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# Excision Repair Characteristics of *recB*$^-$*res*$^-$ and *uvrC*$^-$ Strains of *Escherichia coli*

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An *Escherichia coli* strain carrying the *recB21* and *res-1* mutations showed an abnormally low level of colony-forming ability although it grew essentially normally in liquid medium. The *recB21 res-1* strain showed little, if any, of the ultraviolet (UV)-induced deoxyribonucleic acid (DNA) breakdown characteristic of the *res-1* mutant. Nevertheless, the double mutant was far more sensitive to UV than either the *res-1* or the *recB21* strain. When compared with a wild-type strain, the rate of release of dimers from UV-irradiated DNA was very slow in the *recB21 res-1*, but normal in the *res-1 recB*$^+$ or *recB21 res*$^+$ mutants. However, the ratio of dimer-to-thymine released into the acid-soluble fraction was three times higher than the wild type in *recB21 res*$^+$ and *recB21 res-1* and only one-tenth as high as the wild type in *res-1 rec*$^+$. Alkaline sucrose gradient centrifugation revealed occurrence of single-strand incision of UV-irradiated DNA and the restitution of nicked DNA at a similar rate in the *recB21 res-1* and *recB21 res*$^+$ strains. Mutants *uvrC*$^-$ showed increased amounts of nicks in their DNA with increasing incubation time after UV irradiation, although no detectable amounts of dimers were excised from UV-irradiated DNA. From these results, it is concluded that the increased sensitivity of the *res-1* strain to UV light is due to a reduced ability to excise dimers from UV-irradiated DNA and that the high rate of UV-induced breakdown of DNA is not the primary cause. A possible role of *uvrC* gene in the excision repair is discussed.

Immediately after the discovery of the excision repair system in *Escherichia coli* (4, 35), it was predicted that there must be several kinds of enzymes involved in excision repair (34), and some of them might be functioning in a pathway common to excision repair and genetic recombination (12). In fact, several different mutants deficient in deoxyribonucleic acid (DNA) repair were isolated, and various enzymatic defects were identified. Ultraviolet (UV)-sensitive mutants of *Micrococcus luteus* (21) and mutant T4*uv* of bacteriophage T4 (39) have been shown to lack endonucleases specific for UV-irradiated DNA. Mutants of *E. coli* deficient in polynucleotide ligase are UV sensitive (8, 31). Mutants of T4 phage which produce heat-labile DNA polymerase or ligase become UV sensitive at higher temperatures (1). Recombination-deficient mutants *recB*$^-$ and *recC*$^-$ of *E. coli* lack an adenosine triphosphate-dependent nuclease activity (2, 9, 24). De Lucia and Cairns (6) have described an *E. coli* mutant *polA1*, which has little or no DNA polymerase I activity. The *polA1* mutation is also responsible for the increased sensitivity of the strain to irradiation by UV light as well as to methyl methane sulfonate (MMS) (10). The UV sensitivity associated with the defect in DNA polymerase I has been shown to be due to reduction in the efficiency of excision repair (26, 32).

We (17) have also found that previously isolated (16) X-ray sensitive mutants of *E. coli* B lack DNA polymerase I activity. The genetic analysis of those mutants has led to the conclusion (17) that these mutants are defective in the repair synthesis of the excision repair process and that DNA polymerase I is partly responsible for the repair synthesis of excised portions. The *res* mutation caused elevated spontaneous mutability (19) and also was responsible for the slow joining of newly replicated DNA chains in the mutant (30). Analysis of repair of radiation damage in *res*$^-$ cells may, therefore, help further our understanding of the mechanisms involved in repair. However, extensive breakdown of DNA after UV irradiation in these mutants makes it difficult to study biochemi-

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cally the nature of the repair defect of the mutant. To circumvent this problem of degradation, a *recB21 res-1* double mutant was constructed. This paper deals with the characteristics of defective DNA excision repair in the *recB21 res-1* and the *uvrC*$^-$ mutants. It will be shown that DNA polymerase I is involved, in vivo, in excising dimer coupling some way with the product of *uvrC*$^+$ gene.

## MATERIALS AND METHODS

**Bacterial and phage strains.** The origin and characteristics of the bacterial strains used are described in Table 1. Phages used were T1 and P1*kc*.

**Media.** The growth medium consisted of 8 g of nutrient broth (Difco) and 4 g of NaCl, per liter of water. The PA medium used for colony counting was made of peptone agar (10 g of polypeptone, 2.5 g of NaCl, 15 g of agar, per liter of water adjusted to *p*H 7.0) enriched by 0.4 g of beef extract (Wako Pure Chemical Co.). M9 medium (5.8 g of $Na_2HPO_4$, 3 g of $KH_2PO_4$, 1 g of $NH_4Cl$, 5 g of NaCl, 0.1 g of $MgSO_4$, 4 g of glucose, and 1 liter of water) used for labeling DNA contained 100 $\mu$g of adenosine per ml, 2.5 mg of Casamino Acids per ml, and 5 $\mu$g of thiamine per ml. After being washed, labeled cells were suspended in M9$^-$ medium (M9 medium without glucose). T1 buffer contained 0.62 g of $Na_2HPO_4$, 0.3 g of $KH_2PO_4$, and 4 g of NaCl per liter of water to which 0.6 ml of 1 M $MgSO_4$, 0.5 ml of 1 M $CaCl_2$, and 1 ml of 1% gelatin were added after autoclaving. Selection of recombinants was made on a minimal agar (minimal E agar supplemented with 0.4 g of glucose and a combination of required amino acids of 20 $\mu$g/ml each). Streptomycin, when necessary, was added at a concentration of 100 $\mu$g/ml.

