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# *Escherichia coli* Gene That Controls Sensitivity to Alkylating Agents

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A new type of *Escherichia coli* mutant which shows increased sensitivity to methyl methane sulfonate but not to UV light or to gamma rays was isolated after mutagenesis with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. The mutant is unable to reactivate phage λ*vir* or double-stranded φX174 DNA (replicative form) that had been treated with methyl methane sulfonate. The mutant is sensitive to other alkylating agents, such as ethyl methane sulfonate, mitomycin C, and *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, as well. It grows normally and exhibits almost normal recombination proficiency. The mutant possesses normal levels of DNA polymerase I, exonuclease I, exonuclease V, endonuclease specific for methyl methane sulfonate-treated DNA, and 3-methyladenine-DNA glycosidase activities. The genetic locus responsible has been named *alk* and is located near *his* on the chromosome.

Alkylating agents produce various alkylated purine and pyrimidine adducts in DNA, as well as forming phosphotriesters and inducing apurinic sites and strand breaks (9). Some of such damages may be related to killing of cells and induction of mutation and cancer, although it has not been possible to specify the exact reaction sites resulting in particular biological effects.

DNA lesions induced by alkylating agents appear to be subjected to cellular repair mechanisms. It has been shown that *polA* and *recA*, *-B*, or *-C* mutants of *Escherichia coli* are sensitive to methyl methane sulfonate (MMS) and other alkylating agents (4, 5). Since these mutants are sensitive to UV and gamma radiation as well, it is likely that they are defective in some common steps in DNA repair. To analyze the precise process for repair of alkylated DNA and also to correlate the reaction products with the biological consequences, it seemed necessary to isolate mutants that are specifically sensitive to alkylating agents.

In this paper we describe the isolation and characterization of an *E. coli* mutant that exhibits increased sensitivity to MMS but not to UV or to gamma rays. The mutation was found to reside on a gene that has not been described before. The gene was named *alk* and is located near *his* on the *E. coli* chromosome.

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## MATERIALS AND METHODS

**Bacteria.** The bacterial strains used in these experiments are listed in Table 1. MS22, a histidine-requiring mutant of MS20, was isolated after mutagenesis with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG).

**Chemicals.** MMS and MNNG were purchased from Tokyo Kasei Kogyo Co., Tokyo, and ethyl methane sulfonate came from Eastman Kodak Co. Mitomycin C was donated from Kyowa Hakko Kogyo Co. Ltd., Tokyo.

**Media.** Nutrient broth (16) was used in most of the experiments. M9S medium contained 5.8 g of $Na_2HPO_4$, 3 g of $KH_2PO_4$, 5 g of NaCl, 1 g of $NH_4Cl$, 0.247 g of $MgSO_4 \cdot 7H_2O$, and 0.0147 g of $CaCl_2 \cdot 2H_2O$ per liter of water. The compositions of L broth and EMB sugar medium were described elsewhere (15). The MMS-containing plate was prepared immediately before use by spreading 0.1 ml of 1% MMS solution on the whole surface of nutrient agar (20 ml per plate) which had been incubated overnight at 37°C.

**Isolation of MMS-sensitive mutants.** Exponentially growing cells of *E. coli* W3623 were treated with 100 μg of MNNG per ml at 37°C for 15 min in 50 mM tris(hydroxymethyl)aminomethane (Tris)-maleate buffer, pH 6.0 (1). The cells were washed, resuspended in nutrient broth, and incubated overnight at 37°C. After appropriate dilution, bacteria were plated on nutrient agar, and colonies formed were transferred to MMS-containing plates by replica plating. After incubation overnight at 37°C, MMS-sensitive colonies were picked up from the master plate and purified.

**MMS sensitivity.** A 50 mM solution was prepared by dissolving 0.085 ml of MMS in 20 ml of M9S medium immediately before use. For the quantitative determination, bacteria were treated with various concentrations of MMS in M9S medium at 37°C. At times

