Effect of Near-UV Light on *Escherichia coli* in the Presence of 8-Methoxypsoralen: Wavelength Dependency of Killing, Induction of Prophage, and Mutation

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Received for publication 28 December 1977

Wavelength dependency of photo-inactivation and photoinduced reverse mutation of *Escherichia coli* sensitized with 8-methoxypsoralen, and wavelength dependency of photoinduction of lambda prophage from the sensitized lysogen were measured in a range of 298 to 400 nm. The most efficient sensitization for these biological effects was observed between 320 and 340 nm. In the presence of 8-methoxypsoralen, the induced mutation frequency per lethal hit was highest at 298 nm in the range examined and was gradually decreased with increasing wavelength to a minimum frequency at 345 nm. This finding may be a reflection of the production of more than one type of lesions which have different efficiencies for mutation compared with the killing efficiency.

Irradiation of cells or viruses with near-UV light causes a larger killing effect in the presence of psoralen or some of its derivatives (8, 10, 22–24) than in its absence. The main lesion for killing has been believed to be the diaduct formation (DNA cross-linking) (6, 7, 9, 10, 22), and the lesion is repaired by sequential enzyme reactions controlled by the *uvr* and *rec* genes (3, 9), but it is not photo-reactivable (16). Suzuki et al. (24) obtained evidence that the damaged T4 phage can be slightly repaired by mechanisms controlled by the *px* and *y* genes but not by the *v* gene. Near-UV light induces mutations more strongly in the presence of psoralen than in its absence (2, 13, 16, 21, 22, 25). Clinical application of the sensitization with psoralen to phototheraphy of psoriasis and mycosis fungoides (20) is developing. In most clinical applications and basic research, various types of fluorescent black-light lamps have been used as a light source. Only a few studies on wavelength dependency of the reaction have been reported. Dall’Acqua et al. (11) measured the yield of photoadducts of tritiated 8-methoxypsoralen (8-MOP) at wavelengths corresponding to some emission peaks of a high-pressure mercury lamp by using a grating monochromator. In their experiment, a maximal yield of the photoadducts was obtained at 312 nm. Suzuki et al. (23) measured wavelength dependency of the photo-inactivation of bacteriophages T4 and lambda sensitized with 8-MOP by means of a Xe arc lamp coupled with a grating monochromator, and found the maximal sensitization at about 330 nm. In both studies, the wavelength inducing the maximal sensitization was shifted to a longer side from the absorption peak (302 nm). In the present study, wavelength dependency of the photosensitized killing of *Escherichia coli* was measured to determine whether this kind of bathochromic shift is also true for bacterial cells.

When *E. coli* cells are exposed to treatments such as far-UV light, X-rays, mitomycin C, nitrogen mustard, a coordinate expression of inducible functions or "SOS" functions results (19, 29). It is possible that the inhibition of DNA synthesis by these agents initiates the induction pathway. Photoinduction is an expression of SOS functions as well as of induction of mutation. It has been proven that the treatment of *E. coli* with 8-MOP and near-UV light induces lytic phage development in the lysogen (14) and base-change mutagenesis (16). The present paper will describe the results of the wavelength dependency of 8-MOP sensitized photokilling and photoinduction of mutation of *E. coli* as well as of photoinduction of prophage lambda.

**MATERIALS AND METHODS**

**Bacterial strains and growth media.** *E. coli* Hs3OR *uvrA argF*(Am) (18), kindly supplied by S. Kondo, Osaka University, was used in the experiments of killing and mutation. This strain shows a higher sensitivity and a higher mutability upon the treatment with 8-MOP plus near-UV light (16). A derivative of *E. coli* K-12, JC1557 (4), a wild type for repair, was also used for the experiment of killing. For the induction of prophage, the *λ* lysogen of strain AB1157 (15) was used. Cultures of Hs3OR, AB1157, and AB1157(λ) were grown in a nutrient broth medium (8 g of nutrient broth [Difco] and 5 g of NaCl per liter, pH 7.0). JC1557
was grown in M9 medium (1) supplemented with Casamino Acids and required amino acids. Strains Hs30R and AB1157(α) reached a concentration of 2 × 10^8 to 3 × 10^8 cells per ml in the nutrient broth medium during the 3.5-h incubation period at 37°C. JC1557 reached a concentration of 4 × 10^7 to 7 × 10^7 cells per ml in the supplemented M9 medium during the same incubation time.