**Host cell reactivation capacity of MMS-treated T1 phage.** Phage T1 suspension at a concentration of $2 \times 10^{10}$ particles/ml in T1 buffer was prepared from the lysate of bacterial culture infected with T1 phage by differential centrifugation and resuspension. To 0.9 ml of the phage solution, 0.1 ml of 5% MMS (Eastman Organic Chemicals; vacuum redistilled before use) was added, and the solution was kept at room temperature for the treatment. At intervals, 0.1-ml samples were withdrawn and diluted immediately into 9.9 ml of ice-cold T1 buffer to stop the reaction. The MMS-treated phage samples were mixed with host bacterial cells ($2 \times 10^8$/ml), which had been starved in T1 buffer, at multiplicity of infection 0.1, and the infection was allowed to proceed for 10 min at 37 C. Unadsorbed phage particles were removed by centrifugation at 6,000 $\times$ *g* for 15 min in the cold, and the infected cells were resuspended in cold T1 buffer. Infective centers of the complex were assayed by the standard soft agar layer method.

**Identification of the recombinants Hcr$^-$ for MMS-treated T1 phage.** Equal volumes of exponential-phase Hfr donor OK2102 (*res-1*, *thi*$^+$, *str-s*) and F$^-$ recipient AB2470 (*recB21*, *thi*$^-$, *str-r*) were mixed and incubated at 37 C. Mating was interrupted with a Vortex mixer after 7 min, and diluted samples were plated on selective medium containing streptomycin (100 $\mu$g/ml). The selective plates were incubated for 42 hr at 37 C, and the recombinants selected for *thi*$^+$ *str-r* markers were transferred onto master plates. A part of each colony on the master plate was suspended in 0.5 ml of T1 buffer in a small test tube at a concentration of about $10^9$ cells/ml. A sample of T1 phage solution, which was treated by MMS to give about 10% survival, was added to the cell suspension to the concentration of $10^6$ phages/ml. About 1 $\mu$liter of the mixture was streaked on PA medium and incubated for 24 hr at 37 C. The clones which supported the formation of only a few plaques were selected. This selection method was based upon the fact that the mutant carrying *res*$^-$ gene showed

TABLE 1. *Relevant characteristics of bacterial strains used*[a]

| Strain | Sex | Genotype of DNA repair | | | Hcr for T1 phage inactivated by | | References |
|---|---|---|---|---|---|---|---|
| | | *uvr* | *rec* | *res* | UV | X ray or MMS | |
| H/r30R | F$^-$ | + | + | + | + | + | 38 |
| R15 | F$^-$ | + | + | *res-1* | + | − | 17 |
| OK2102 | Hfr[b] | + | + | *res-1* | + | − | 17 |
| OK2701 | F$^-$ | + | *recB21* | *res-1* | + | − | This paper |
| OK2001 | F$^-$ | + | + | + | + | + | 17 |
| OK2603 | Hfr | *uvrC* | + | + | − | + | 17 |
| AB1886 | F$^-$ | *uvrA* | + | + | − | + | 13 |
| AB1884 | F$^-$ | *uvrC* | + | + | − | + | 13 |
| AB2470 | F$^-$ | + | *recB21* | + | + | + | 12 |
| N12-2 | F$^-$ | *uvrC* | + | + | − | + | 28 |

[a] Abbreviations: UV, ultraviolet light; MMS, methyl methane sulfonate; Hcr, host-cell reactivation; +, wild-type; −, deficient.

[b] The point of origin of transfer locates around 74 min on the standard *E. coli* map, and transfer of chromosome markers is in the order *ilv-res-thi*.

phenotype Hcr⁻ (host cell reactivation-minus) for MMS-treated T1 phage (17).

**Labeling of cells.** Bacteria were grown for 3 hr in M9 medium supplemented with 15 μCi of thymidine-*methyl*-$^3H$ (5 Ci/mmole) per ml and 100 μg of adenosine per ml. Labeled cells were centrifuged and washed twice; unincorporated radioactivity was minimized by incubating the cells for 1 hr in M9 medium in the presence of 100 μg of nonradioactive thymidine per ml.

**Sedimentation in alkaline sucrose.** Sedimentation of DNA in alkaline sucrose was carried out by the method of McGrath and Williams (22) with minor modifications. Labeled cells were washed and suspended in cold M9⁻ medium at a concentration of 2 × $10^8$ cells/ml. One part of the cell suspension was subjected to UV irradiation in an iced petri dish, the other part served as a nonirradiated control. Both the samples were supplemented with glucose, Casamino Acids, and thiamine and incubated at 37 C. At intervals, samples were taken from the irradiated suspension and were kept in an ice-water bath. A 0.1-ml sample of the suspension was lysed by pipetting it onto 0.2 ml of 0.5 M NaOH containing 0.2% sodium dodecyl sulfate which had been put on top of a 4.8-ml 5 to 20% alkaline sucrose gradient. To attain complete lysis, the gradient was kept standing at room temperature not less than 20 min, then centrifuged at 30,000 rev/min for 90 min at 20 C in a Hitachi model 55PA centrifuge. Fractions were collected on filter paper discs and immersed in 5% trichloroacetic acid. The discs were washed three times with acid and twice with ethanol, and dried. Radioactivities in the discs were counted in POP-toluene counting solution by a liquid scintillation counter.