TABLE 1. *Bacterial strains*

| Strain | Sex | Genotype or phenotype | Source or derivation |
|---|---|---|---|
| W3623 | $F^-$ | *trp gal strA* | Hirota |
| MS20 | $F^-$ | As W3623 but $MMS^s$ (*alk-1*) | MNNG treatment of W3623 |
| MS30 | $F^-$ | As W3623 but *recB* or *-C* | MNNG treatment of W3623 |
| MS40 | $F^-$ | As W3623 but *polA* | MNNG treatment of W3623 |
| MS22 | $F^-$ | As MS20 but *his* | MNNG treatment of MS20 |
| AB1157 | $F^-$ | *thr-1 leu-6 proA2 his-4 argE3 thi-1 lacY1 galK2 ara-14 xyl-5 mtl-1 tsx-33 strA31 supE44* | Adelberg |
| AB2277 | $F^-$ | *ilv-145 metE46 his-4 trp-3 pro-2 thi mtl-1 mal-1 ara-9 gal-2 lac-114* $T_1^r$ *str* | Adelberg |
| JC1553 | $F^-$ | *argG6 metB1 his-1 leu-6 recA1 mtl-2 xyl-7 malA1 gal-6 lacY1 str-104 tonA2 tsx-1* $\lambda^r$, $\lambda^-$ *supE44* | Clark |
| MS23 | $F^-$ | As AB1157 but $his^+$ *alk-1* | P1(MS20) × AB1157; $His^+$ selection |
| BW2001 | $F^-$ | As AB1157 but *xth* | Weiss (22) |
| JC7695 | $F^-$ | *endA gal thi* | Clark (8) |
| JC7694 | $F^-$ | As JC7695 but *sbcB15* | Clark (8) |
| BW7 | $F^-$ | $Mit^s$ (Mitomycin C sensitive) | Weiss (21) |
| BW46 | $F^-$ | As BW7 but *xonA1* | Weiss (21) |
| W2252 | HfrC | *metB*, injection order; *purE lac leu* | Hirota |
| MS21 | HfrC | $MMS^s$ (*alk-1*) | W2252 × MS20; $Met^+$ $Gal^+$ recombinant |
| X64 | HfrH | *lacZ-X64*, injection order; *thr leu lac* | Newton |
| BE5408 | Hfr | Prototroph, injection order; *his uvrC trp* | This paper |
| ED1039 | Hfr | *thi*, injection order; *uvrC trp* | Broda |
| KL98 | Hfr | Prototroph, injection order; *dsdA arsC* | Low |
| KL16 | Hfr | *thi rel*, injection order; *lysA thyA* | Low |
| CGSC4326 | F'150 | JC1553/F'$his^+$ $zwf^+$ | Bachmann |
| CGSC4246 | F'131 | JC1553/F'$his^+$ $supD43^+$ | Bachmann |
| CGSC4280 | F'129 | JC1553/F'$dsdA^+$ $his^+$ | Bachmann |

the cells were plated on nutrient agar after appropriate dilution and incubated overnight at 37°C.

As a convenient test, sensitivity was determined on plates with filter disks containing MMS. A loopful of an overnight culture was streaked from the periphery to the center on the surface of an L broth plate. A filter paper (6 mm in diameter) was placed in the center of the plate, and 10 μl of undiluted MMS solution was applied to the paper. After incubation overnight at 37°C, the degree of growth inhibition was compared. This test allowed the rapid examination of sensitivity of many strains on a single plate and was used for genetic mapping.

**Radiation sensitivity.** Exponentially growing cells were harvested, washed, and resuspended in M9S medium. A suspension of cells ($5 \times 10^7$ cells per ml) was irradiated with UV (Toshiba 15W germicidal lamp) or gamma rays.

**Host cell reactivation.** Phage λ*vir* ($3 \times 10^8$ plaque-forming units per ml) was suspended in M9S medium containing 50 mM MMS and incubated at 37°C. At various times, the suspension was diluted, and 0.1 ml of the dilution was plated on a nutrient broth agar plate with 0.2 ml of exponentially growing cells and 2.5 ml of nutrient broth top agar. After incubation overnight at 37°C, the number of plaques was counted.

φX174 RFI (replicative-form) DNA was prepared by the procedure described by Tanaka and Sekiguchi (17). RFI ($5 \times 10^5$ plaque-forming units per ml) was incubated in 10 mM Tris-hydrochloride–1 mM ethylenediaminetetraacetic acid (pH 7.8) containing 0 to 80 mM MMS at 37°C for 30 min. After treatment, each of the reaction mixtures was transferred to a Visking tube and dialyzed against two changes of 200 ml of 10 mM Tris-hydrochloride–1 mM ethylenediaminetetraacetic acid (pH 7.8) at 4°C for 16 h. The infectivity of the DNA was determined as follows. A 0.1-ml volume of DNA solution was added to 0.3 ml of a suspension of $CaCl_2$-treated cells ($10^{10}$ cells per ml) in 0.05 M $CaCl_2$, and the mixture was kept in an ice bath for 15 min and then incubated at 37°C for 5 min. At the end of the incubation period, the mixture was plated on a nutrient broth agar plate with 0.3 ml of exponentially growing culture of *E. coli* C ($2 \times 10^8$ cells per ml) and 3 ml of nutrient broth top agar. $CaCl_2$-treated cells were prepared according to Mandel and Higa (14).

**Conjugation.** Bacteria were grown in L broth to about $2 \times 10^8$ cells per ml. Mixtures of 10 $F^-$ cells to 1 Hfr cell were incubated at 37°C with slow reciprocal shaking for 120 min. Samples were withdrawn, mixed vigorously for 1 min, and plated on selective agar media.

For F duction, F'$his^+$ and $F^-$ cells were mixed at a ratio of 1:1 and shaken at 37°C for 120 min. The mixture was plated on selective agar containing tryptophan to allow the growth of the F ductants. The presence of F' was further confirmed by the cells' sensitivity to Qβ, MS2, or fd phages.

**Transduction.** Transduction was performed as described by Lennox (10), except that P1*vir* was used at a multiplicity of infection of about 0.2.

