REPAIR DEFICIENCY IN ESCHERICHIA COLI UV-SENSITIVE MUTATOR STRAIN uvr502

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SUMMARY--The effect of ultraviolet irradiation (UV) has been studied in Escherichia coli mutator UV-sensitive mutant uvr502, its uvrA6 derivative and wild-type strain. The uvr502 mutant is about 5 times more UV-sensitive than the uvr^+ isogenic strain, but 3 times less sensitive than the uvrA6 single mutant. Cells of the uvr502 mutant are unable to rejoin the fragments of parental DNA formed after UV as a result of incision. The double mutant uvrA6 uvr502 as well as the single uvrA6 mutant irradiated with UV is unable to introduce breaks into parental DNA. The extent of postreplication repair is essentially normal in the uvr502 cells. There is no significant difference between the uvr^+ and uvr502 cells in the rate and extent of UV-induced DNA degradation.

At least two repair systems are involved in the enzymatic dark repair of DNA in UV-irradiated E. coli. In excision repair an incision is made near a pyrimidine dimer or other distortion of the double helix. Oligonucleotides containing the defect are removed and the excised material is then replaced with normal DNA by repair replication, with the undamaged complementary strand as template. Finally the joining of the repaired fragment to the parental DNA strand occurs. The second system, called postreplication repair, allows cells to survive with the unrepaired pyrimidine dimers in the parental DNA. Gaps left in the daughter DNA strands opposite dimers after the replication of dimer-containing DNA are repaired.

The genetic control of these repair pathways has been extensively studied. Cells carrying a mutation in either the uvrA or uvrB gene are unable to carry out excision repair, possibly because of deficiency in the first step of this process (1). Both dimer excision and repair replication are most probably performed by DNA polymerase I (2-6), coded by the polA gene (7,8). It was suggested that the recA gene is responsible for postreplication repair (9). This suggestion was confirmed by the finding of complete inability of the recA mutant to carry out this process (10). However recently direct and indirect evidence has accumulated suggesting

involvement of the recA-mediated repair in the excision repair pathway, presumably at steps affected by the polA1 mutation (5,6).

Although genetic control of some steps of DNA repair has been determined, there are genetic loci controlling radiation sensitivity whose repair functions are still obscure. In this communication we present the results of experiments designed to determine which repair pathway is affected in the UV-sensitive mutator strain uvr502, by assaying the final (rejoining) steps of excision and postreplication repair.

MATERIALS AND METHODS

The bacterial strains used are listed in Table I.

Medium was medium A(11), containing 1% tryptone (Difco), 0.2% glucose supplemented with 20μg/ml thymidine.

Labeling of parental DNA. Overnight cultures were diluted 1:30 with pre-warmed medium containing 20μCi/ml thymidine (4.3 Ci/mmole). After 90 min incubation at 37° the cells were washed on membrane filters (HUFS, Chemapol, ChSSR), resuspended and incubated 30 min in the medium without thymidine and then 30 min with 20μg/ml thymidine at 37°. The bacteria were collected on the filter and resuspended in 1/15 M phosphate buffer, pH 7.0. The suspension (1-3 x 10^7 cells/ml) was divided into two parts, one of which was UV-irradiated and another was used as control.

UV-irradiation. The suspensions were cooled in ice water and exposed to UV at a dose rate of 0.5 ergs/mm^2/sec, primarily at 254 nm. The cells were irradiated with a dose of 300 ergs/mm^2 for the studies of excision repair and degradation of DNA and with a dose of 25 ergs/mm^2 for measurements of postreplication repair.

Excision repair. Sedimentation through alkaline sucrose gradients of ^{3}H-labeled parental DNA from bacteria incubated in nutrient broth at 37° 0, 10 and 60 min after irradiation was used as a measure of their ability to perform excision repair.

Postreplication repair. Overnight cultures were diluted 1:30 into fresh

medium and incubated for 90 min at 37°. The cells were collected on filters, washed, resuspended in buffer and irradiated. The bacteria were then incubated in medium supplemented with 100μCi/ml ^{3}H-thymidine for 10 min at 37°. Then the cells were harvested by filtration and washed, and the DNA sedimented through alkaline sucrose either immediately or after 60 min further incubation in nonradioactive medium at 37°.

Methods of sedimentation and measurement of DNA degradation have been described previously (12,13).

RESULTS

Table I shows that the uvr502 mutant is about 5 times more UV-sensitive

TABLE I

	Bacterial Strains[a]			
	uvrA	uvr(502)	D_{37} UV dose (ergs/mm^2)[b]	Relative UV Sensitivity
KS112	+	+	92	1
KS113	6	+	6	15
KS114	+	502	20	4.6
KS115	6	502	4	23

[a] Construction of strains is described elsewhere (12). All are F$^-$, thyA, metF, rha, lac Y14Str^R.

