JOURNAL OF BACTERIOLOGY, Aug. 1979, p. 454-459
0021-9193/79/08-0454/06$02.00/0

Vol. 139, No. 2

Inactivation of *Escherichia coli* by Near-Ultraviolet Light and 8-Methoxypsoralen: Different Responses of Strains B/r and K-12

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Received for publication 15 May 1979

A series of *Escherichia coli* K-12 AB1157 strains with normal and defective deoxyribonucleic acid repair capacity were more resistant to treatment with 8-methoxypsoralen (8-MOP) and near-ultraviolet light (NUV) than a comparable series of strains from the B/r WP2 family although sensitivities to 254-nm ultraviolet light were closely similar. The difference was most marked with strains deficient in both excision and postreplication repair (*uvrA recA*). The hypothesis that the internal level of 8-MOP was lower in K-12 than B/r *uvrA recA* derivatives was ruled out on the basis of fluorometric determinations of 8-MOP content and the similar inactivation curves for phage T3 treated intracellularly within the two strains. The demonstration of liquid holding recovery with AB2480 but not WP100 (both *recA uvrA* strains) and the somewhat greater resistance of the former strain to inactivation by captan revealed the presence in the K-12 strain of a deoxyribonucleic acid repair system independent of the $recA^+$ and $uvrA^+$ genes. The presence of this repair system did not, however, affect the survival of T3 phage treated with 8-MOP plus NUV and probably has a relatively small effect on survival of AB2480 under normal conditions. Experiments in which 8-MOP monoadducts were converted to cross-links by a second NUV exposure in the absence of 8-MOP indicated that the level of potentially cross-linkable monoadducts immediately after 8-MOP + NUV is about eightfold lower in K-12- than in B/r-derived strains. It is therefore suggested that the photoproduct yield in the former is well below that in the latter. In agreement with this is the observation that, during the first 10 min after treatment, deoxyribonucleic acid synthesis was just over five times more sensitive to inhibition by 8-MOP plus NUV in WP100 than in AB2480. We assume that 8-MOP in K-12 bacteria is hindered in some way from adsorbing to cellular (though not to phage T3) deoxyribonucleic acid. Consistent with this, 8-MOP has been shown to act as an inhibitor of a component of repair of 254-nm ultraviolet light damage in WP2 but not in AB1157.

Furocoumarins such as 8-methoxypsoralen (8-MOP) in the presence of UV light of around 340 nm (near-UV [NUV] cause damage to the DNA of bacteria and other organisms. First, binding to DNA occurs in a weak noncovalent manner at a limited number of sites (7), a proportion of which may then use the energy of a photon to form a covalently bound adduct to one of the pyrimidine bases (6). The energy of a second photon may then be used at a proportion of adducts to allow a reaction between the other end of the psoralen molecule and the other DNA strand, forming an interstrand cross-link (4). At low doses, monoadducts greatly outnumber cross-links, but the proportion of cross-links increases with increasing dose. In a recent study using members of the B/r family we concluded that a very small number of cross-links, possibly one, could on average kill excision-deficient bacteria (strain WP2 *uvrA*), whereas bacteria of a derivative strain, WP100 (*uvrA recA*), were killed at doses well below those at which a significant number of cross-links should have been formed, and might well be killed by a very small number of monoadducts, possibly one (manuscript in preparation; see also 13). We now present results showing that bacteria from the K-12 AB1157 family are markedly more resistant than those derived from B/r WP2 and suggesting that this difference is primarily due to a difference in DNA photoproduct yield for a given 8-MOP plus NUV exposure.

MATERIALS AND METHODS

Microorganisms. Bacterial strains are shown in Table 1. The B/r strains required tryptophan, and the K-12 strains were auxotrophic for threonine, arginine, proline, leucine, histidine, and thiamine. Bacteriophage T3 was obtained from N. Symonds.

