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Deoxyribonucleic Acid Damage by Monofunctional Mitomycins and Its Repair in *Escherichia coli*

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Exposure of *Escherichia coli* to the antibiotic mitomycin C (MTC) at a concentration of 0.5 μg/ml caused cross-linkage between complementary strands of deoxyribonucleic acid (DNA). Derivatives of mitomycin, 7-methoxymitosene (7-MMT) and decarbamoyl mitomycin C (DCMTC), at a level as high as 20 μg/ml formed no cross-links between DNA strands. Ultraviolet light-sensitive mutants of *E. coli* K-12 bearing *uvrA*, *uvrB*, *uvrC*, or *recA* mutations were more sensitive to the lethal action of 7-MMT and of DCMTC than was the wild-type strain. Treatment of wild-type cells with these antibiotics resulted in the production of single-strand breaks in DNA, which were repaired upon incubation in a growth medium. Such breaks in DNA were not produced in the *uvrA* and the *uvrB* mutants. In the *uvrC* mutant, single-strand breaks were produced by 7-MMT or by DCMTC, but these breaks were not repaired upon incubation. These results are discussed in connection with the mechanism for removal of pyrimidine dimers in ultraviolet-irradiated bacteria.

The mitomycins are a group of alkylating antibiotics that have strong activity against bacteria as well as against tumor cells (7, 23, 26). Mitomycin C (MTC), the most thoroughly studied among them, has effects very similar to those of ultraviolet (UV) light on bacteria and phages. It causes preferential inhibition of deoxyribonucleic acid (DNA) synthesis (22), degradation of DNA during post-treatment incubation (3, 16, 25), and induction of temperate phage (13, 15, 17). It also has a cross-resistance relationship with UV irradiation (3, 6, 16). MTC is known to cause the formation in vivo and in vitro of a cross-linked DNA (9, 10), whereas UV irradiation causes the formation of thymine dimers in DNA (4, 21). From studies on UV-sensitive mutants of bacteria, it was assumed that DNA damage produced by exposure to either MTC or UV light was repaired by a similar molecular mechanism (3). However, it is still unclear whether the enzyme for removing UV damage in DNA participates in the excision of MTC cross-linked DNA.

Although MTC forms cross-links between complementary strands of DNA, the majority of mitomycin residues attach to DNA by a monofunctional alkylation in which one antibiotic molecule is attached to a single base (27), and nothing is known about the action of mitomycin residues attached monofunctionally to one strand of DNA. For the purpose of understanding the repair mechanism of cross-linked DNA on the one hand, and of understanding the action of mitomycin residues attached to DNA by a monofunctional alkylation on the other, 7-methoxymitosene (7-MMT; 1, 2, 11) and decarbamoyl mitomycin C (DCMTC; 12), which have been assumed to act as a monofunctional alkylating agent (24), were used in this study. We present evidence that they produce DNA damage which results in the production of DNA strand breaks. The DNA strands at the induced breaks appear to be joined by excision-repair enzymes.

MATERIALS AND METHODS

Bacterial strains. The following strains of *Escherichia coli* K-12 were employed: AB1157 (*thr*$^-$, *leu*$^-$, *pro*$^-$, *arg*$^-$, *thi*$^-$, *lac*$^-$, *gal*$^-$, *ara*$^-$, *xyl*$^-$, *mtl*$^-$, *tsx*^{r}, *str*^{r}, F$^-$), AB1886 (AB1157 *uvrA*), AB1885 (AB1157 *uvrB*), AB1884 (AB1157 *uvrC*), AB2463 (AB1157 *recA*), and 1100 (*end-1*$^-$). Abbreviations used are as follows: *thr*, threonine; *leu*, leucine; *pro*, proline; *his*, histidine; *arg*, arginine; *thi*, thiamine; *lac*, lactose; *gal*, galactose; *ara*, arabinose; *xyl*, xylose; *mtl*, mannitol; *tsx*, T6 phage; *str*, streptomycin; end-1, endo-

nuclease 1; −, incapable of producing or using; and *r*, resistant; *uvr* denotes the gene affecting host-cell reactivation and UV sensitivity; *rec* designates the gene affecting genetic recombination and UV sensitivity.

Media. Nutrient broth contained (per liter of distilled water) 10 g of polypeptone and 10 g of beef extract. It was adjusted to *p*H 7.2 with NaOH. For nutrient agar, nutrient broth was solidified with 1.5% agar. M medium contained 10.5 g of K_2HPO_4, 4.5 g of KH_2PO_4, 0.05 g of $MgSO_4$, 1.0 g of $(NH_4)_2SO_4$, 0.47 g of sodium citrate, 2.0 g of glucose, and water to a volume of 1 liter. Enriched M medium (EM) consisted of M medium with 2.5 g of Casamino Acids and 0.1 mg of thiamine added per liter. M buffer was M medium with glucose omitted. SSC consisted of 0.15 M NaCl and 0.015 M sodium citrate, *p*H 7.0.