[b] The increment of dose necessary to reduce survival by 63% in the exponential region of the curve, calculated from the data of Ref. 12.

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to inactivation of colony-forming ability than the uvr$^+$ strain, and 3 times more resistant than uvrA6. The double mutant uvrA6 uvr502 is only 1.5 times more UV-sensitive than the uvrA6 single mutant, suggesting that the uvr502 mutation affects some step of the excision repair pathway. To test this suggestion we compared the ability of the uvr$^+$ and uvr502 strains to repair single-strand breaks introduced into parental DNA after UV-irradiation by determination of single-strand molecular weight of DNA at different times after UV. Figure 1 a, b shows that the distribution of single-strand

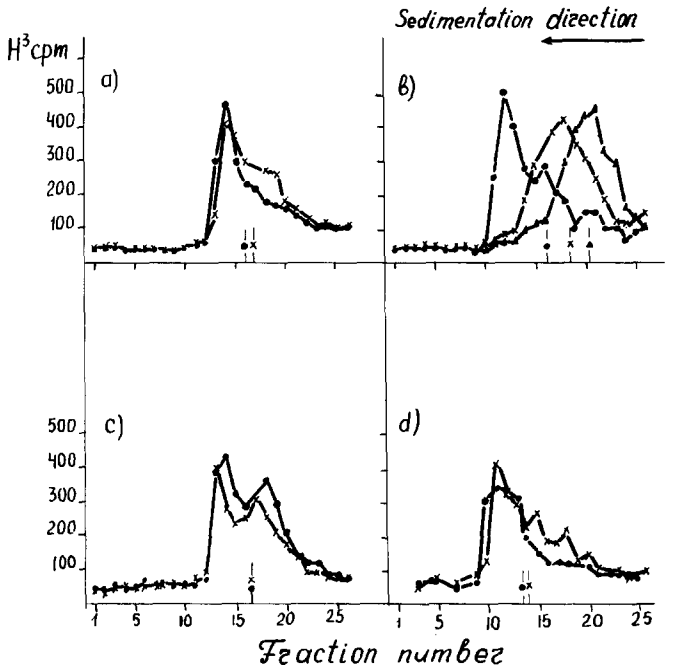

FIG. 1. Sedimentation profiles of parental DNA in E. coli KS112 uvr$^+$ (a), KS114 uvr502 (b), KS113 uvrA6 (c) and KS115 uvrA6 uvr502 (d). ●: unirradiated control; X: 300 ergs/mm^2 UV and 10 min incubation at 37° after irradiation; ▲: 300 ergs/mm^2 and 60 min incubation after irradiation. The alkaline sucrose gradients were centrifuged in a Spinco SW39 rotor at 30,000 rpm at 20° for 90 min (a, b, c) and for 100 min (d). The position of the weight average molecular weight for each sample is indicated at the bottom of the profiles.

molecular weight of DNA from the uvr502 mutant incubated 10 min after UV is significantly lower than that of the uvr$^+$ strain. When the uvr502 cells were incubated 60 min after UV, further reduction of the single-strand molecular weight was observed. These data show that the uvr502 mutant is incision proficient but deficient in some later step(s) of excision repair. To confirm this point the ability of the uvrA6 and uvrA6 uvr502 strains to introduce breaks into their DNA after UV was measured. As expected both strains were unable to initiate excision repair (Fig. 1 c,d).

Next we examined the ability of the uvr502 mutant to perform postreplication repair (Fig. 2). The DNA from the uvr502 as well as from the uvr$^+$ cells labeled with ^{3}H-thymidine for 10 min after UV sediments more slowly in

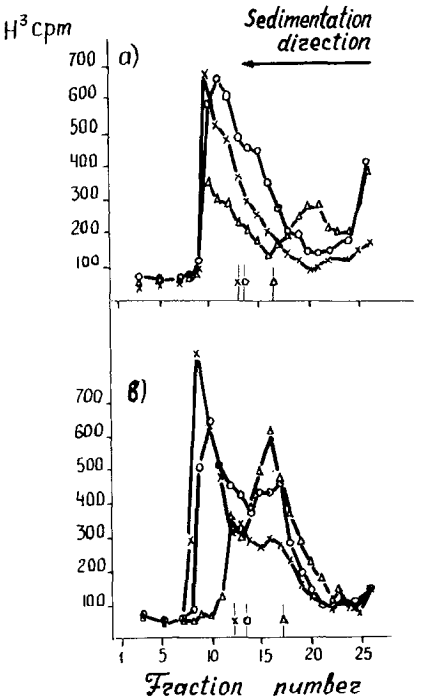

FIG. 2. Sedimentation profiles of newly synthesized DNA in UV-irradiated E. coli KS112 uvr$^+$ (a) and KS114 uvr502 (b). O: unirradiated cells and 10 min pulse of ^{3}H-thymidine; Δ: 25 ergs/mm^2 and then 10 min pulse of label; X: 25 ergs/mm^2, 10 min pulse of label and then 60 min incubation in non-radioactive medium at 37°. The position of the weight average molecular weight for each sample is indicated at the bottom of the profiles.