**Assay of thymine-containing dimers.** $^3H$-labeled cells were suspended in cold M9⁻ medium at a concentration of 2 × $10^8$ cells/ml. The suspension was irradiated by UV light with a dose of 1,000 ergs/mm² in an iced petri dish and then incubated at 37 C after being supplemented with glucose, Casamino Acids, and thiamine. At intervals, 0.5-ml samples were withdrawn, centrifuged in the cold, and washed with 1 ml of M9⁻ medium. The supernatant and washing fluids were combined and analyzed for radioactive materials released into the extracellular medium. The washed cells were acidified by perchloric acid (PCA) at a final concentration of 0.5 N, centrifuged in the cold, and separated into acid-soluble and acid-insoluble fractions. To each fraction, PCA was added to give final concentration of 6 N, and the mixture (total volume of 1 ml) was heated at 100 C for 3 hr. The analysis of radioactive materials in the extracellular medium was carried out as follows. After heating at 100 C for 10 min in 0.5 N PCA, the medium was charged onto an activated charcoal column. The column was washed with 100 ml of water to remove inorganic salts in the medium and then eluted with 100 ml of 50% ethanol-2% $NH_4OH$ at 45 C. The effluent was evaporated and hydrolyzed in 1 ml of 6 N PCA at 100 C for 3 hr. The amounts of thymine monomers and thymine-containing dimers were determined by using Dowex-1 column chromatography as described by Sekiguchi et al. (33). After hydrolysis, a drop of bromothymol blue solution was added, and the hydrolysate was neutralized with KOH. The precipitate was removed by centrifugation and washed with cold water. The supernatant fluid and the washing were combined, and $NH_4OH$ was added to a final concentration of 0.02 M. The mixture was applied onto a column (1 by 10 cm) of Dowex-1 (×8, 200–400 mesh) previously equilibrated with 0.02 M $NH_4OH$. The column was washed with 20 ml of 0.02 M $NH_4OH$ and 10 ml of 0.02 M $NH_4OH$-0.016 M formic acid (*p*H 8.8), successively. Dimers and monomers were recovered in the first 20 ml and the next 30 ml of the effluent, respectively. Ten milliliters of the dimer fraction and 1 ml of the monomer fraction were transferred into counting vials and dried. The residue was dissolved in 1 ml of water, and the radioactivity was determined by adding to it 15 ml of Bray scintillation solution and counting in a liquid scintillation counter.

**Photoreactivation.** $^3H$-labeled cells were starved for 1 hr, washed twice, suspended in M9⁻ medium, and irradiated with UV light. One part of the irradiated sample was subjected to the photoreactivating treatment as described previously (20) at room temperature in a quartz cell of 1-cm light path with monochromatic radiation of wavelength 420 nm. The other part was kept at room temperature during the photoreactivation treatment and served as an untreated control. Total dose given for the photoreactivation was $10^6$ ergs/mm² at dose rate of 2.5 × $10^4$ ergs per mm² per min.

**Irradiation.** For UV irradiation, bacteria were suspended, unless otherwise stated, in phosphate buffer (0.065 M; *p*H 7.0) at a concentration of about 2 × $10^8$ cells/ml and exposed to UV radiation from two 15-w low-pressure mercury germicidal lamps. The dose rates were 0.8 erg per mm² per sec for sensitive strains and 9 ergs per mm² per sec for resistant strains. X irradiation was carried out with a Toshiba X-ray machine operated at 180-kv peak and 25 ma with a 1.0-mm aluminum filter.

## RESULTS

**Construction of a recB21 res-1 mutant.** Since *res⁻* mutants are Hcr⁻ for MMS-treated T1 phage, this character was used to confirm the transfer of *res⁻* gene into a *recB⁻* recipient. HfrR-1 strain OK2101 (*res-1*, *thi⁺*, *str-s*) was crossed with strain AB2470 (*recB21*, *thi⁻*, *str-r*), and *thi⁺*, *str-r* recombinants were selected. Five out of 100 *thi⁺*, *str-r* recombinants turned out to be Hcr⁻ for MMS-treated T1 phage. One of the clones, OK2701, was purified and subjected to a test of its genotype. Strain OK2001 (*argA⁻*, *metE⁻*, *lysA⁻*, *cysC⁻*) was infected with P1 phage grown on strain OK2701, and *argA⁺* or *metE⁺* transductants were selected and examined for their sensitivity to UV and X rays. Twenty out of 35 *argA⁺* transductants and 6 out of 100 *metE⁺* transductants showed, respectively, UV and X-ray sen-

sitivities characteristic of the *recB21* and the *res-1* strains. These frequencies of cotransduction of the *recB* gene with *argA* and the *res* gene with the *metE* gene are in agreement with those previously reported (7, 17). All the five clones of MMS-Hcr$^-$ strains showed much reduced ability in forming colonies and very slow growth on the solid medium, although the increase of turbidity of mutants in the liquid medium was essentially normal. Microscope observation of growing cells showed few filamenteous cells. The growth rates of strains AB2470 and OK2701 in the liquid medium measured by optical density and by the numbers of colonies formed on PA medium are presented in Table 2.

**Phenotypic characteristics of the recB21 res-1 strain: (i) Sensitivities to UV and X rays.** Survivals of the *recB21 res-1* double mutants at different doses of UV and X radiation were compared with those of the single mutants *recB21* and *res-1* in Fig. 1a and b. Strain OK2701 (*recB21 res-1*) was about six times more sensitive to UV in the low-dose range than either strain AB2470 (*recB21*) or strain OK2101 (*res-1*) although it became almost as resistant as the *recB21* and the *res-1* strain in the high-dose range. The X-ray sensitivity of OK2701 was slightly, but significantly, higher than OK2102, the most X-ray sensitive strain of examined component single mutants.