Growth media. All strains of bacteria were grown with shaking at 37°C to about 2×10^8/ml in the following medium, containing (per liter): Na_2HPO_4, 6 g; KH_2PO_4, 3 g; NaCl, 0.5 g; NH_4Cl, 1 g; $CaCl_2$, 11 mg; $MgSO_4$, 0.25 g; gelatin, 1 mg; glucose, 4 g; tryptophan, 10 mg; Casamino Acids (Difco), 4 g. All the K-12 strains grew marginally more slowly in this medium than the analogous B/r derivatives. Bacteria were suspended in phage buffer (1) at a density of around 10^5/ml (unless otherwise stated) before irradiation. Where appropriate, 8-MOP (Sigma) was added to a final concentration of 20 μg/ml, and the suspension was allowed to stand at room temperature for 10 min before irradiation. Viable counts of all strains were made after appropriate dilution on Davis and Mingioli (8) minimal salts plus 0.4% (wt/vol) glucose, 0.25 μg of tryptophan per ml, 0.4% (wt/vol) Casamino Acids, and 1.5% Davis New Zealand agar.

For experiments involving bacteriophage T3, bacteria were grown in L-broth and phage assays were made in L-agar with a lawn of WP2 unless otherwise stated. Phage were treated with 8-MOP plus NUV in phage buffer at a density of 10^5/ml. Where appropriate, phage adsorption was allowed to occur with a 100-fold-greater concentration of bacteria during a 10-min incubation in phage buffer at room temperature. All experiments were carried out at least three times.

Irradiation. Samples were irradiated 10.5 cm from a Philips 20 watt "Black-light" lamp, the emission from which was distributed roughly symmetrically about the transmission maximum of the lamp filter at 350 nm. The fluence through the sample was 4.63×10^{-4} W cm^{-2}. For short exposures a shutter apparatus was placed above the sample which reduced the incident fluence to 1.85×10^{-4} W cm^{-2}. Samples were irradiated at room temperature (20 to 22°C), and all operations were carried out in low-intensity yellow light to prevent unwanted photoreactions. UV light of predominantly 254 nm was obtained from a Hanovia low-pressure mercury lamp calibrated with a Latarjet meter.

Determination of 8-MOP content of cells. Bacteria that had been incubated for 10 min at room temperature at a density of ca. 2×10^8/ml in phage buffer containing 20 μg of 8-MOP per ml were centrifuged and suspended in 1/10 volume of distilled water. A microscopic total count was performed at this stage. After 10 min to allow 8-MOP to diffuse out of the bacteria, the suspension was passed through a membrane filter, and the filtrate was analyzed for 8-MOP content with a calibrated fluorimeter (excitation wavelength 341 nm, emission wavelength 500 nm). Cell weight was estimated by passing a suspension containing a known number of bacteria through a weighed prewetted membrane filter and then reweighing.

DNA synthesis. Bacteria were grown to about 10^8/ml in M9 salts plus 0.4% (wt/vol) glucose, 10 μg of tryptophan per ml, and 0.4% (wt/vol) Casamino Acids, and 8-MOP was added to a final concentration of 20 μg/ml. After 10 min, portions were exposed to various fluences of NUV and 0.2 ml samples were added to tubes containing 0.01 ml of an aqueous solution containing 4 μCi of [^{3}H]thymidine and 20 μg of deoxyadenosine. After 10 min of incubation at 37°C, 0.1-ml aliquots were transferred to filter paper disks, which were immediately washed twice in cold trichloroacetic acid and twice in ethanol before counting.

TABLE 1. *E. coli strains*

Strain	Repair genotype	Source or reference
K-12		
AB1157	*uvr*$^+$ *rec*$^+$	(10)
AB1886	*uvrA rec*$^+$	(10)
AB2480	*uvrA recA*	(10)
B/r		
WP2	*uvr*$^+$ *rec*$^+$	E. Witkin
WP2 *uvrA*	*uvrA rec*$^+$	(9)
WP100	*uvrA recA*	E. Witkin

RESULTS

Inactivation of K-12 and B/r strains. The inactivation of nominally similar wild-type and DNA repair-deficient strains of K-12 and B/r after exposure to NUV in the presence of 20 μg of 8-MOP per ml is shown in Fig. 1. It can be seen that for all three pairs the K-12 strain was more resistant than the B/r and that the difference was most marked (a six- to sevenfold difference in inactivation rate) for the *uvrA recA* pair, which are generally considered to be deficient in all dark repair of DNA damage. Whereas the difference between the wild-type and excision-deficient pairs could be postulated to be due to different levels of recombination, excision repair, or both, since psoralen-NUV damage is clearly susceptible to both these repair pathways (see references 5 and 11), such an explanation cannot hold for the *uvrA recA* double mutants, and our attention has therefore been concentrated on this pair. In contrast to the results with 8-MOP plus NUV, the survival of the *uvrA recA* pair at 254 nm UV was essentially identical (Fig. 2). The other pairs also showed closely similar inactivation curves for UV. (Data for the wild-type pair can be seen in Fig. 9.).