alkaline sucrose gradients than DNA from unirradiated cells. Incubation of the cells of both strains after labeling led to a similar increase in the rates of sedimentation of the labeled DNA, which became comparable with those of unirradiated controls. It appears, therefore, that the uvr502 cells are able to repair the majority of gaps formed in DNA synthesized after exposure to 25 ergs/mm^2 of UV.

Since according to the data of Figure 1 the breaks introduced by incision in the uvr502 cells remain unrepaired for at least 60 min, we looked for DNA degradation which might be initiated at such breaks. The extent of DNA degradation even after 100 min of incubation was at most 10%, and only slightly exceeded that in the uvr$^+$ cells. We conclude that the ends of

unrepaired single-strand breaks in the DNA of the UV-irradiated uvr502 cells are not available for extensive exonucleolytic hydrolysis.

DISCUSSION

The finding that the UV-sensitivity of the double mutant uvrA6 uvr502 is only 1.5 times more than that of the uvrA6 single mutant suggests that the uvr502 mutation affects UV-sensitivity by reducing the efficiency of excision repair and does not alter the postreplication repair pathway. This suggestion was confirmed in direct experiments. The uvr502 mutant was found to be deficient in the rejoining of single-strand breaks appearing in parental DNA after UV and proficient in postreplication repair. Since the uvr502 mutation affects neither incision nor polynucleotide ligase activity (14), the reaction affected by this mutation is probably between the initial (incision) and final (rejoining) steps of the excision repair pathway.

Preliminary data show that the UV-irradiated uvr502 cells are able to excise pyrimidine dimers from their DNA (as detected by the immunological measurement of dimers remaining in DNA), although the final extent of the reaction was not determined precisely. Thus the $uvr(502)^{+}$ gene product seems to be required for repair synthesis following excision.

We have not found any measurable increase in single-strand molecular weight of DNA from UV-irradiated uvr502 cells at least during 60 min of post-irradiation incubation; on the contrary, by 60 min a further decrease in molecular weight was observed. These data indicate that the uvr502 cells are unable to repair all the single-strand breaks that appear in their DNA after UV. However, the uvr502 mutant is less UV-sensitive than the uvrA6 mutant, which is totally lacking in excision ability. The reason for this intermediate UV-sensitivity of the uvr502 mutant is not clear at present. Probably the rate of induction of breaks in the uvr502 cells is higher than that of the joining process, so that the latter cannot be detected during the first 60 min after UV.

Mutants $recA^{-}$ and $polA^{-}$ are also known to be deficient in the repair of

single-strand breaks after UV (3,15). Thus DNA polymerase I as well as the recA$^+$ and uvr(502)$^+$ gene products are required for the completion of excision repair. The polA$^-$ bacteria are not totally deficient in excision repair (3), because repair replication can be performed by some enzymatic activities dependent upon the recA$^+$ allele (6). In contrast, UV-irradiated recA$^-$ and uvr502 mutants appear to be more deficient in strand rejoining. Thus it could be concluded that neither DNA polymerase I nor recA$^+$-mediated repair are able to carry out the rejoining of all single-strand breaks introduced into DNA of uvr502 in the course of excision. This may be explained on the basis of at least two possibilities: i) the uvr(502)$^+$ gene product modifies the ends of endonuclease-produced single-strand breaks, making them available for polymerisation; ii) the enzymes carrying out the second step of excision repair work only in a complex with the uvr(502)$^+$ gene product.

Recently we have shown that the double mutant uvr502 polA is inviable (16). Since the uvr502 single mutant is viable, it was suggested that DNA polymerase I may effectively substitute for the missing uvr(502)$^+$ gene product in performing some function(s) essential for cell growth. Probably DNA polymerase I is also able to substitute for the uvr(502)$^+$ gene product in the repair of breaks in UV-irradiated cells. However, the apparent deficiency of the uvr502 cells in rejoining of the breaks suggests that this substitution, if it occurs, is much less efficient than that of DNA polymerase I in carrying out a vital function in the unirradiated uvr502 cell.

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