**Enzyme assays.** Bacteria were grown at 37°C in L broth (for endonuclease II and *N*-glycosidase assays) or yeast extract-phosphate-glucose medium (21) (for exonuclease I assay) and harvested in the logarithmic phase of growth. Cells were collected by a refrigerated centrifuge, resuspended in buffer (at a concentration of $10^{10}$ cells per ml), and disrupted in an ultrasonic disintegrator. After centrifugation at 10,000 × *g* for 20 min, the supernatant fluid was taken as a cell-free extract.

Preparation of $^{32}$P-labeled T4 phage DNA has been described (23). [$^{3}$H]adenine-labeled DNA, prepared from *E. coli* B *ade* grown in the presence of [2-$^{3}$H]-adenine, was alkylated as described by Lindahl (11). Immobilization of DNA in acrylamide gel beads was performed according to Wada and Kishizaki (19).

Exonuclease I was assayed according to Yajko et al. (21). Endonuclease II was measured by the procedure of Yajko and Weiss (22), using heavily alkylated DNA gel beads. *N*-glycosidase activity, which releases 3-methyladenine from DNA, was assayed according to Lindahl (11).

## RESULTS

**Isolation of MMS-sensitive mutants.** Strain W3623 was mutagenized by MNNG, and MMS-sensitive mutants were isolated by the replica-plating method. We selected six MMS-sensitive mutants with varied MMS sensitivity and examined their properties. One mutant, named MS20, was as resistant to UV as the parental strain, although the rest of the MMS-sensitive mutants examined were also sensitive to UV. Figure 1 compares sensitivity of MS20 and some other mutants to MMS, UV, and gamma rays. Unlike other mutants (*polA* and *recB* or *-C* mutants), MS20 was specifically sensitive to MMS. In addition, MS20 was more sensitive than its parent W3623 to ethyl methane sulfonate, MNNG, and mitomycin C (Fig. 2), all known alkylating agents.

The growth rate of MS20 was slightly lower than that of the parental strain W3623. However, strains to which the MMS-sensitive mutation (*alk*) was transferred by P1 transduction exhibited normal growth rates in L broth at 30, 37, and 42°C. Thus, the mutation does not seem to affect the growth rate of cells.

Strain MS20 possessed normal levels of DNA polymerase I and exonuclease V (*recBC* enzyme) activities (data not shown). The recombination proficiency of MS20 was almost normal when examined in a cross with HfrH (X64) strain (data not shown). These properties distinguish MS20 from *polA* and *recA, -B,* or *-C* mutants, which were also selected by the initial screening procedure.

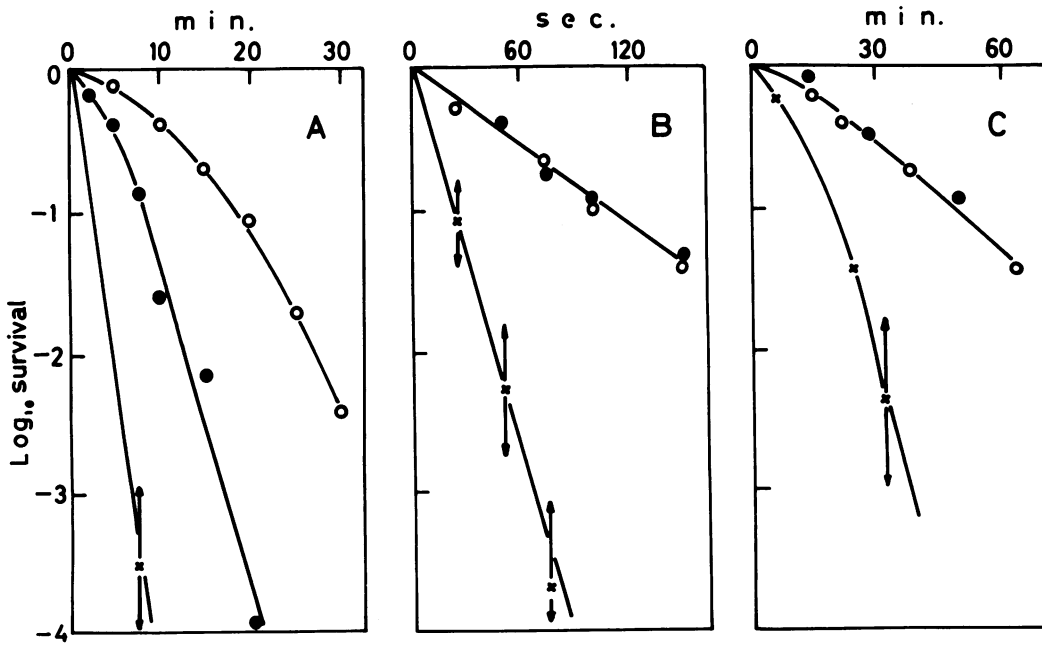


FIG. 1. *Survival of E. coli strains exposed to MMS, to UV, and to gamma rays. (A) Treatment with MMS; cells were suspended in M9S medium containing 50 mM MMS and incubated at 37°C for various times. (B) Irradiation with UV; cells were irradiated at room temperature (dose rate: 1.5 J/m$^2$ per s). (C) Irradiation with gamma rays; 1,900 Ci of $^{60}$Co was used as a source of gamma rays, and cells were irradiated (dose rate: 612 R/min).* (○) *W3623;* (●) *MS20 (alk);* (×) *MS30 (recB or recC), MS40 (polA).*