**(ii) Host cell reactivation of UV- and MMS-treated T1 phage.** It is characteristic of *res*$^-$ mutants that they have an impaired ability to reactivate T1 phage irradiated by X rays, although they retain a normal Hcr for UV-irradiated T1 phage (16). The reduced Hcr capacity of *res*$^-$ strains can be demonstrated more markedly for T1 phage inactivated with MMS (17). The survivals of T1 phage on OK2701 after treatment with MMS and UV were compared with those on three other strains AB2470 (*recB21*), AB1886 (*uvrA*$^-$), and OK2102 (*res-1*) (Fig. 1c and d). The *recB21* strain was Hcr$^+$ for UV and MMS damages, and the *uvrA*$^-$ strain was Hcr$^-$ for UV damage, but Hcr$^+$ for MMS damage; the *res-1* and *recB21 res-1* strains were Hcr$^+$ for UV damage but Hcr$^-$ for MMS damage.

TABLE 2. *Difference in growth rate and colony-forming ability between OK2701 (recB21 res-1) and AB2470 (recB21)*

| Incubation time (hr) | Turbidity of bacterial cultures[a] (660 nm) | | No. of colony formers[a] ($\times 10^{-7}$/ml) | |
|---|---|---|---|---|
| | AB2470 | OK2701 | AB2470 | OK2701 |
| 0 | 0.012 | 0.012 | 0.57 | 0.025 |
| 1 | 0.022 | 0.018 | 0.60 | 0.045 |
| 2 | 0.082 | 0.061 | 1.50 | 0.058 |
| 3 | 0.310 | 0.230 | 5.40 | 0.140 |
| 4 | 0.626 | 0.530 | 19.90 | 0.17 |
| 5 | 0.670 | 0.600 | 27.60 | 0.220 |

[a] Samples of 0.1 ml each withdrawn from overnight cultures were inoculated into 10 ml of fresh M9$^+$ medium and incubated at 37 C. At 1-hr intervals, samples were taken to measure the turbidity and to score for colony formers. Colonies were counted after incubation of cells on PA agar at 37 C for 48 hr.

**(iii) UV-induced DNA breakdown.** Stability of cellular DNA in the *recB21 res-1* mutant after irradiation by 1,000 ergs/mm$^2$ UV dose was compared with those of the single mutants *recB21* and *res-1* (Fig. 2). Extensive breakdown of DNA after UV irradiation, which is characteristic of the *res*$^-$ strain, was almost completely suppressed by the presence of *recB*$^-$ gene in OK2701 (*recB21 res-1*). However, the presence of gene *recB21* did not decrease, but increased, UV sensitivity of the *res-1* strain (Fig. 1a). Therefore, the high level of UV-induced DNA breakdown is not the cause of the high UV sensitivity of the *res-1* mutant.

**Dimer excising activities in recB21 res-1 and res-1 mutants.** Excision rates of pyrimidine dimers from DNA in UV-irradiated cells of strains H/r30R, OK2701 (*recB21, res-1*), AB2470 (*recB21*), and R15 (*res-1*) (the parental strain for the *res-1* gene in OK2701) were measured by the column chromatography method described by Sekiguchi et al. (33). Cells possessing DNA labeled with $^3$H-thymidine were exposed to UV at 1,000 ergs/mm$^2$ and then incubated. At intervals, fractions were withdrawn, and the radioactivity in the dimer and in the thymine portions of acid-soluble and acid-insoluble fractions was determined (Table 3). As can be seen from a comparison of radioactivity in dimers in acid-soluble fraction and extracellular medium to radioactivity in the total thymine in Table 3, rate of excision of dimers from DNA was about five to eight (*see* Table 4) times slower in the *recB21 res-1* strain but normal in the *res-1* and *recB21* strains when compared to the wild-type strain H/r30R. Significant amounts of dimer, however, might be released from DNA associated with DNA degradation. Therefore, an evaluation was made for the dimers released through degradation process. Estimation of dimer to thymine ratios in acid-soluble fraction and extracellular medium is difficult because a considerable amount of thymine was released into the acid-soluble