Isotopic labeling of cells. Overnight cultures were diluted 20-fold into EM medium containing 5 μCi of ^{3}H-thymidine per ml (specific activity, 11.0 Ci/mmole) or 2 μCi of ^{14}C-thymine per ml (specific activity, 50 mCi/mmole) and 250 μg of deoxyadenosine per ml. The cultures were incubated at 37 C with aeration for about 4 hr until there were about 3×10^8 cells/ml. The cells were harvested by centrifugation, suspended in EM medium containing 20 μg of nonradioactive thymidine, and incubated at 37 C to deplete their metabolic pools of labeled thymine. After 60 min, they were centrifuged, washed, and suspended in an appropriate medium.

CsCl gradient sedimentation of DNA. Suspensions containing approximately 5×10^9 cells per ml were harvested by centrifugation. The cells were washed with 0.1 M tris(hydroxymethyl)aminomethane (Tris) buffer (*p*H 8.0) and resuspended in 0.03 M Tris buffer (*p*H 8.0) plus 0.5 M sucrose. They were then treated with 0.01 volume of lysozyme (5 mg/ml in 0.03 M Tris, *p*H 8.0), 0.04 volume of ethylenediaminetetraacetate (EDTA; 0.1 M in 0.03 M Tris, *p*H 8.0), and 0.02 volume of trypsin (5 mg/ml) for 30 min at 37 C, and were lysed with sodium dodecyl sulfate (SDS; final concentration, 0.4%). The DNA was twice extracted by shaking with SSC-saturated phenol and was dialyzed twice with 0.1 SSC.

For preparative equilibrium sedimentation, 3.90 g of CsCl was added directly to 3.0 ml of the aqueous phase from the DNA extraction, and the mixture was centrifuged in the SW50L rotor of a Spinco model L2 ultracentrifuge for 45 hr at 37,000 rev/min and 19 C. Four-drop fractions were collected on filter-paper discs 2.1 cm in diameter. They were then washed with ice-cold 5% trichloroacetic acid and with ethanol. After drying under a heat lamp, the filters were counted by use of an Aloka scintillation counter with a scintillation fluid made up of 4 g of 2,5-diphenyloxazole and 0.1 g of 1,4-bis-2-(5-phenyloxazolyl)-benzene per liter of toluene.

Sedimentation in alkaline sucrose gradients. The method of McGrath and Williams (14) was used for sedimenting DNA in alkaline sucrose gradients. Spheroplasts were produced at ice temperature as described above except that the trypsin and SDS treatments were omitted. After 5 min in ice, the spheroplasts (10 μliters, about 10^7) so obtained were lysed in 0.2 ml of 0.5 N NaOH containing 0.1% SDS in the surface layer of a 4.6-ml gradient of 5 to 20% (w/v) sucrose containing 10^{-3} M EDTA and 0.9 M NaCl, adjusted to *p*H 12 with NaOH. The tubes were centrifuged in the SW50L rotor for 90 min at 30,000 rev/min at 19 C in a Spinco L2 centrifuge. After centrifugation, six-drop fractions were collected on filter discs by puncturing the tube bottom, and radioactivity was counted as described above.

DNA breakdown after treatment with antibiotics. Labeled bacteria at a concentration of about 3×10^8 cells/ml were exposed to various concentrations of the antibiotics for 30 min at 37 C in M buffer. A sample of cells was taken for measurement of viable cells; the remaining cells were centrifuged, suspended in EM medium plus thymidine (20 μg/ml), and aerated at 37 C. Total radioactivity was determined by counting 50-μliter samples of the labeled culture. The cells were acidified by addition of one-tenth volume of 50% trichloroacetic acid and were kept cold for 20 min. After centrifugation, 0.1 ml of the supernatant fluid was counted for radioactivity.

Chemicals. MTC, 7-MMT, and DCMTC were kindly supplied by Kyowa Hakko Kogyo Co. Ltd., Tokyo, Japan. ^{3}H-thymidine (specific activity, 11.0 Ci/mmole) and ^{14}C-thymine (specific activity, 50 mCi/mmole) were obtained from Daiichi Kagaku Co., Tokyo, Japan. Chemical structures of mitomycins are shown in Fig. 1.

RESULTS

Binding of mitomycins with DNA. Interstrand cross-links in DNA can be demonstrated by ultracentrifugation of heat-denatured DNA in a CsCl density gradient (9). The cross-linked DNA has been shown to have a density characteristic of native DNA when heated at a temperature above the melting