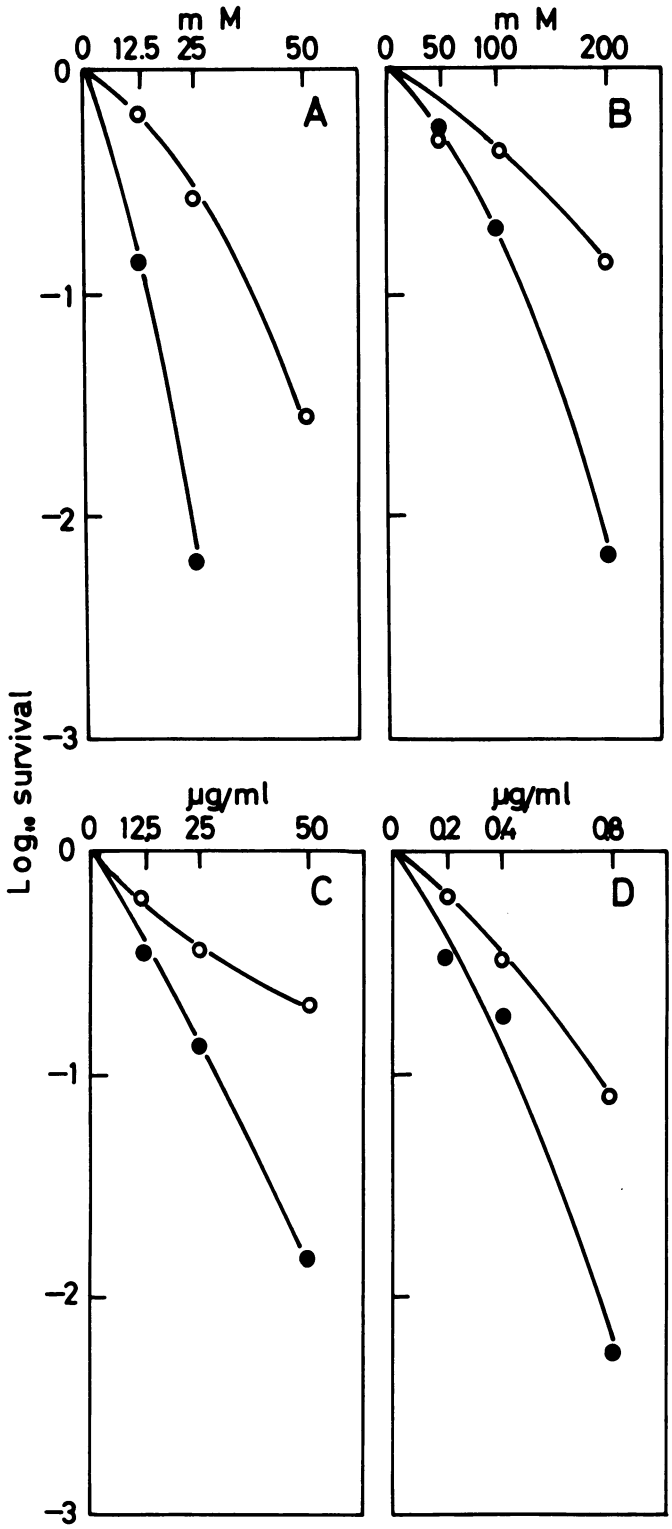


FIG. 2. *Survival of W3623 and MS20 strains after treatment with various alkylating agents. (A) Bacteria were suspended in M9S medium containing 0, 12.5, 25, or 50 mM MMS and incubated at 37°C for 30 min. (B) Bacteria were suspended in M9S medium containing 0, 50, 100, or 200 mM ethyl methane sulfonate and incubated at 37°C for 60 min. A 50 mM solution was prepared by dissolving 0.1 ml of ethyl methane sulfonate in 20 ml of M9S medium immediately before use. (C) Bacteria were suspended in 0.1 M citrate buffer (pH 5.5) containing 0, 12.5, 25, or 50 μg of MNNG per ml and incubated at 37°C for 60 min. (D) Bacteria were suspended in M9S medium containing 0, 0.2, 0.4, or 0.8 μg of mitomycin C per ml and incubated at 37°C for 20 min.* (○) *W3623 (alk⁺);* (●) *MS20 (alk).*

**Location of the mutation on the genetic map.** The locus of the mutation responsible for the increased sensitivity to alkylating agents (denoted as *alk*) on the chromosome was determined as follows.

**(i) Conjugation.** An HfrC strain carrying the *alk* mutation was constructed by crossing MS20 and W2252 HfrC and selecting Gal$^+$ Met$^+$ recombinants. The strain, MS21 (*str*$^+$ *alk*), was mated with AB1157 (*str alk*$^+$ and multiauxotrophic properties), and the resulting two types of recombinant, *arg*$^+$ *str* and *his*$^+$ *str*, were tested for their unselected markers. The results (Table 2) indicate that the *alk* mutation is linked to *his* more closely than to the other markers.