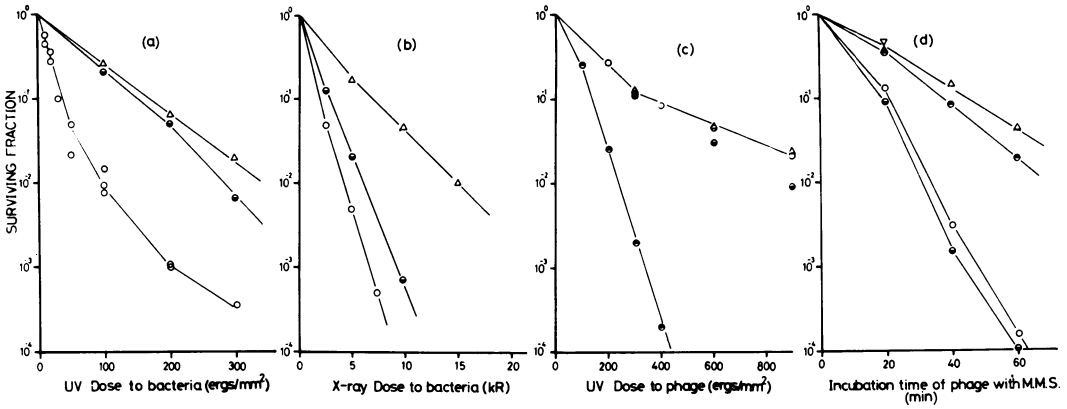


FIG. 1. *a and b, Fractions of surviving colonies of the double mutant recB⁻ res⁻ and the component single mutants recB⁻ and res⁻ after irradiation with various doses of UV and X-rays. Overnight cultures of bacteria were washed and suspended in phosphate buffer at a concentration of 2 × 10⁸ cells per ml. Samples of 1 ml were irradiated, and cells were plated on peptone agar and incubated at 37 C for about 48 hr. Symbols: △, AB2470 (recB21); ◒, OK2102 (res-1); and ○, OK2701 (recB21 res-1). c and d, Fraction of surviving infective centers of T1 phage inactivated by UV light (c) and MMS (d). Phage particles are assayed on the three strains of E. coli used in 1(a) and (b) plus an uvrA⁻ strain. Overnight cultures of host bacteria were washed, starved for 1 hr at 37 C, and suspended in T1 buffer at a concentration of 2 × 10⁹ cells/ml. The cell suspensions were mixed with T1 phage having been treated with UV or MMS at a multiplicity of infection of about 0.1. The infection was allowed to take place for 15 min at 37 C. After removal of unadsorbed phage particles by centrifugation in the cold, the complex was plated in soft agar with indicator bacteria of strain R15 to count plaque formers. Symbols: △, AB2470 (recB21); ◒, OK2102 (res-1); ○, OK2701 (recB21 res-1); and ◓, AB1886 (uvrA⁻).*

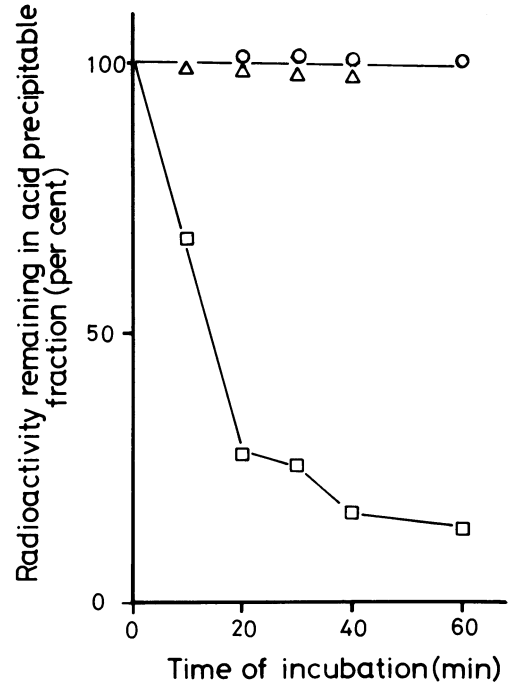


FIG. 2. *UV-induced degradation of cellular DNA in the recB⁻ res⁻, recB⁻ res⁺, and recB⁺ res⁻ strains. Ratios of radioactivity remaining in PCA-insoluble fractions to the total material were calculated from Table 3, for the three strains OK2701, R15, and AB2470. These were normalized to unity at 0 min incubation time. The experimental procedures were described in Materials and Methods. Symbols: ○, OK2701 (recB21 res-1); □, R15 (res-1); and △, AB2470 (recB21).*

fraction in unirradiated cells. After correcting for these control radioactivity counts, relative amounts of dimer and thymine in acid-soluble fractions were estimated from Table 3 and are summarized in Table 4. The dimer-to-thymine ratios averaged over the post-UV incubation from 10 to 60 min were, respectively, 1.4% for the H/r30R, 5% for the *recB21 res-1*, 0.13% for *res-1*, and 5.1% for the *recB21* strain. The value 0.13% for *res-1* is very close to that of total dimer-to-thymine ratio of the cellular DNA (see the last column of Table 3). This strongly suggests that the dimers released from DNA in *res-1* cells would be the result of a nonspecific breakdown of DNA after UV irradiation.

**Sedimentation of DNA in alkaline sucrose gradient.** Excision of dimers in vivo must be accompanied by a structural alteration of the cellular DNA. Therefore, mutants defective at various steps of the excision repair process would show different states of structur-

TABLE 3. *Balance sheet for excision of dimers in H/r30R (rec⁺ res⁺), OK2701 (recB21 res-1), AB2470 (recB21), and R15 (res-1)*