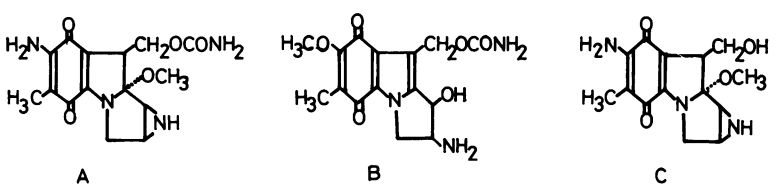

FIG. 1. *Chemical structures of mitomycins. A, mitomycin C; B, 7-methoxymitosene; C, decarbamoylmitomycin C.*

point and quenched at 0 C, whereas the DNA without cross-links has a density characteristic of heat-denatured DNA under the same conditions. To test cross-link formation by 7-MMT and by DCMTC with DNA bases, *E. coli* K-12 strain 1100 was grown in medium containing ^{3}H-thymidine and exposed to these antibiotics. DNA was extracted, denatured by heating at 100 C for 5 min, and rapidly cooled at 0 C. The sample was centrifuged in a CsCl gradient with a ^{14}C-labeled nonheated normal *E. coli* DNA as a reference. As shown in Fig. 2, there was a significant difference in the banding pattern of DNA from cells treated with MTC and that from cells treated with its derivatives. The heat-denatured DNA from cells treated with 2 μg of MTC/ml (at a survival of 4.1×10^{-4}) banded in the position occupied by the normal DNA (Fig. 2C). With the denatured DNA from cells exposed to 0.5 μg of MTC/ml (survival, 10^{-1}), about 40% of DNA banded at the density characteristic of the native or renatured DNA (Fig. 2B). In contrast, with the DNA from cells treated with 7-MMT (survival, $<1.3 \times 10^{-3}$; Fig. 2D) and DCMTC (survival, $<4.1 \times 10^{-4}$; Fig. 2E) at 20 μg/ml, a concentration 40 times high as that of MTC, only 8% of total radioactivity was recovered from the position normally occupied by the renatured DNA. However, under the experimental conditions, 7% of the heat-denatured normal *E. coli* DNA banded at the same position (Fig. 2A). There-

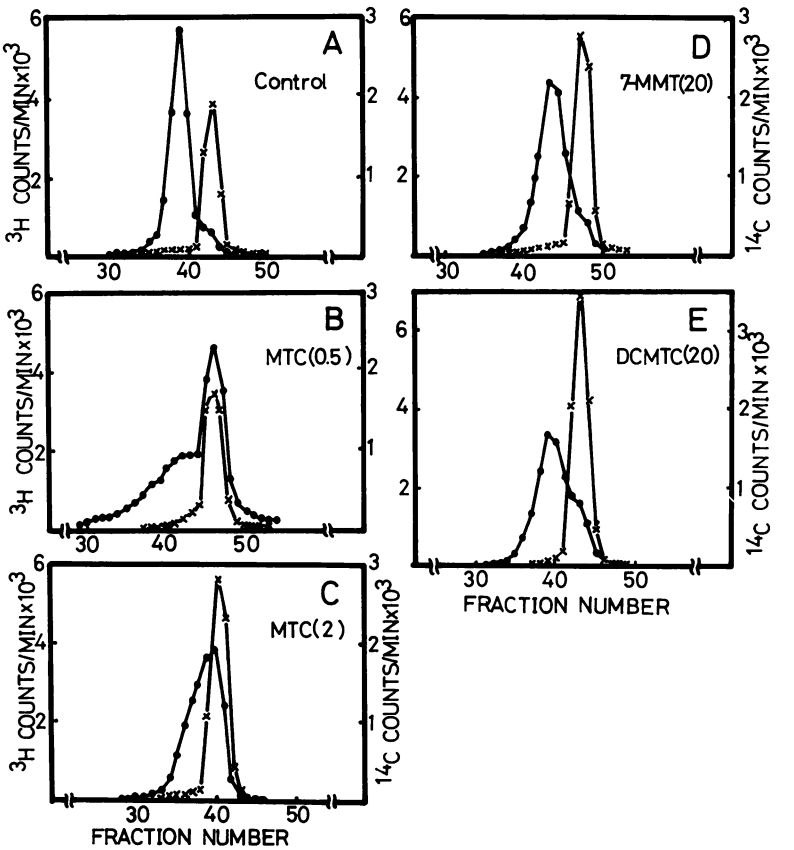

FIG. 2. *Preparative equilibrium sedimentation of DNA prepared from cells exposed to mitomycins. DNA was extracted from E. coli 1100 not exposed to antibiotic (A) or exposed to antibiotics for 60 min in M buffer as follows: mitomycin C (MTC), 0.5 μg/ml (B); MTC, 2 μg/ml (C); 7-methoxymitosene (7-MMT), 20 μg/ml (D); and decarbamoyl mitomycin C (DCMTC), 20 μg/ml (E). All of the DNA samples were denatured for 5 min at 100 C in 0.1 SSC and rapidly chilled at 0 C. ^{14}C-labeled normal E. coli 1100 DNA was added as a reference. The figure illustrates the distribution of ^{3}H (heated antibiotic-treated DNA, ●) and ^{14}C (nonheated normal DNA, ×) after centrifugation in a CsCl gradient at 37,000 rev/min for 45 hr. Survival of cells after the various treatments was as follows: 0.5 μg of MTC/ml, 10^{-1}; 2 μg of MTC/ml, 4.1×10^{-4}; 20 μg of 7-MMT/ml, $<1.3 \times 10^{-3}$; and 20 μg of DCMTC/ml, $<4.1 \times 10^{-4}$.*

fore, we concluded that 7-MMT and DCMTC form few, if any, cross-links between complementary strands of DNA.