TABLE 2. *Recombination of MS21 with AB1157*[a]

| Recombinants | No. of recombinants examined | % of recombinants scored as | | | | |
|---|---|---|---|---|---|---|
| | | Pro$^+$ | Thr$^+$ Leu$^+$ | Arg$^+$ | His$^+$ | MMS$^s$ |
| Arg$^+$ Str$^r$ | 95 | 87 | 90 | · | 1 | 2 |
| His$^+$ Str$^r$ | 98 | 86 | 77 | 74 | | 50 |

[a] A mating mixture was prepared by mixing exponentially growing donor and recipient cells in L broth in a ratio of 1:10. After shaking for 120 min at 37°C, the mating was interrupted, and the mixture was diluted and plated onto minimal plates supplemented with nutritional requirements (except arginine or histidine) and streptomycin (100 μg/ml). The plate was incubated for 48 h at 37°C. Arg$^+$ Str$^r$ and His$^+$ Str$^r$ recombinants were tested for unselected markers.

In the next experiments, MS20 was crossed with Hfr strains which transfer their chromosomes from the region near *his* (for the origin and the direction of the transfer, see Fig. 3), and selection was made for *trp*$^+$ *str*. In the crosses with KL16, KL98, and BE 5408, a number of recombinants received the donor *alk*$^+$ character, whereas the cross with ED1039 yielded no *alk*$^+$ recombinant (Table 3). This indicates that the *alk* mutation is located between *his* and *uvrC* on the chromosome (Fig. 3).

**(ii) F duction.** MS22 (*his alk*) was mated with strains carrying various F′ episomes that carry genes in the *his* region of the chromosome, and *his*$^+$ F ductants obtained were then tested for their MMS sensitivity. All the F ductants receiving F′131 and F′150 were MMS$^r$, whereas those with F′129 were MMS$^s$ (Table 4). Therefore, the mutation site is between *supD* and *his* (Fig. 3). This result indicates also that the *alk* allele is recessive to the wild-type allele.

**(iii) P1 transduction.** A P1 lysate prepared on strain MS20 (*his*$^+$ *alk*) was applied to AB1157 (*his alk*$^+$) as a recipient. Of 100 *his*$^+$ transductants examined, 14 exhibited the *alk* character, indicating that the mutation is 14% cotransducible with *his*. In another experiment, in which the transduction was performed on AB2277, a 12.5% cotransduction with *his* was obtained.

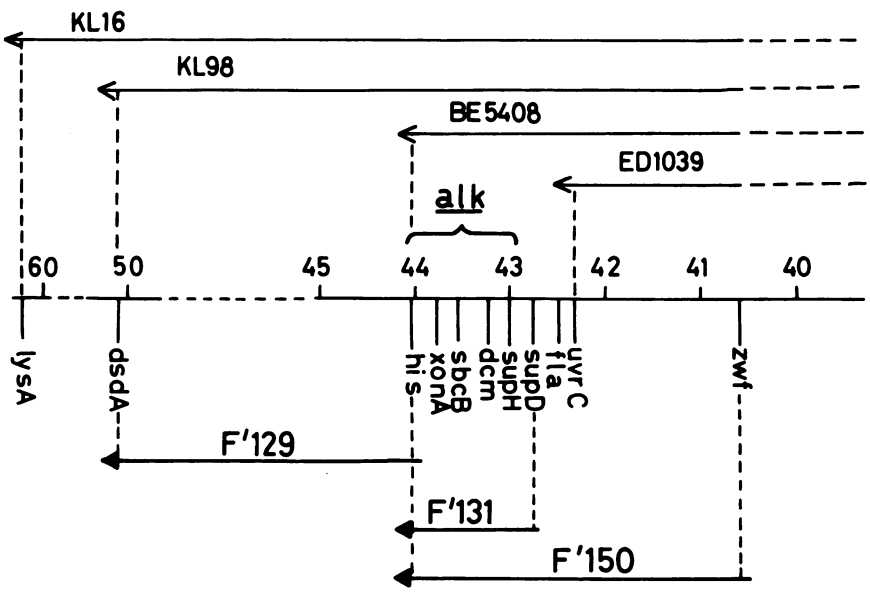


FIG. 3. *The position of alk and other genes and the regions covered by F′ factors. The origins and the directions of transfer of the chromosomes of Hfr strains KL16, KL98, BE5408, and ED1039 are also shown. The positions of genes other than alk are from Bachmann et al. (2).*

TABLE 3. *Recombination of MS20 with various Hfr strains*[a]

| Hfr | No. of recombinants scored | No. of MMS-sensitive cells | No. of MMS-resistant cells |
|---|---|---|---|
| KL16 | 100 | 72 | 28 |
| KL98 | 64 | 59 | 5 |
| BE5408 | 100 | 63 | 37 |
| ED1039 | 98 | 98 | 0 |

[a] MS20 was crossed with various Hfr strains. The mating was interrupted at 60 min (for a cross with KL16), 45 min (with KL98), or 30 min (with BE5408 and with ED1039). In each cross, $Trp^+$ $Str^r$ recombinants were selected, and their MMS sensitivity was determined.