| Strain and UV dose | Incubation time (min) | Radioactivities in dimers of fractions (counts/min) | | | | Radioactivities in thymine of fractions ($10^3$ counts/min) | | | | Ratio of dimers to thymine (%) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | PCA soluble[a] | Extracellular[b] | PCA insoluble[a] | Total | PCA soluble[a] | Extracellular[b] | PCA insoluble[a] | Total | |
| OK2701 | | | | | | | | | | |
| Control | 0 | 12 | | 324 | 336 | 27.4 | | 716 | 743.4 | 0.045 |
| | 60 | 13 | | 215 | 228 | 26.5 | | 695 | 721.5 | 0.032 |
| 1,000 ergs/mm² | 0 | 53 | | 1,340 | 1,393 | 25.4 | | 735 | 760.4 | 0.182 |
| | 10 | 105 | | 1,260 | 1,365 | 28.1 | | 666 | 694.1 | 0.196 |
| | 20 | 99 | | 1,210 | 1,301 | 21.8 | | 650 | 671.8 | 0.194 |
| | 30 | 117 | | 1,510 | 1,627 | 24.0 | | 894 | 918.0 | 0.177 |
| | 40 | 216 | | 1,250 | 1,466 | 29.0 | | 688 | 717.0 | 0.204 |
| | 60 | 230 | | 1,070 | 1,300 | 44.6 | | 653 | 697.6 | 0.187 |
| R15 | | | | | | | | | | |
| Control | 0 | 13 | 4.6 | 366 | 384 | | 8.0 | 732 | 740.0 | 0.052 |
| | 60 | 25 | | 354 | 379 | 25.1 | 36.8 | 715 | 776.9 | 0.049 |
| 1,000 ergs/mm² | 0 | 40 | | 1,160 | 1,200 | 57.2 | 4.1 | 714 | 775.3 | 0.155 |
| | 10 | 255 | 51 | 864 | 1,119 | 129.6 | 103.1 | 484 | 716.5 | 0.155 |
| | 20 | 368 | 111 | 488 | 967 | 166.2 | 241.8 | 187.5 | 595.5 | 0.162 |
| | 30 | 423 | 149 | 393 | 965 | 173.0 | 291.0 | 156.0 | 620.0 | 0.156 |
| | 40 | 428 | 230 | 342 | 970 | 132.0 | 387.2 | 100.0 | 619.2 | 0.166 |
| | 60 | 400 | 256 | 320 | 976 | 106.8 | 434.7 | 86.8 | 627.3 | 0.155 |
| AB2470 | | | | | | | | | | |
| 1,000 ergs/mm² | 0 | 60 | | 1,690 | 1,750 | 17.4 | | 925 | 942.4 | 0.186 |
| | 10 | 347 | | 1,260 | 1,610 | 21.8 | | 939.5 | 956.3 | 0.168 |
| | 20 | 782 | | 960 | 1,740 | 33.4 | | 900 | 933.4 | 0.187 |
| | 30 | 824 | | 1,040 | 1,860 | 36.8 | | 895 | 931.8 | 0.199 |
| | 40 | 770 | | 850 | 1,620 | 33.6 | | 890 | 923.6 | 0.177 |
| | 60 | 996 | | | | 43.5 | | | | |
| H/r30R | | | | | | | | | | |
| Control | 0 | 14 | | 460 | 474 | 35.3 | | 1,150 | 1,185 | 0.039 |
| | 60 | 19 | | 420 | 439 | 48.0 | | 1,050 | 1,100 | 0.036 |
| 1,000 ergs/mm² | 0 | 32 | 18 | 2,300 | 2,356 | 65.0 | 22.6 | 1,120 | 1,207.6 | 0.193 |
| | 10 | 235 | 120 | 1,770 | 2,125 | 49.3 | 20.5 | 893 | 962.8 | 0.22 |
| | 20 | 545 | 310 | 1,330 | 2,185 | 56.0 | 54.6 | 935 | 1,146 | 0.196 |
| | 30 | | | 750 | | 43.2 | 50.4 | 956 | 1,051 | |
| | 40 | 1,050 | 540 | 400 | 1,990 | 75.5 | 108.0 | 1,000 | 1,184 | 0.176 |
| | 60 | 714 | 520 | 410 | 1,644 | 69.5 | 116.0 | 900 | 1,086 | 0.157 |

[a] Samples were separated into dimer and thymine fractions in Dowex I column chromatography.
[b] Radioactive materials released from cells into extracellular medium; these were separated into dimer and thymine fractions in Dowex I column chromatography after removing inorganic salts in medium.

ally altered DNA. Analysis of DNA in an alkaline sucrose gradient was carried out to see what is the fate of single-strand scissions or gaps induced by the repair process during post-UV incubation. Strains examined were wild-type strain H/r30R, excision-defective mutant AB1886 (*uvrA*⁻), and AB1884 (*uvrC*⁻), the double mutant OK2701 (*recB21 res-1*), and AB2470 (*recB21*). Preliminary experiments with strain H/r30R revealed that amounts of single-strand breaks (as measured by appearance of slowly sedimenting DNA) depend upon the post-UV incubation time with a maximal amount appearing after about 20 min of incubation, and that 60 min is enough to return the DNA profile to that of nonirradiated cells. Therefore, most experiments were done with samples at 0, 20, and 60 min postincubation. Like the wild-type strain H/r30R (data not presented), strain AB2470 (*recB21*) showed a significant change in sedimentation profile of UV-irradiated DNA during the post-UV-incu-

bation: single-strand breaks were detected at 20 min and had rejoined by 60 min (Fig. 3a). No such change in the profile of irradiated DNA was found with strain AB1886 (*uvrA⁻*) (Fig. 3b). The strain OK2701 (*recB21 res-1*) (Fig. 3d) showed an incubation-time-dependent change in DNA profiles very similar to that of strain AB2470 (recB21). On the other hand, strain AB1886 (*uvrC⁻*) showed a peculiar change in DNA profiles (Fig. 3c); this excision-defective mutant showed an increased number of nicks as post-UV-incubation time increased. Similar features were observed with two other *uvrC⁻* strains of different origin, N12-2 and OK2603. The number of single-strand breaks accumulated during 60 min of post-UV incubation in all the three *uvrC⁻* mutants were estimated from a sedimentation profile of DNA (Table 5). Photoreactivation of irradiated cells before incubation restored the sedimentation profiles almost completely to the profile of nonirradiated DNA. These results indicate that the accumulation of single-strand breaks in DNA in these *uvrC⁻* mutants is induced by action of a dimer-specific nuclease during post-UV incubation. However, it should be noted that the estimated number of nicks induced in 60 min corresponds to only about one-tenth of the total number of pyrimidine dimers calculated from the yield of 6.7 pyrimidine dimers per erg per mm² per bacterial chromosome.

## DISCUSSION

The double mutant *recB21 res-1* grew very poorly on solid agar, but grew normally in liquid when compared to mutant *recB21*. The double mutant, however, exhibits a high spontaneous loss of colony-forming ability (Table 2). The cause of this elevated spontaneous lethality of the *recB21 res-1* strain is still obscure. It may be useful to note that a combination of genes *recA⁻* or *recB⁻* and *polA* (11, 25) is lethal if the two mutations are nonleaky. These facts suggest that the double mutant may have a suppressor which makes the cell escape partially from the lethality. In fact, we have detected a very low level of DNA polymerase I activity in the lysate of *recB21 res-1* mutant (T. Kato, *unpublished data*).