Effect of 7-MMT and DCMTC on UV-sensitive mutants of E. coli. As it has been shown by Boyce and Howard-Flanders (3) that UV-sensitive mutants of *E. coli* are more sensitive to the lethal action of MTC than are wild-type bacteria, experiments were performed to see whether the mutants were also sensitive to 7-MMT and DCMTC, which had been shown to cause no cross-links between DNA strands. Bacteria grown to logarithmic phase were exposed to 7-MMT or to DCMTC for 30 min at 37 C, and plated on nutrient agar for counting of the surviving cells. Figure 3 presents survival curves of the bacteria as a function of the concentration of the antibiotics. It is apparent that both 7-MMT and DCMTC have a much greater lethal effect on the UV-sensitive mutant strains, AB1886 (*uvrA*), AB1885 (*uvrB*), AB1884 (*uvrC*), and AB2463 (*recA*), than on the wild-type parent strain, AB1157. Since UV irradiation causes production of pyrimidine dimers on DNA and the UV-sensitive mutants used in this experiment are assumed to be defective in removal of such dimers from DNA (3, 4, 8, 21), the result indicates that the antibiotics act directly on the DNA molecule, and the increased sensitivity of the mutant strains to the antibiotics might depend on each strain's ability to repair its damaged DNA.

Sucrose gradient centrifugation of DNA. Wild-type strains of *E. coli* have mechanisms for the removal of UV photoproducts from this DNA. The first step is assumed to be an incision in a strand near a pyrimidine dimer. This is followed by release of nucleotides, including the dimers, by excision-repair enzymes. In UV-sensitive strains, however, such a mechanism is apparently lacking (3, 4, 8, 21). Since strains bearing the *uvrA*, *uvrB*, and *uvrC* mutation were more sensitive to the lethal action of 7-MMT and of DCMTC than was the wild type, we considered the possibility that the repair process operative in wild-type cells might produce DNA strand breaks, and that sensitive mutant strains may be defective in various stages of such a repair process. To test this, bacterial strains were labeled in their DNA by growth in medium containing ^{3}H-thymidine. Spheroplasts were prepared from cells immediately after treatment and after 60 min of incubation. The distribution of the radioactivity of the DNA in each of these samples was then determined by zone centrifugation in an alkaline sucrose gradient. As shown in Fig. 4, the DNA isolated from wild-type cells, immediately after exposure to the anti-

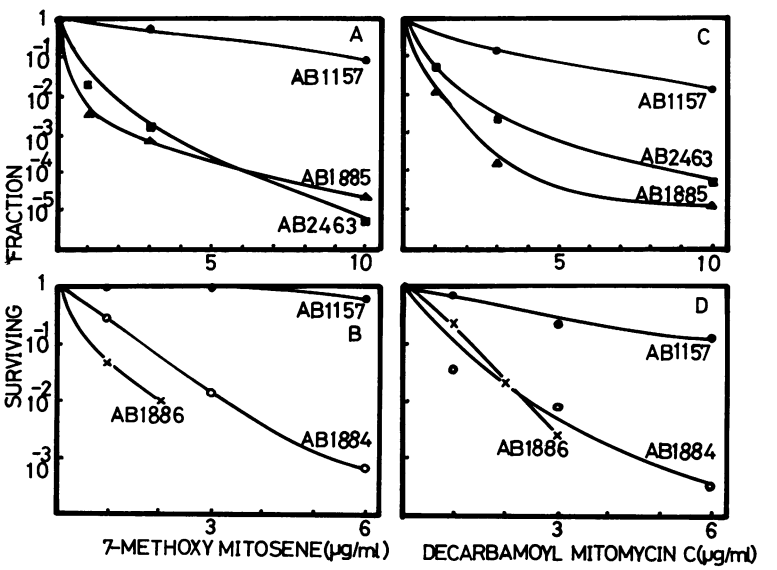

FIG. 3. *Survival of cells after exposure to various concentrations of 7-methoxymitosene (7-MMT) and decarbamoyl mitomycin C (DCMTC). Cells grown in EM medium were harvested in logarithmic phase, washed twice, and suspended in M buffer. They were exposed to various concentrations of 7-MMT and DCMTC at 37 C for 30 min. After appropriate dilutions, cells were plated on nutrient agar, incubated for about 20 hr at 37 C, and scored for the number of visible colonies formed. A and B, treated with 7-MMT; C and D, with DCMTC.*

biotics sedimented at a lower velocity as compared with the nontreated control preparation, and the DNA from incubated cells sedimented at the same position as the control DNA. However, DNA from untreated control cells and that from the antibiotic-treated cells were sedimented at the same velocity by centrifugation in a neutral sucrose gradient (Fig. 5). These results indicated that there were single-strand breaks in the DNA of the antibiotic-treated *E. coli*, and that the resulting breaks in the polynucleotide strand were repaired by some mechanism.