TABLE 4. *Complementation test by F duction*[a]

| F' | No. of F ductants examined | MMS sensitivity | |
|---|---|---|---|
| | | Sensitive | Resistant |
| 129 | 9 | 9 | 0 |
| 131 | 6 | 0 | 6 |
| 150 | 3 | 0 | 3 |

[a] MS22 was mated with strain 4326 (F'150), 4246 (F'131), or 4280 (F'129) for 120 min at 37°C, and $His^+$ cells were selected. The cells that were sensitive to Qβ, MS2, or fd (male-specific phage) were tested for their MMS sensitivity on filter-disk plates.

Thus, it was concluded that the *alk* mutation is located between 43 and 44 min on the *E. coli* chromosome (Fig. 3). We propose the gene symbol *alk* (for *alk*ylating agent-sensitive) for this locus.

**Host cell reactivation.** There is a possibility that the *alk* mutant might be a permeability mutant that takes up MMS or other chemicals more efficiently than does the wild-type strain. Therefore, we next examined whether phage or DNA pre-exposed to MMS can be reactivated by the mutant. In these experiments AB1157 (*alk*$^+$) and its P1 transductant, MS23 (*alk*), were used; they are isogenic for all the loci except the *alk-his* region.

As shown in Fig. 4A, the *alk* mutant was less capable of reactivating MMS-treated phage λ*vir*. On the other hand, the mutant exhibited normal host cell reactivation capacity for UV-irradiated λ*vir* (data not shown).

A similar experiment was performed using infective phage DNA. Replicative-form DNA (RFI) of φX174 was treated with MMS, and infectivity was determined on $CaCl_2$-treated cells. As shown in Fig. 4B, more plaques were formed on AB1157 (*alk*$^+$) than on MS23 (*alk*). These results strongly suggest that the *alk* mutant is not a permeability mutant but may be defective in a process that is essential for the

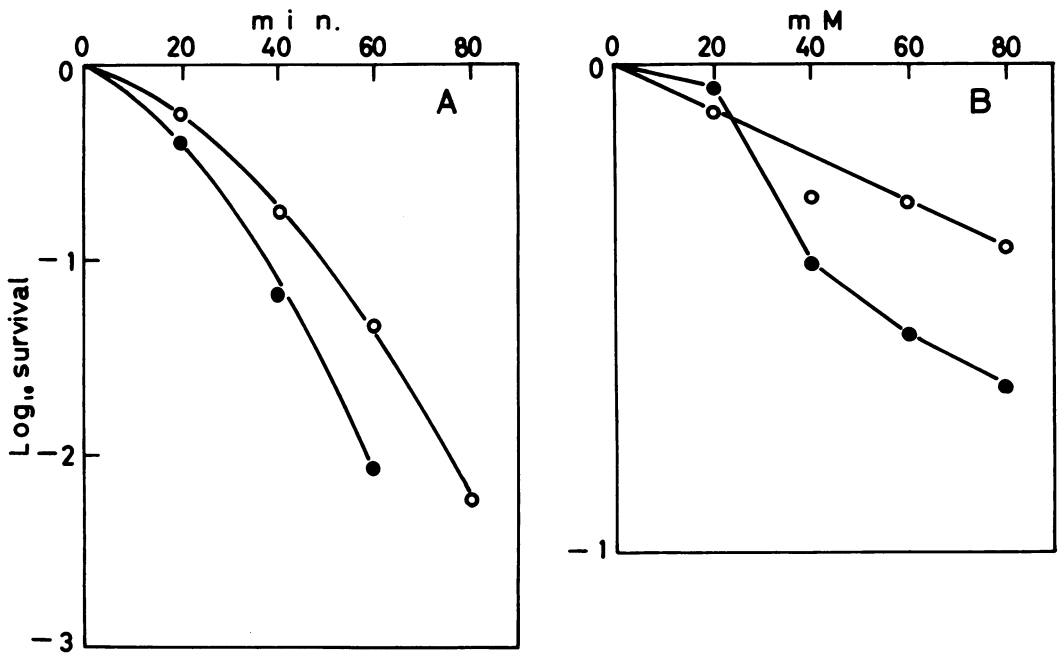


FIG. 4. *Host cell reactivation. (A) Surviving fraction of λvir exposed to MMS. λvir (3 × 10$^8$ plaque-forming units per ml) was incubated at 37°C in M9S medium supplemented with 50 mM MMS. At times the suspension was diluted and plated with bacteria. (B) Surviving fraction of φX174 RFI. DNA of φX174 (5 × 10$^5$ plaque-forming units per ml) was incubated at 37°C in 10 mM Tris-hydrochloride–1 mM ethylenediaminetetraacetic acid (pH 7.8) with 0 to 80 mM MMS for 30 min, and then dialyzed overnight. The infectivity of DNA was determined with $CaCl_2$-treated cells. (○) AB1157 (alk$^+$); (●) MS23 (alk).*

repair of MMS-induced damages in its own or phage DNA.