As shown with strain *recB21 res-1*, the absence of functional *recB⁺* product prevented the *res-1* strain from extensive breakdown of DNA after UV irradiation (Fig. 2), whereas the UV sensitivity of the double mutant *recB21 res-1* was not decreased but rather increased beyond that of either single mutant *recB21* or *res-1* (Fig. 1). Therefore, the high sensitivity of the *res-1* strain to UV irradiation is not correlated with UV-induced degradation of DNA. For this reason, one of the hypotheses previously proposed (17)—that the *res-1* mutant may be sensitive to UV because of extensive breakdown of DNA—seems to be untenable. Willetts and Clark (37) have noted a similar lack of correlation between DNA degradation and UV sensitivity in strain *recA⁻* with *recA⁻ recB⁻* (or *recC⁻*) double mutants.

TABLE 4. *Comparison of dimer-excising and thymine-releasing ratio among the four strains recB⁻ res⁻, recB⁻, res⁻, and recB⁺ res⁺* [a]

| Genotype | | Abundance in PCA-soluble and extracellular fractions | | |
|---|---|---|---|---|
| *recB* | *res* | Dimer | Thymine | Ratio of dimer to thymine (%) |
| + | + | 1.0 | 79 | 1.4 |
| − | + | 0.92 | 18 | 5.1 |
| + | − | 0.97 | 760 × 0.97 | 0.13 |
| − | − | 0.14 | 20 × 0.14 | 5.0 |

[a] Relative ratios of $^3H$ radioactivities in each fraction at the same incubation time have been normalized to unity for dimers of the wild-type strain and averaged over the post-UV-irradiation incubation from 10 to 60 min. Correction was made for those of unirradiated control samples.

The most pronounced characteristic of the double mutant *recB21 res-1* was the reduced excision of dimers from DNA as compared with the wild-type or *recB21* strains (Tables 3 and 4). It may, therefore, be concluded that the *res-1* mutation is responsible for the impaired excision repair ability of the *recB21 res-1* mutant and that DNA polymerase I (17, 30), the product of *res-1* gene, is involved at least partly in excision of dimers in vivo. Yet, an argument that combination of *recB21* and *res-1* mutations reduces in some way the excision activity still remains. Boyle et al. (5) reported that strain *polA1* is able to excise dimers, at a rate only slightly slower than the wild-type strain. Katsuki and Sekiguchi (Abstr. 42nd Annu Meet. of Genet. Soc., Japan, 1970) have also made a preliminary report that a *pol⁻* strain isolated by Ogawa (27) has a very low excision capacity. These facts may mean that mutations at different sites in the *pol* locus frequently lead to quantitatively different levels of deficiency in the excising dimers in vivo. In fact, the *polA4**, a radiation-resistant revertant of the *polA4*, has been reported to continue to lack DNA polymerase I activity in vitro (3).

Sedimentation profiles of DNA from UV-irradiated *uvrC⁻* mutants revealed the accu-

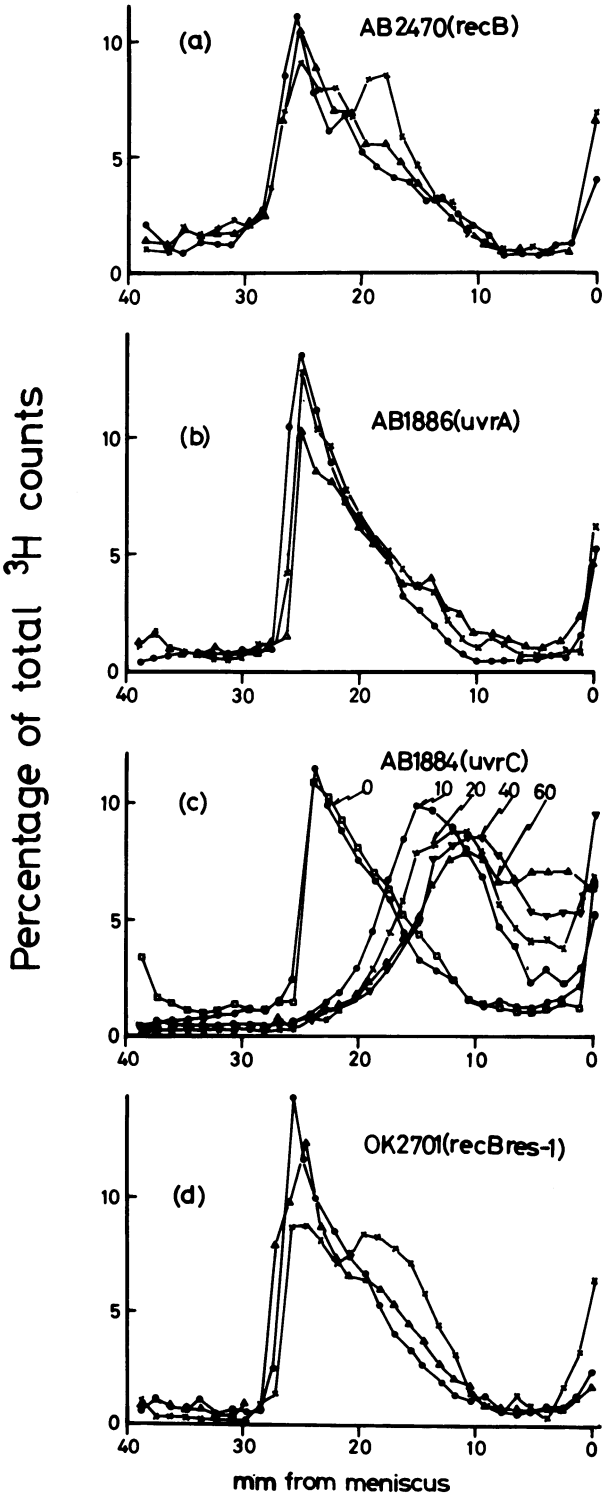


FIG. 3. *Sedimentation profiles of DNA in an alkaline sucrose gradient from cells of four strains irradiated with 400 ergs/mm² of UV light. Cells labeled with ³H-thymidine were starved for 1 hr at 37 C in M9⁻ medium and exposed to UV light at 0 C. After supplementing with glucose and Casamino Acids, nonirradiated and irradiated cells were incubated at 37 C. At 20 and 60 min after the initiation of post-UV incubation, samples of the three strains were withdrawn for analysis by sucrose gradient centrifugation. Samples of the strain uvrC⁻ were analyzed at shorter intervals as indicated in the figure. Symbols: ○, control cells with 60 min of incubation; ×, irradiated cells after 20 min post-UV incubation; and △, cells after 60 min of post-UV incubation.*