However, when *uvr* mutants were exposed to

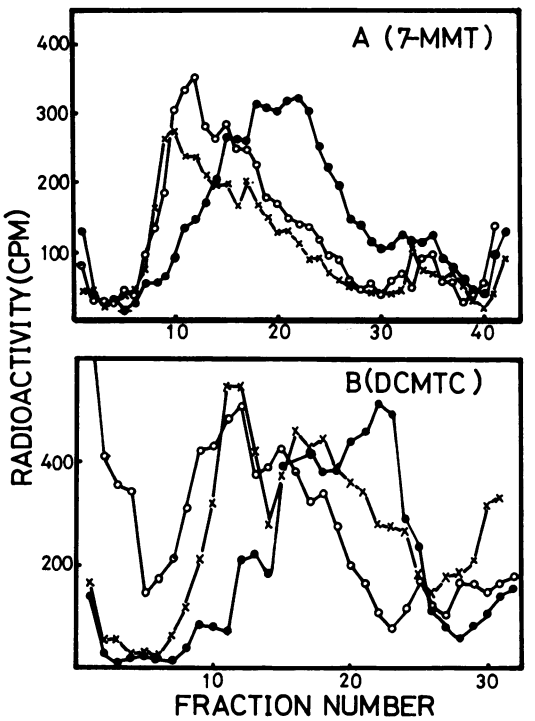

FIG. 4. *Alkaline sucrose centrifugation of DNA of bacteria treated with 7-methoxymitosene (7-MMT) and decarbamoyl mitomycin C (DCMTC). Wild-type E. coli 1100 (3 ml) grown to about 3 × 10⁸ cells/ml was labeled with ³H-thymidine as described in Materials and Methods, collected by centrifugation, and suspended in 3 ml of M buffer. A 1-ml portion of the cells was used as a control, and the rest were treated with 2 μg of 7-methoxymitosene (7-MMT)/ml (survival, 9.2 × 10⁻¹ and 2 μg of decarbamoyl mitomycin C (DCMTC)/ml (survival, 2.3 × 10⁻¹) for 30 min at 37 C. After centrifugation, half of the antibiotic-treated cells were taken at zero time and the rest were incubated for 60 min in EM plus thymidine (20 μg/ml) medium. The cells were centrifuged, concentrated to 0.4 ml, and converted to spheroplasts. A 20-μliter portion was lysed on 0.2 ml of a 0.5 N NaOH layer containing 0.1% SDS on the top of the sucrose gradient. A, 7-MMT-treated; B, DCMTC-treated cells; ○, nontreated control; ●, antibiotic-treated; ×, antibiotic-treated and incubated.*

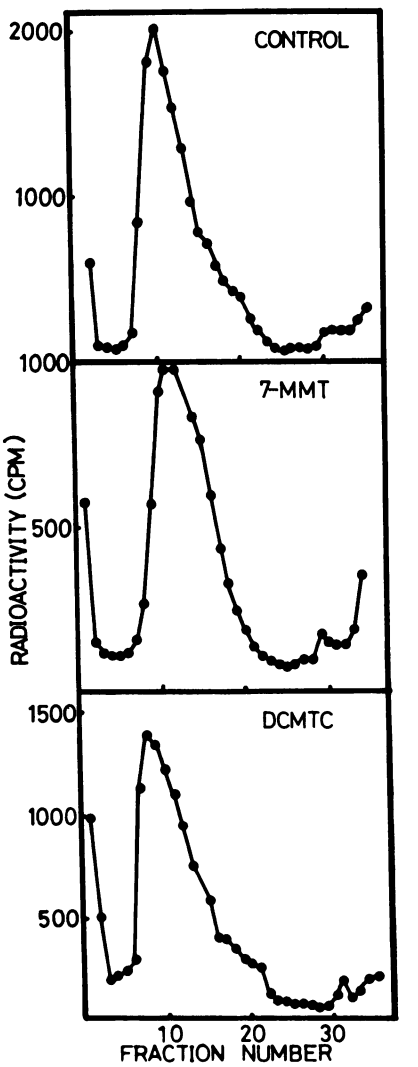

FIG. 5. *Neutral sucrose centrifugation of DNA of bacteria treated with 7-methoxymitosene (7-MMT) and decarbamoyl mitomycin C (DCMTC). DNA was prepared from the spheroplasts used for alkaline sucrose centrifugation in Fig. 4. After two cycles of freezing and thawing of the spheroplasts, the lysates were treated with trypsin (200 μg/ml) for 30 min at 37 C. SDS was added to a final concentration of 1% and the mixture was incubated further for 15 min at 37 C. The lysates were diluted 20-fold with SSC, and a 0.2-ml portion was layered on a sucrose gradient (5 to 20% in SSC). After centrifugation for 110 min at 33,000 rev/min in a SW50L rotor at about 15C, fractions were collected on filter discs and radioactivity was counted as described above.*

2 μg of 7-MMT or DCMTC/ml, a concentration which was sufficient to cause the appearance of breaks in DNA of the wild-type bacteria, the DNA from the *uvrB* mutant sedimented at the same velocity as the nontreated control DNA, and the DNA from the *uvrA* mutant sedimented slightly higher than the control preparation (Fig. 6). In the *uvrC* mutant, DNA isolated immediately after treatment with the antibiotics or after 60 min of incubation in a growth medium sedimented at a lower velocity than the nontreated control DNA (Fig. 7). These results indicate that, when the cells were exposed to 7-MMT or DCMTC, single-strand breaks were produced in the DNA of the *uvrC* mutant, but not in that of the *uvrA* and the *uvrB* mutants, and that such breaks in the DNA of the *uvrC* mutant were not repaired upon incubation in the growth medium.