In the experiments shown in Fig. 1 the bacteria were incubated with 8-MOP for 10 min before exposure to NUV. In fact, the preincubation period had little effect on the difference between WP100 and AB2480; most of the lethal effect upon irradiation was found with as little as 30 s of incubation with 8-MOP prior to NUV.

We considered two possible explanations for the greater resistance of the K-12 strains to 8-

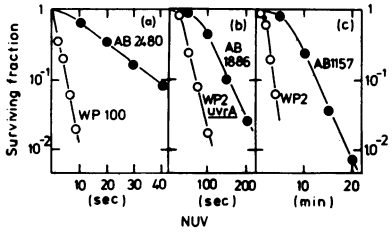

FIG. 1. *Survival of E. coli strains (a) WP100* (○) *and AB2480* (●); *(b) WP2 uvrA* (○) *and AB1886* (●); *and (c) WP2* (○) *and AB1157* (●) *as a function of exposure to NUV in the presence of 20 μg of 8-MOP per ml (representative experiments). Fluences: (a) 1.85 × 10^{-4} W cm^{-2}; (b) and (c) 4.63 × 10^{-4} W cm^{-2}.*

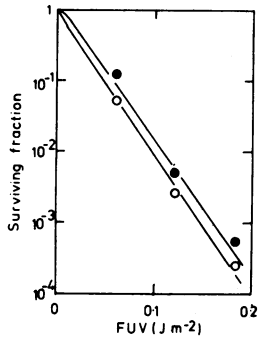

FIG. 2. *Inactivation of E. coli WP100* (○) *and AB2480* (●) *as a function of fluence of 254-nm UV (representative experiment).*

MOP plus NUV. Either fewer adducts are formed in the K-12 strains by a given exposure to NUV, or K-12 strains possess some DNA dark-repair capacity over and above that dependent upon the *recA*$^+$ and *uvrA*$^+$ genes.

Survival of phage T3. Essentially the same inactivation curve was found with both AB2480 and WP100 for phage T3 exposed to NUV while suspended in phage buffer containing 20 μg of 8-MOP per ml, either before or after complexing with the appropriate bacteria (Fig. 3). This experiment indicates that under these conditions the photoproduct yield in the DNA of T3 is similar whether it is situated within AB2480 or WP100 complexes or within the phage coat and that the free intracellular concentration within the complexes is similar to the extracellular concentration.

The experiment further suggests that AB2480 possesses no repair capacity for T3 DNA that is not also possessed by WP100, although it is possible that a slow repair process might not be revealed with T3 because of the rapidity with which the phage DNA is replicated.

8-MOP content of AB2480 and WP100. An assay was developed for 8-MOP in solution

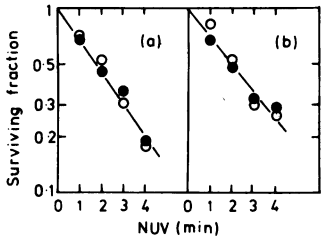

FIG. 3. *Survival of phage T3 as a function of exposure to NUV at 4.63 × 10^{-4} W cm^{-2} in the presence of 20 μg of 8-MOP per ml. (a) Phage suspended in buffer plus 8-MOP during irradiation and plated on WP100* (○) *or AB2480* (●); *(b) phage complexed with WP100* (○) *or AB2480* (●) *and suspended in buffer plus 8-MOP before irradiation (representative experiment).*

based on its ability to fluoresce at 500 nm when exposed to light of 341-nm wavelength. Surprisingly, the 8-MOP content of AB2480 bacteria suspended in a 20-μg/ml solution of 8-MOP proved to be more than twice that of WP100 bacteria (Table 2). Bacteria of the two strains differed little in wet weight; WP100 was marginally heavier. The internal concentration in AB2480 was about 2.6 times that in WP100 on a weight basis. This result effectively eliminates any explanation for the greater resistance of AB2480 to 8-MOP plus NUV based on a lower internal content of the psoralen. It is also clear that the internal concentration is much greater than the external concentration. Since the similar sensitivity of phage T3 DNA within or without the cell indicates that the concentration of free 8-MOP is also similar, it would appear that the excess 8-MOP within the cells is complexed to macromolecules.