Treatments of bacteria with 8-MOP and near-UV light. 8-MOP (Taisho Pharmaceutical Co.) was dissolved in ethanol at a concentration of 1.2 mg/ml; this was kept in the dark as a stock solution. The medium of the log-phase culture was replaced with physiological saline by centrifugation and was diluted 100 times with the saline containing 8-MOP (12 μg/ml) when the killing effect was examined. For the prophage induction, lysogen AB1157(α) was mixed with 8-MOP to make a suspension of 2 × 10^6 cells per ml in a λ dilution buffer (5 g of NaCl, 95 mg of MgCl2, and 10 mg of gelatin in 1 liter of 10^-2 M tris(hydroxymethyl)aminomethane-hydrochloride buffer, pH 7.4). A 0.5-ml portion was irradiated with monochromatic light in a quartz cuvette (10-mm light path and 10-mm width) by a Spectro-Irradiator (Japan Spectroscopic Co., Tokyo, model CRM-FM) (23). A lid of a plastic petri dish was used as a filter to eliminate stray light of wavelengths shorter than 290 nm. The incident beam has a band width of 20 nm for the 10-mm width of a cuvette. The culture without 8-MOP was also irradiated for comparison. Fluence of the monochromatic light was measured with a vacuum thermopile coupled with a microvoltmeter. All the solutions after mixing with 8-MOP were handled under reduced light except during the irradiation.

Determination of survival fractions. The irradiated samples were plated at appropriated dilutions on agar plates (17.5 g of antibiotic medium 3 (Difco), and 15 g of agar per liter for Hs30R and JC1557, and 10 g of peptone (Difco), 1 g of yeast extract (Difco), 2.5 g of NaCl, and 12 g of agar per liter for AB1157(α), respectively).

Prophage induction. A 0.1-ml portion of diluted suspension of the treated lysogen, AB1157(α), was mixed with 0.2 ml of an overnight culture of an indicator strain, AB1157, in 2.5 ml of a top agar medium (10 g peptone [Difco], 1 g of yeast extract, 2.5 g of NaCl, and 5 g of agar pet liter, pH 7.0) at 45°C, and overlaid on a hard agar medium (containing 1.2% agar). After incubation overnight, the number of infective centers was counted.

Scoring of phenotypic revertants. Phenotypic revertants of arginine auxotrophs were scored by the method of Witkin (28) and Kondo et al. (17). A 0.2-ml portion of the irradiated suspension (1.7 × 10^7 to 3.3 × 10^8/ml with 12 μg of 8-MOP/ml) was spread on a 0.5% SEM (semi-enriched) agar plate (minimal E agar supplemented with 0.4% glucose, 0.5% liquid nutrient broth, and 0.5% NaCl). After incubation for 2 days at 37°C, colonies of arginine prototrophs were counted. The arginine auxotroph does not form an observable colony on this agar medium. An induced mutation frequency was calculated as (total revertants/treated plate − spontaneous revertants/control plate)/survivors. Preexisting spontaneous revertants in the stock culture were excluded by single colony isolation every third or fourth day.

RESULTS

Lethal effect. Figure 1 shows the survival curves of JC1557 (5 × 10^6 cells per ml) irradiated with monochromatic light in the range of 302 to 380 nm in the absence and presence of 8-MOP. The surviving fraction decreased with exponential kinetics, and the slope was larger for cells in the presence of 8-MOP than in its absence. Figure 2 shows the plot of inactivation cross-section against wavelength. The cross-section, k, was calculated by taking the reciprocal of the fluence to yield the survival fraction of 0.37 (F_37). The values of F_37 were obtained by interpolation or extrapolation, assuming that the survival curve is a simple exponential one. Even if the sensitizer is absent, near-UV light of wavelengths shorter than 310 nm elicits a definite effect. The curves with the same shape were also obtained for a much more sensitive strain, Hs30R, at a concentration of 2.5 × 10^6 bacteria per ml (Fig. 3). The differences between two curves in Fig. 2 and 3 were plotted versus the wavelength to determine the wavelength dependency of the sensitization for killing (Fig. 4). An apparent peak of the sensitization was 320 to 330 nm.