DNA degradation by mitomycins. It has been shown that MTC causes extensive degradation of DNA in wild-type bacteria but less degradation in the *uvr* strains (3, 16). To test the effect of monofunctional mitomycins on DNA degradation, DNA was labeled with ^{3}H-thymidine and exposed to various doses of 7-MMT or of DCMTC. After centrifugation, the cells were incubated in EM medium supplemented with nonradioactive thymidine to minimize reincorporation of radioactive nucleotides released. After 2 hr, radioactivity in the acid-soluble fraction was counted. In each experiment, MTC was used as a control. In Fig. 8, degradation of DNA as well as survival of wild-type bacteria are plotted against the concentration of mitomycins. DNA of MTC-treated cells was more extensively degraded than that of cells treated with 7-MMT or DCMTC. MTC at 1 μg/ml caused a reduction in survival equivalent to that caused by either 7-MMT or DCMTC at 10 μg/ml.

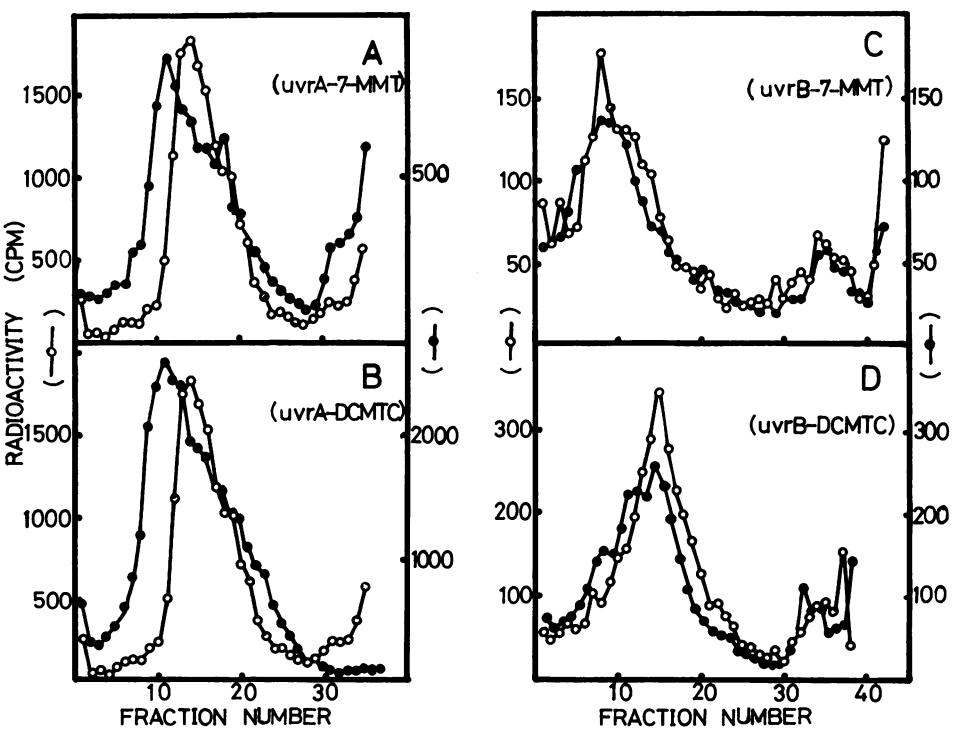

FIG. 6. *Alkaline sucrose centrifugation of DNA of uvrA and uvrB mutants exposed to 7-methoxymitosene (7-MMT) and to decarbamoyl mitomycin C (DCMTC). Amounts of 2 ml of E. coli AB1886 (uvrA) and AB1885 (uvrB) were labeled by growth in medium containing 2.5 μCi of ^{3}H-thymidine/ml. The cells (about 3 × 10^8 cells/ml) were harvested by centrifugation, and divided into two parts. Portions (1 ml) were treated with 7-MMT (2 μg/ml) and with DCMTC (2 μg/ml) for 30 min at 37 C; the rest was used as a control. Spheroplasts were prepared by the procedures described in Materials and Methods, and 20-μliter portions (about 10^7 spheroplasts) were used for zone centrifugation in sucrose gradients. A, 7-MMT-treated AB1886 (survival, 3.8 × 10^{-3}); B, DCMTC-treated AB1886 (survival, 1.9 × 10^{-1}); C, 7-MMT-treated AB1885 (7.4 × 10^{-2}); D, DCMTC-treated AB1885 (9.2 × 10^{-2}). Symbols: ○, nontreated control; ●, antibiotic-treated cells.*