LHR. The existence of prereplication dark repair of DNA may sometimes be revealed by delaying replication for a period so that more repair can occur (for review see 14). An increased survival obtained by holding bacteria in buffer for a period after irradiation is termed liquid holding recovery (LHR).

To test whether some repair activity over and above Uvr$^+$-dependent excision and *recA*$^+$-dependent recombination repair exists in K-12 strains, LHR experiments were carried out with the *uvrA recA* strains AB2480 and WP100 after 8-MOP plus NUV treatment (Fig. 4). The chief problem in interpreting these experiments is that the viable count for both strains increases during incubation in buffer due to residual division. With WP100 the increase in viable count over 18 h was the same for both untreated cells and those given 8-MOP plus NUV, i.e., there was no LHR. With AB2480 the treated sample

TABLE 2. *8-MOP content in AB2480 and WP100 cells suspended in a 20-μg/ml solution*

Cells	Avg 8-MOP per cell ($g \times 10^{-16}$)				Avg wt of cell ($g \times 10^{-12}$)			Internal concn (μg/g)
	Expt 1	Expt 2	Expt 3	Mean ± SD[a]	Expt 2	Expt 3	Mean	
AB2480	11.8	12.8	9.02	11.2 ± 0.16	3.7	4.0	3.85	290
WP100	4.69	5.74	4.86	5.1 ± 0.46	4.4	4.8	4.6	110

[a] SD, Standard deviation.

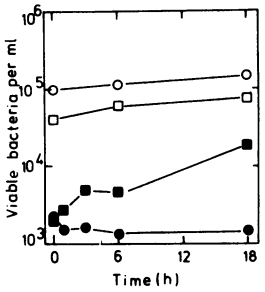

FIG. 4. *Viability of irradiated (solid symbols) and unirradiated (open symbols) E. coli WP100 (circles) and AB2480 (squares) as a function of time of post-irradiation incubation in buffer at 37°C. Irradiation consisted of exposure to 4 or 40 s, respectively, of NUV at 4.63 × 10^{-4} W cm^{-2} in the presence of 20 μg of 8-MOP per ml (representative experiment).*

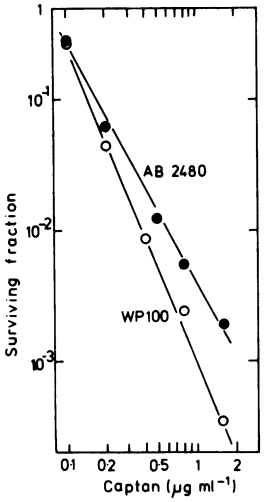

FIG. 5. *Survival of E. coli WP100 and AB2480 after incubation with captan in buffer for 1 h at a density of 10^7/ml as a function of concentration (representative experiment).*

consistently showed a greater increase (about fivefold) than the untreated, which we regard as LHR and thus presumptive evidence for the presence of a repair process in this strain that is absent in WP100.

Inactivation by captan. The LHR experiments suggested that AB2480 might possess a *uvrA*$^+$-independent repair pathway for large DNA adducts. If this were so, it might be expected also to deal with adducts of similar size produced in other ways. We have examined the inactivation of AB2480 and WP100 by captan, a large alkylating agent which reacts at the N-7 position of guanine and which is similar to 8-MOP plus NUV in producing damage susceptible to the *uvrA*$^+$- and *recA*$^+$-dependent repair pathways (3). We found (Fig. 5) that AB2480 was significantly more resistant to captan than WP100, consistent with the prediction from the LHR experiment.