Induction of lambda prophage. As lambda prophage induction by 8-MOP plus near-UV light...
Fig. 2. Variation of the inactivation cross-section of JC1557 with wavelength. The bacteria (4 x 10^6 to 7 x 10^7 cells per ml) were irradiated with a given fluence, 3.4 kJ/m², at each wavelength (298 to 400 nm) in the absence (○) and presence (●) of 8-MOP (12 μg/ml). The cross-section, k, was calculated by taking the reciprocal of the fluence to yield a 37% survival, assuming that the survival curve is a simple exponential one. Bars and arrows indicate the range of standard deviation in the repeated experiments (8 to 12 times at each wavelength). Values represented by the circles in parentheses are not significant.

light was proven in our previous study (14), its wavelength dependency was also measured by irradiation of the lysogen with monochromatic light covering 298 to 380 nm. Figure 5 shows the kinetics of prophage induction with survival curves at several representative wavelengths in the absence and presence of 8-MOP. Maximal fractions induced attained 10 to 20% in the wavelength range between 298 and 365 nm in the presence of 8-MOP. In the absence of 8-MOP, the prophage induction was observed in the wavelength range below 320 nm, but the maximal fraction was decreased at 315 and 320 nm. If the reciprocal of the fluence required for induced fraction of 5 x 10^{-2} per treated cells is plotted against the wavelength, the curves shown in Fig. 6 are obtained. The difference spectrum between the curves in the presence and absence of 8-MOP is also illustrated with a broken line. It demonstrates that the most efficient wavelength for the sensitization of prophage induction is between 320 and 345 nm.

Mutagenic effect. To examine wavelength dependency of the near-UV light induced mutagenesis, a culture of Hs30R was irradiated with monochromatic light of various wavelengths in the absence and presence of 8-MOP. The numbers of the induced Arg^+ revertant per 10^9 bacteria that survived at 298, 310, and 330 nm are listed in Table 1. Figures 7 and 8 demonstrate the frequencies of mutation induced with monochromatic light in the absence and presence of 8-MOP, respectively. Data at 315, 320, and 330 nm in the presence of 8-MOP are not shown in Fig. 8 to avoid complication, but they are between the curves of 310 and 345 nm. In the
EFFECT OF 8-MOP PLUS NEAR-UV LIGHT ON E. COLI 357

absence of 8-MOP, the number of mutant colonies induced with monochromatic light longer than 345 nm was too small to be detected at the maximum fluence employed. If the reciprocal of fluence to yield the induced mutation frequency of $5 \times 10^{-6}$ per survivor is plotted against the wavelength, the curves shown in Fig. 9 are obtained. The mutation frequencies in the absence and presence of the 8-MOP are nearly the same at both wavelengths of 298 and 302 nm, explaining that DNA damage induced with light itself of shorter wavelengths acts as mutagenic. The difference spectrum between both curves suggests that the sensitized mutagenicity is highest between 310 and 330 nm. To attempt another type of analysis of the data of mutation, we plotted the mutation frequency induced in the presence of 8-MOP against the surviving fraction. Curves at representative wavelengths are shown in Fig. 10. From a series of such curves, the revertant frequency at a surviving fraction of 0.37 at various wavelengths could be read out and plotted against the wavelength. The induced mutation frequency per lethal hit is the highest at 298 nm in the wavelength range examined, and is gradually decreased with increasing wavelength to attain a minimal value at 345 nm (Fig. 11), indicating that the damage produced with 345-nm light causes killing action over mutagenic action.

DISCUSSION

In a range of shorter wavelengths, especially below 310 nm, near-UV light alone causes the lethal and mutagenic effects and induces lambda prophage growth as have been reported. These effects can be explained with DNA damage, such as pyrimidine dimers (26), spore product-like photoproducts (5), and/or single-strand breaks.

FIG. 4. Wavelength dependency of the sensitization. Difference between the inactivation cross-section in the presence of 8-MOP (12μg/ml) and the cross-section in the absence of 8-MOP was plotted against the wavelength. Both curves were obtained from Fig. 2 and 3. Values represented by circles in parentheses are not significant.