**Enzyme activities.** Among many loci within the region where *alk* is, *sbcB* and *xonA* must be considered, since it was reported that *sbcB* and *xonA* mutants are defective in exonuclease I activity (8, 21).

MS20 possesses a normal level of exonuclease I, whereas the *xonA* and *sbcB* mutants are deficient in enzyme activity (Table 5). The following properties of *xonA* and *sbcB* mutants also distinguish them from MS20. (i) The *xonA* and *sbcB* mutants show normal sensitivity to MMS. (ii) *sbcB* and *xonA* are 30 to 50% and 41 to 62% cotransducible with *his*, respectively (8, 21). Thus, the *alk* gene seems to differ from the *xonA* and the *sbcB* genes.

Since an endonuclease specific for alkylated DNA (endonuclease II) has been implicated in the repair of alkylated DNA, endonuclease II activity was determined by the procedure of Friedberg and Goldthwait (3, 19, 22), which measures the release of radioactive materials from alkylated DNA gel beads. However, no difference was observed between the activities of MS20 and the wild-type strain (Fig. 5A).

3-Methyladenine is the major alkylated base found in [$^3$H]adenine-labeled DNA after treatment with MMS. On incubation with an *E. coli* extract, 3-methyladenine was rapidly released from the DNA (7, 11). Figure 5B shows the release of 3-methyladenine by MS20 and W3623 extracts; there is essentially no difference in the activity. 1-Methyladenine was also released from the DNA, but its amount was too small to compare exactly the activity in the two types of extracts.

TABLE 5. *Exonuclease I activity of mutants*[a]

| Strain | Mutation | Exonuclease I (units/mg of protein) |
|---|---|---|
| W3623 | *alk*$^+$ | 20.3 |
| MS20 | *alk* | 20.4 |
| BW7 | *xonA*$^+$ | 18.2 |
| BW46 | *xonA* | 3.1 |
| JC7695 | *sbcB*$^+$ | 21.5 |
| JC7694 | *sbcB* | 9.0 |

[a] Reaction mixture contained 12.6 μmol of glycine-NaOH (pH 9.5), 1.2 μmol of $MgCl_2$, 1.2 μmol of 2-mercaptoethanol, 7.6 nmol of nucleotide of [$^{32}$P]T4 DNA ($1.5 \times 10^4$ cpm/nmol), and an extract of each strain (0 to 40 μg of protein) in 0.2 ml. The reaction was performed at 42°C for 30 min, and the radioactivity released into the acid-soluble fraction was determined. One unit was defined as the activity which catalyzed the release of 1.0 nmol of nucleotide in 30 min (21).

## DISCUSSION

In the present studies we have isolated a mutant, MS20, that is specifically sensitive to alkylating agents. The mutant exhibits increased sensitivity to MMS and other alkylating agents, but is as resistant to UV and gamma radiation as the parental strain. Lack of the host cell reactivation capacity for MMS-treated phage or infectious DNA strongly suggests that the mutant is defective in some step(s) in the pathway for repair of alkylated DNA.

A simple way of accounting for the existence of such mutants is to suppose that the repair of MMS-induced damages in DNA requires a specific reaction that is not necessary for the repair of UV- or gamma ray-induced damages. For instance, this step may be catalyzed by an enzyme which recognizes MMS-induced damages and initiates the repair reactions. Enzymes with such specificities have been found in *E. coli*; these include endonucleases specifically active on alkylated or depurinated DNA (6, 12, 18) and *N*-glycosidases that release alkylated bases from DNA (7, 11). Although preliminary attempts to find an enzyme deficiency in the *alk* mutant have not been successful, this type of mutant should help in elucidation of the mechanism of repair of DNA damaged by alkylating agents.

Searches for mutants specifically sensitive to MMS have been the subjects of a number of previous studies. Ljungquist et al. (13) isolated a mutant that is very sensitive to MMS but only slightly sensitive to UV. The mutant, AB3027, was later found to be a double mutant, with mutations in both *polA* and *xthA* genes. It was demonstrated that the mutant is defective in DNA polymerase I and an endonuclease specific for apurinic DNA. It has been reported that the mutant is also defective in exonuclease III activity (22).

Yajko and Weiss (22), on the other hand, isolated strains deficient in exonuclease III by nonselective screening of mutagenized *E. coli* clones. They found that three independently isolated strains having mutations in the *xthA* gene are defective in both exonuclease III and endonuclease II. One of the mutants, BW2001, was found to be sensitive to MMS, though the other two were not sensitive. The *alk* mutant isolated in the present studies is apparently different from the *xthA* mutants, since the *alk* mutation was located at 43 to 44 min on the revised map of the *E. coli* chromosome whereas *xthA* resides at 38 min on the chromosome (2, 20).