Conversion of monoadducts to cross-links. When excision-deficient bacteria (e.g., strain WP2 *uvrA*) are treated with a small dose of 8-MOP plus NUV, washed free of 8-MOP, and exposed to a second longer dose of NUV, a proportion of monoadducts in the DNA are converted to cross-links with a consequent dramatic increase in lethality (13). Strain WP100 shows a negligible effect in such an experiment (unpublished data), presumably because the monoadducts are as lethal as cross-links. Strain AB2480 also failed to show any effect on survival of a second cross-linking NUV dose (Fig. 6). The NUV itself caused inactivation, but the rate was similar whether or not the bacteria received an initial 8-MOP plus NUV treatment. This result enables two fairly firm conclusions to be drawn. First, if monoadducts were actually present immediately after 8-MOP plus NUV treatment in AB2480 to the same extent as in WP100, conversion to cross-links should have prevented the possibility of any subsequent repair and should have increased sensitivity to that shown by WP100. The data rule out this possibility; monoadducts appear to be formed in AB2480 at a lower rate than in WP100 for a given 8-MOP plus NUV treatment. Second, although present at a lower level, monoadducts are still lethal for AB2480, since conversion to cross-links does not enhance their lethality. It would therefore appear that in practice the presumptive repair process identified on the basis of the LHR and captan experiments either plays only a small role in determining the survival of AB2480 after 8-MOP plus NUV or does not operate on the restricted class of monoadducts capable of forming cross-links.

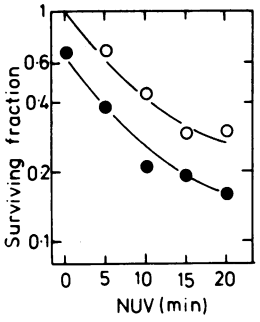

FIG. 6. *Survival as a function of exposure to NUV at 4.63 × 10^{-4} W cm^{-2} in the absence of 8-MOP of E. coli AB2480 bacteria which were (●) or were not (○) previously exposed to 2.5 s of NUV at 4.63 × 10^{-4} W cm^{-2} in the presence of 20 μg of 8-MOP per ml. Initial cell density was 10^8/ml. 8-MOP was removed after the initial exposure by 10^4-fold dilution in buffer and a 10-min holding period at room temperature.*

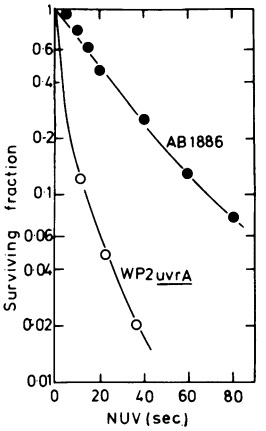

FIG. 7. *Survival of E. coli WP2 uvrA and AB1886 as a function of exposure to NUV at 1.85 × 10^{-4} W cm^{-2} at a density of 10^8 bacteria per ml in the presence of 20 μg of 8-MOP per ml, followed by exposure to 40 min of NUV at 4.63 × 10^{-4} W cm^{-2} after removal of 8-MOP by 10^4-fold dilution and holding at room temperature for 10 min (representative experiment).*

In similar experiments carried out with the two *uvrA* strains, the K-12 strain (AB1886) proved to be about eight times more resistant than the B/r (WP2 *uvrA*) (Fig. 7). We argue elsewhere that WP2 *uvrA* is killed by a single cross-link under conditions such as those where all available monoadducts have been converted into cross-links (manuscript in preparation). The present data indicate either that the yield of monoadducts available for cross-linking in AB1886 exposed to 8-MOP plus NUV is one-eighth of that in WP2 *uvrA* (a result consistent with that from the *uvrA recA* pair), or that AB1886 is capable of repairing seven out of eight cross-links.

Inhibition of DNA synthesis. If the photoproduct yield is indeed considerably lower after 8-MOP plus NUV treatment in K-12 than in B/r strains, this should manifest itself in the inhibition of DNA synthesis at early times after treatment. Figure 8 shows the inhibition by 8-MOP plus NUV of [^{3}H]thymidine uptake during the first 10 min after exposure. AB2480 is just over five times more resistant than WP100, in general agreement with this prediction.