FIG. 5. Surviving fraction (○, □) and phage-induced fraction (△, △) of irradiated cells, AB1157(λ). The cells (2 × 10^9/ml) were irradiated with monochromatic light of various wavelengths (298, 302, 315, 320, and 345 nm) in the absence (upper panels, open symbols) and presence of 8-MOP (12μg/ml) (lower panels, closed symbols).
When 8-MOP is present in a suspension of cells, the near UV light-induced biological effects are sensitized, and the sensitization is dependent on wavelength. The lethal effect was most strongly enhanced with light between 320 and 330 nm in the presence of 8-MOP. The wavelength dependency obtained was very similar to those of the inactivation of phage T4 and lambda (23). The difference in wavelengths of the action peak and the absorption peak (302 nm) may be, in part, accounted for by a bathochromic shift of the absorption band based on intercalation. This shift of the absorption maximum of 8-MOP, as well as 5-MOP (shifted by 10.5 nm when bound to DNA), has been reported (12). How-

**Fig. 6.** Phage-inducibility of AB1157(A) cells which were irradiated with monochromatic light of various wavelengths in the absence (○) and presence (●) of 8-MOP (12 μg/ml). The reciprocal of the fluence to yield the induced fraction of $5 \times 10^{-2}$ was plotted against the wavelength. The difference between the value obtained in the presence and that obtained in the absence of 8-MOP (△) was also plotted.

**Fig. 7.** Induced mutation frequency of Hs30R ($1.6 \times 10^8$ to $3.3 \times 10^8$ cells per ml) irradiated with monochromatic light of various wavelengths (298 to 345 nm) in the absence of 8-MOP.
### Table 1. Near-UV light-induced mutation of Hs30R

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<th>Induced revertants/10&lt;sup&gt;6&lt;/sup&gt; survivors</th>
<th>Surviving fraction (%)</th>
<th>Fluence (J/m²)</th>
<th>No. of induced revertants/plate&lt;sup&gt;a&lt;/sup&gt;</th>
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<sup>a</sup> Number of bacteria plated (per 0.2 ml): 4.6 × 10<sup>7</sup>, 3.4 × 10<sup>7</sup>, and 6.6 × 10<sup>7</sup> for 298, 310, and 330 nm without 8-MOP, and 5.1 × 10<sup>7</sup>, 3.3 × 10<sup>7</sup> and 5.9 × 10<sup>7</sup> for 298, 310, and 330 nm with 8-MOP, respectively. Average number of spontaneous plate mutation = 21.

<sup>b</sup> —, The number should be zero. However, numerically, it deviated around zero as a result of the subtraction.
Fig. 8. Induced mutation frequency of Hs3OR (1.6 x 10^6 to 3.0 x 10^6 cells/ml) irradiated with monochromatic light of various wavelengths (298 to 400 nm) in the presence of 8-MOP (12 μg/ml).

Fig. 9. Mutagenicity of Hs3OR by the irradiation with monochromatic light of various wavelengths in the absence (○) and presence (●) of 8-MOP (12 μg/ml). Reciprocal of the fluence to yield the mutation frequency of 5 x 10^-6 per survivor was plotted against the wavelength. The difference between the value obtained in the presence and that obtained in the absence of 8-MOP (△) was also plotted.
The sensitized induction of phage growth and mutation was also dependent on wavelength. The most efficient wavelength for the induction was observed to be in a range corresponding to that of the sensitization of killing. Although ambiguity is still involved, it could be reasonable, assuming that both of the lytic growth of prophage and the mutation are triggered by DNA damage. Most curves of the induced fraction versus fluence have a peak (Fig. 5). The decline after passing a peak is due to inactivation of phage production capacity. The maximal induction of prophage in the presence of 8-MOP is nearly the same over the range of wavelengths up to 365 nm. When the mutation frequency per lethal hit in the presence of 8-MOP is plotted against the wavelength, it is at a minimum at 345 nm and unexpectedly increases at wavelengths longer than 350 nm. It is assumed that certain type(s) of highly mutagenic but less lethal damage is produced in the range of longer wavelengths. Recently, Seki et al. (22) reported that, although cross-links are the major damage causing the cells to become lethal, monoadducts are responsible for mutation. Much more reliable conclusions may be possible by determining action spectra for production of two types of lesions, i.e., cross-links and monoadducts.

ACKNOWLEDGMENTS

We are grateful to M. Ohkido, Department of Dermatology, Tokai University School of Medicine, for encouragement. He permitted us to use the Spectro-Irradiator of his laboratory. We thank M. Kitakami and M. Sano of our laboratory for their technical assistance in part of the present study. This work was supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan.

LITERATURE CITED