mulation of slowly sedimenting DNA during post-UV incubation (Fig. 3c). One of the simplest interpretations of this finding is that single-strand incisions are induced during post-UV incubation by a UV-specific endonuclease, which would be under the control of *uvrA* or *uvrB* genes, or both. Some lines of evidence supporting this notion are (i) kinetics of single-strand break accumulation in the strain *uvrC⁻* during post-UV incubation (Fig. 3c) were qualitatively similar to that of dimer excision (Table 3); (ii) photoreactivation of UV-irradiated cells prior to post-UV incubation eliminated the occurrence of single-strand breaks in a *uvrC⁻* strain (Table 5); (iii) a double mutant *uvrA⁻ urvC⁻* showed few single-strand breaks during 60 min of post-UV incubation (*unpublished data*); and (iv) the *uvrC⁻* mutant was unable to excise dimers, in spite of possessing a normal DNA polymerase I activity. These results suggest that there may exist an intermediate step in vivo between the incision and excision of dimers, and that the product of the *uvrC* gene might be involved in this postulated intermediate step. UV-damaged DNA-specific endonuclease has been isolated from *Micrococcus luteus* (21) and T4 phage infected *E. coli* cells (39). The *Micrococcus* enzyme catalyzes the formation of single-strand incision on the 5′ site of the dimers in UV-irradiated DNA, leaving a 3′-phosphomonoester and 5′-hydroxyl end group. A similar enzyme has not yet been detected in *E. coli* cells. However, if we assume the existence of *Micrococcus*-type enzyme in *E. coli*, then the presence of 3′-phosphate ends blocks translation of the nicks by DNA polymerase I (18, 23) and it would require participation of an intermediate step for preparing 3′-hydroxyl ends from the 3′-phosphate ends.

In UV-irradiated *recB21 res-1* cells, the initial incision and modification of the end group of incised single-strand DNA would take place, but the absence of functional DNA polymerase I blocks excision of dimers which normally follows. These gaps, however, are eventually rejoined if adenosine triphosphate-dependent nucleases are absent (Fig. 3d). X-ray induced single-strand breakage, which is known to produce various end groups (14), can also be repaired by DNA polymerase I in vivo (15, 36). The product of *uvrC* gene could not be involved in the repair of X-ray-induced single-strand breakage, for *uvrC⁻* mutant is apparently able to repair those damages as effectively as the wild-type strain (T. Kato, *unpublished observation*). This also supports the hypothesis of the intermediate mentioned above, which is specific for excision of UV-induced DNA damage. However, if mutants *uvrC⁻* or *res⁻* are completely lacking the excision ability, those strains should be as UV sensitive as strain *uvrA⁻*. This is not the case. One of the most plausible interpretations is that there must be another repair pathway in vivo, which might be able to translate nicks (or gaps) induced by UV-specific endonuclease (or after defective excision of dimers) and could repair those portion of DNA in some way. This would also explain why the most repair-deficient mutants, except strains *uvrA⁻* and *recA⁻*, are relatively resistant to UV. A similar conclusion has been reached by Monk et al. (26) studying excision repair of *polA1 uvrA⁻* mutant.

TABLE 5. *Estimated numbers of single-strand breaks in UV-irradiated DNA of various uvrC⁻ strains which were produced in 60-min post-irradiation incubation*

| Strain | UV dose (ergs/mm²) | No.-avg distance[a] of sedimentation of DNA pieces (mm from meniscus) | Molecular wt[b] ($\times 10^{-6}$) | No. of single-strand breaks/chromosome[c] |
|---|---|---|---|---|
| AB1884 | 0 | 17.5 | 120 | |
| AB1884 | 100 | 13.9 | 50.0 | 50 |
| N12-2 | 200 | 10.6 | 24.3 | 103 |
| OK2603 | 240 | 9.8 | 19.5 | 140 |
| AB1884 | 400 | 7.7 | 10.7 | 235 |
| AB1884 | 100 + Phr[d] | 17.0 | | |

[a] The number-average distance was calculated by using the formula: $D_n = \Sigma f_i/f_i/D_i$ where $f_i$ is the fraction of the total radioactivity in the ith fraction and $D_i$ is the distance of the ith fraction.

[b] The molecular weight ($M_2$) was calculated from sedimentation distance $D_2$ using $M_2/M_1 = (D_2/D_1)^\alpha$ (Bergi-Hershey), where $M_1$ and $D_1$ are sedimentation distance and molecular weight of reference DNA. As previously reported (15), we used T1 phage DNA as reference and the following values: $M_1 = 15.5 \times 10^6$, $D_1 = 8.9$ mm and $\alpha = 1/3.4$.

[c] Estimation was based on the molecular weight $2.4 \times 10^9$ for intact double-stranded chromosome of *E. coli*.

[d] Prior to incubation, UV-irradiated cells were subjected to photoreactivation treatment with 420 nm at dose of $10^6$ ergs/mm².


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