Inhibition of repair of 254-nm UV damage by 8-MOP. In WP2, but not WP2 *uvrA*, 8-MOP present in the plating medium enhances the lethal effect of irradiation at 254 nm by removing the shoulder on the inactivation curve (2), presumably by interfering with some aspect of excision repair. No such effect was, however, observed in AB1157 (Fig. 9). One interpretation of this result is that 8-MOP, although present in the K-12 strain, is prevented from interacting with the DNA to the same extent as in the B/r strain.

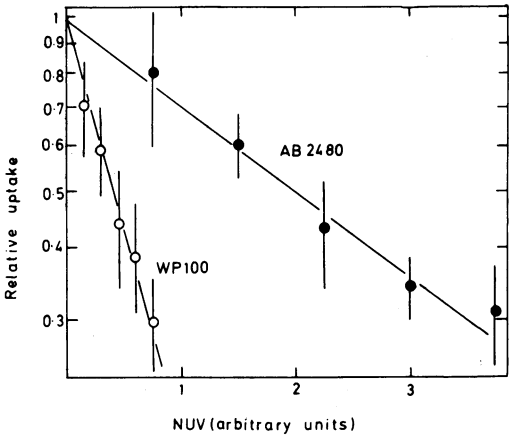

FIG. 8. *Decrease in uptake of [^{3}H]thymidine by WP100 and AB2480 during the first 20 min as a function of exposure to NUV at a density of 10^8 bacteria per ml in the presence of 20 μg of 8-MOP per ml. Because the exposure was given in growth medium, the fluence is given in arbitrary units. Mean of three experiments ± standard errors.*

DISCUSSION

We have found evidence for the presence in AB2480 of a *recA*$^+$ *uvrA*$^+$-independent prereplicative process for the repair of large DNA adducts, but it would appear to be slow in operation and likely to be responsible for a relatively small part of the greater resistance of AB2480. There is evidence (12) for the presence within K-12 bacteria of a nuclease that might conceivably be involved in such a repair path-

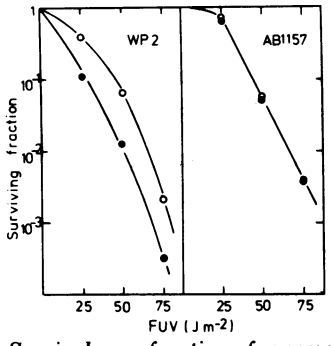

FIG. 9. *Survival as a function of exposure to 254-nm UV of E. coli WP2 and AB1157 plated on medium with (●) and without (○) 40 μg of 8-MOP per ml (representative experiment).*

way, but no evidence that it is normally involved in promoting cell survival. Repair systems frequently confer increased resistance on stationary-phase bacteria in comparison with exponential-phase bacteria. In contrast to this, the sensitivity of AB2480 to 8-MOP plus NUV was somewhat greater in the stationary phase than in the exponential phase, whereas that of WP100 was unchanged (unpublished data). Even in the stationary phase the difference in sensitivity between the strains was still more than fourfold.

The possibility that the internal content of 8-MOP is considerably lower in AB2480 than WP100 seems untenable in the light of the similar T3 inactivation curves in the two strains and the internal levels of 8-MOP as determined fluorometrically. The data seem to point fairly clearly toward the conclusion that the photoproduct yield in the DNA of K-12 bacteria is lower than that in the DNA of B/r bacteria by a factor of between five and eight even though the free 8-MOP concentration and the photoproduct yield in T3 DNA are similar in both cells. We therefore suppose that 8-MOP, although present within the K-12 bacteria, is prevented from close interaction with the DNA of this strain, presumably by a different ionic microenvironment around the DNA or by the presence of other molecules protecting the sites at which adsorption might occur. The fact that 8-MOP fails to inhibit a component of Uvr^+-dependent excision repair in AB1157, in contrast to WP2, is consistent with this interpretation although it does not prove it.

The conclusion that an intercalating mutagen may interact with DNA to a markedly different extent in related strains even within the same species might have profound consequences in environmental mutagenesis and carcinogenesis should it prove to be of more general application.

ACKNOWLEDGMENT

We thank Aubrey Knowles for calibrating the NUV source.

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