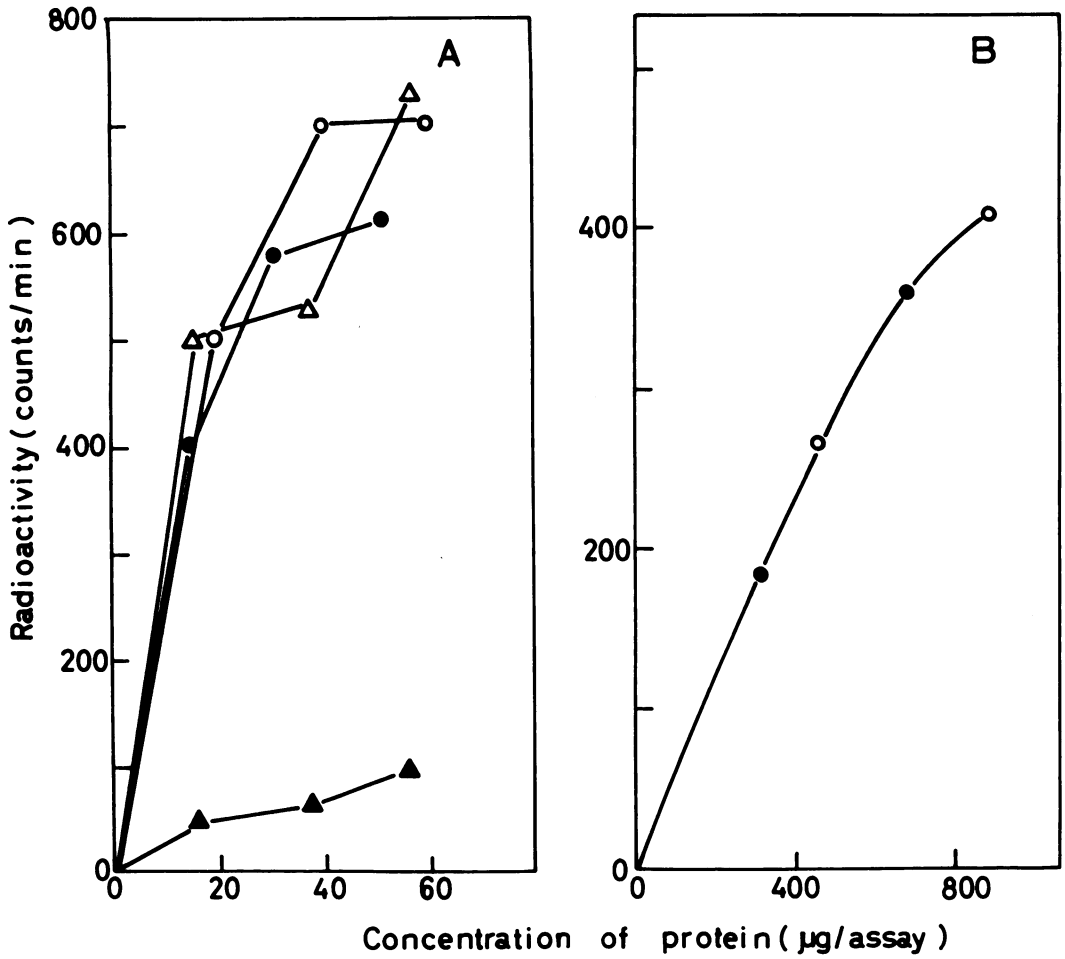


FIG. 5. *Enzyme activities toward alkylated DNA. (A) Endonuclease II. The reaction mixture (0.3 ml) contained 15 μmol of Tris-hydrochloride (pH 8.0), 0.3 μmol of 2-mercaptoethanol, 1.8 μmol of $MgCl_2$, 3.5 nmol of nucleotide of alkylated [$^{32}$P]T4 DNA gel beads (1.3 × $10^4$ cpm/nmol), and an extract (0 to 60 μg of protein). The reaction was performed at 37° C for 15 min, and the radioactivity released from the beads was determined (22). (B) 3-Methyladenine-DNA glycosidase. The reaction mixture (0.2 ml) contained 70 mM Tris-hydrochloride (pH 7.8), 1 mM dithiothreitol, 15 μg of MMS-treated [$^3$H]adenine-labeled DNA (3 × $10^3$ cpm/μg), and an extract (0 to 800 μg of protein). After incubation at 37° C for 20 min, the mixture was chilled and supplemented with 10 μl of 0.25% 3-methyladenine (Cyclo Chemicals), 10 μl of 0.25% 1-methyladenine (Sigma), 10 μl of 0.25% adenine (Seikagaku Kogyo Co.), 40 μl of 4 M NaCl, and 0.5 ml of ethanol (chilled at −10° C). The mixture was kept at −10° C for 1 h and then centrifuged in the cold. A portion of the supernatant fraction (0.4 ml) was concentrated to 30 μl and applied to a Whatman 3MM paper, and the paper was developed for 15 h in saturated ammonium sulfate solution-0.1 M phosphate (pH 7.2)-isopropanol (79:19:2) in a descending manner. After the paper was dried, spots were detected under a UV lamp; each piece containing a spot was eluted with water, and the radioactivity was determined in a liquid scintillation counter. In both assays, background values, obtained from samples without incubation, were subtracted.* (○) *W3623 (alk$^+$);* (●) *MS20 (alk);* (△) *AB1157 (xth$^+$);* (▲) *BW2001 (xth).*

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