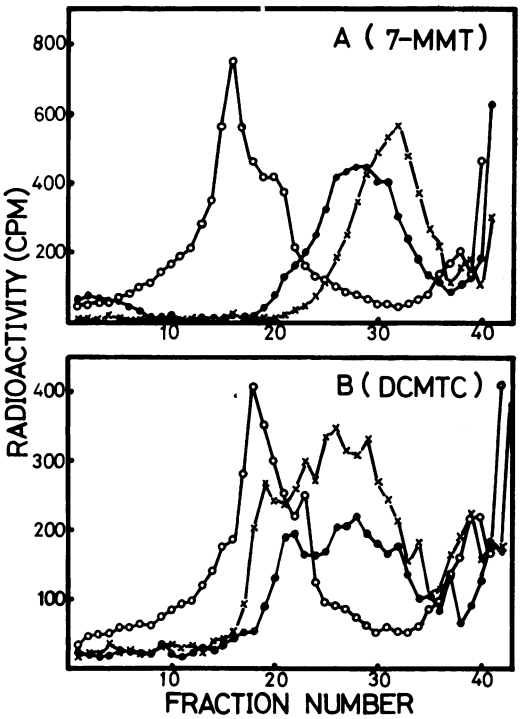

FIG. 7. *Distribution of ^{3}H-labeled DNA of the uvrC mutant exposed to 7-methoxymitosene (7-MMT) and to decarbamoyl mitomycin C (DCMTC). Experimental procedures were the same as described in Fig. 4. Survival of cells treated with 2 μg of 7-MMT/ml was 3.4 × 10^{-1} and with 2 μg of DCMTC/ml was 4.6 × 10^{-1}. Symbols: ○, nontreated control; ●, antibiotic-treated and not incubated; ×, antibiotic-treated and incubated.*

DNA degradation in the wild type and in the *uvrB* mutant is shown in Fig. 9. In this experiment, bacteria were grown in EM medium containing ^{3}H-thymidine and were exposed to 7-MMT. Samples were removed at intervals for assay of radioactivity in the acid-soluble fraction. An appreciable amount of radioactivity was released into the acid-soluble fraction from 7-MMT-treated wild-type bacteria, but the presence of 10 μg of the antibiotic/ml caused little breakdown in the *uvrB* strain.

DISCUSSION

It has been suggested that the aziridine and carbamoyloxy residue of the mitomycin molecule form a covalent cross-link between nucleotides in complementary strands of DNA (24). Derivatives of mitomycin, 7-MMT and DCMTC, have a chemical structure lacking the aziridine moiety or carbamoyloxy side chain, and are thus not expected to cause a cross-linking of complementary strands of DNA. It is shown in Fig. 2 that heat-denatured DNA from *E. coli* exposed to 7-MMT and DCMTC banded at the position normally occupied by the heat-denatured normal DNA in a CsCl density gradient. The results indicate that 7-MMT and DCMTC form few, if any, cross-links between complementary strands of DNA and support the assumption involving two sites of the mitomycin residue for cross-link formation.

When *uvr*$^+$ cells were incubated after 7-MMT or DCMTC treatment, we observed an increase in the sedimentation velocity of DNA molecules in alkaline sucrose centrifugation (Fig. 4). This suggests that any nucleotide which is removed from DNA in the antibiotic-treated bacteria is replaced by the insertion of nucleotides complementary to those of the intact opposite strand by a DNA polymerase, and that these nucleotides are covalently bound to the adjacent nonexcised regions. In the *uvrC* mutant, however, single-strand breaks produced by exposure to the antibiotics were not repaired upon incubation (Fig. 7). Therefore, the *uvrC* mutant seems to contain a mechanism which produces single-strand breaks in DNA, but is defective in some step in the excision process for the mitomycin residue bound to a nucleotide in the DNA molecule.

From the experiments presented in this paper, therefore, we conclude that monofunctional mitomycins, as well as MTC, when attached to DNA by monofunctional alkylation, cause a local distortion in the DNA molecule which is repaired by mechanism such as that which removes the pyrimidine dimers from UV-irradiated DNA.

The monofunctional alkylating agent, methyl methanesulfonate, reacts with DNA primarily by the addition of a methyl group to the purine residues. The alkylated purine moiety tends to split off, leaving an apurinic site, followed by rupture of a phosphodiester bond adjacent to the apurinic site (5). Such methyl methanesulfonate-treated DNA with single-strand breaks in *Bacillus subtilis* can be repaired when the cells are incubated in growth medium for a short time after treatment (18, 19). Although a UV-sensitive mutant of *E. coli* is also sensitive to 7-MMT and to DCMTC (Fig. 3), a UV-sensitive mutant of *B. subtilis* is insensitive to methyl methanesulfonate (20). Therefore, both 7-MMT and methyl methanesulfonate cause the insertion of single-strand breaks in DNA which are repaired upon incubation under appropriate conditions, but the mechanisms by which DNA damage produced by monofunctional

