 260, 7281-7288.
9. Demple, B., Jacobsson, A., Olsson, M., Robins, P., and Lindahl, T. (1982) J. Biol. Chem. 257, 13776-13780.
10. Robins, P., and Cairns, J. (1979) Nature (London) 280, 74-76.
11. Lindahl, T., Demple, B., and Robins, P. (1982) EMBO J. 1, 1359-1363.
12. Hora, J.F., Eastman, A., and Bresnick, E. (1983) Biochemistry 22, 3759-3763.
13. Harris, A.L., Karran, P., and Lindahl, T. (1983) Cancer Res. 43, 3247-3252.
14. Pegg, A.E., Wiest, L., Foote, R.S., Mitra, S., and Perry, W. (1983) J. Biol. Chem. 258, 2327-2333.
15. McCarthy, J.G., Edington, B.V., and Schendel, P.F. (1983) Proc. Nat. Acad. Sci. U.S.A. 80, 7380-7384.
16. McCarthy, T.V., and Lindahl, T. (1985) Nucleic Acids Res. 13, 2683-2698.
17. Jeggo, P. (1979) J. Bacteriol. 139, 783-791.
18. Sedgwick, B. (1983) Mol. Gen. Genet. 191, 466-472.

19. LeMotte, P.K., and Walker, G.C. (1985) J. Bacteriol. 161, 888-895.
20. Nakabeppu, Y., Mine, Y., and Sekiguchi, M. (1985) Mut. Res. 146, 155-167.
21. Teo, I., Sedgwick, B., Kilpatrick, M.W., McCarthy, T.V., and Lindahl, T. (1986) Cell 45, 315-324.
22. Demple, B., Karran, P., Jacobsson, A., Olsson, M., and Lindahl, T. (1983) In Firedberg, E.C., and Hanawalt, P.C. (eds), DNA Repair: A Laboratory Manual of Research Procedures Marcel Dekker, New York, Vol. 2, pp. 41-52.
23. Volkert, M.R., Nguyen, D.C., and Beard, K.C. (1986) Genetics 112, 11-26.
24. Miller, J.H. (1972) Experiments in Molecular Genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
25. Robins, P., Harris, A.L., Goldsmith, I.J., and Lindahl, T. (1983) Nucl. Acids Res. 11, 7743-7758.
26. Wickner, W., Schekman, R., Geider, K., and Kornberg, A. (1973) Proc. Nat. Acad. Sci. U.S.A. 70, 1764-1767.
27. Bradford, M. (1976) Anal. Biochem. 72, 248-254.
28. Volkert, M.R., and Nguyen, D.C. (1984) Proc. Nat. Acad. Sci. U.S.A. 81, 4110-4114.
29. Cairns, J. (1980) Nature (London) 286, 176-178.
30. Mitra, S., B.C. Pal, and Foote, R.S. (1982) J. Bacteriol. 152, 534-537.
31. Ptashne, M. (1986) A Genetic Switch. Gene Control and Phage λ. Cell Press and Blackwell Scientific Publications (Palo Alto, CA.)