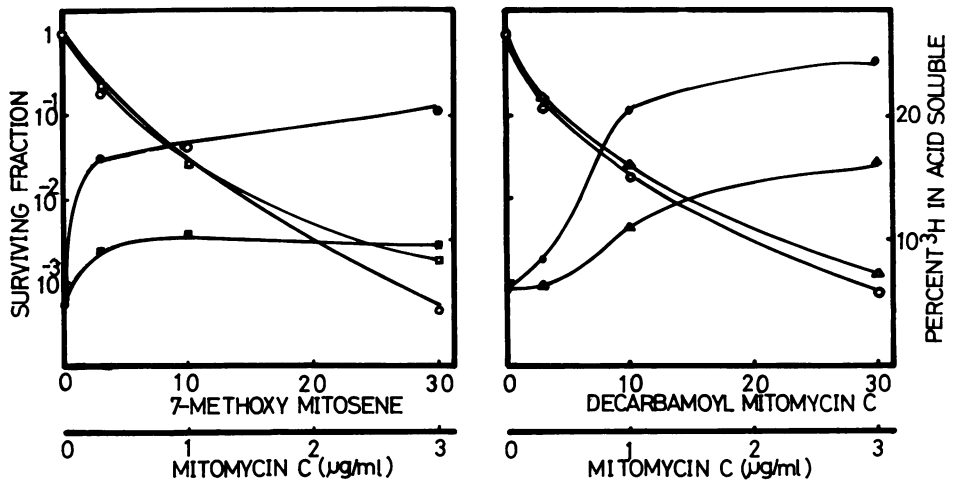

FIG. 8. *Colony-forming ability and DNA breakdown of E coli K-12 treated with mitomycins. The bacteria were labeled by growth in EM medium containing 2.5 μCi of ³H-thymidine per ml. Washed log-phase cells were incubated in EM plus nonradioactive thymidine (20 μg/ml) for 60 min, washed, and suspended in M buffer. They were exposed to various concentrations of the antibiotics for 30 min at 37 C, and a sample was removed for assay of viable cells (○, MTC; □, 7-MMT; △, DCMTC). The cells were centrifuged and incubated in EM plus thymidine (20 μg/ml) for 2 hr at 37 C. Radioactivity was measured as described in the text (●, MTC; ■, 7-MMT; ▲, DCMTC).*

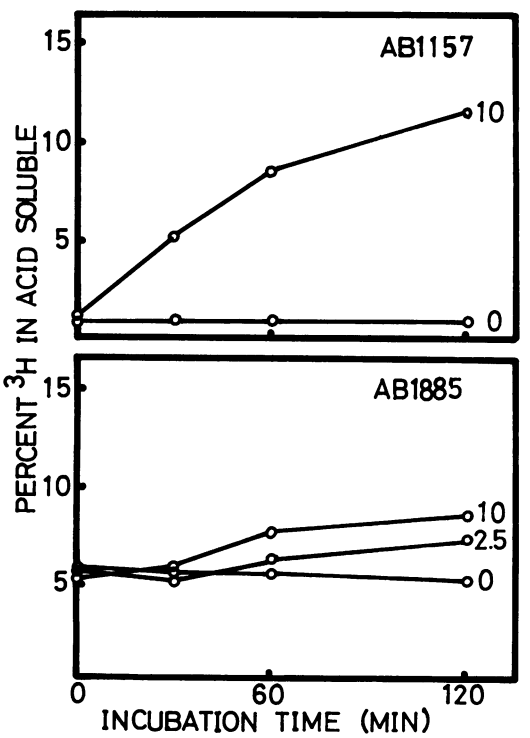

FIG. 9. *Solubilization of DNA in 7-methoxymitosene (7-MMT)-treated cells. Strains AB1157 and AB1885 were labeled by growth in EM medium supplemented with 2.5 μCi of ³H-thymidine per ml plus 250 μg of deoxyadenosine per ml, washed, and then grown for 60 min in EM medium with 20 μg of nonradioactive thymidine per ml. The labeled cells were washed, resuspended in M buffer, and exposed to 7-MMT. After centrifugation, the culture was supplemented with 20 μg of thymidine per ml and incubated at 37 C with aeration. Samples (0.5 ml) were taken at intervals and chilled. The samples were added to 0.25 ml of a culture containing 10^9 cells/ml and 0.25 ml of cold 1 N trichloroacetic acid, and were kept at 0 C for 20 min. The fraction of trichloroacetic acid-soluble radioactivity was determined and plotted against the time of incubation. The survival fraction of AB1157 treated with 10 μg/ml was 2.8×10^{-2}; that of AB1885 treated with 2.5 and 10 μg/ml was 1.1×10^{-2} and 9.6×10^{-5}, respectively.*

mitomycin and by methyl methanesulfonate are repaired may not be completely identical. We explain this difference by assuming that removal of monofunctional mitomycin-induced damage in DNA would require an excision mechanism such as that responsible for removal of the pyrimidine dimers from UV-irradiated DNA, but for methyl methanesulfonate-induced DNA damage no such mechanism is necessary (18, 20).

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