SENSITIVITY OF DNA REPAIR-DEFICIENT STRAINS OF ESCHERICHIA COLI K-12 TO VARIOUS FUROCOUMARINS AND NEAR-ULTRAVIOLET RADIATION

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Abstract—Survival curves were obtained for DNA repair-deficient strains of Escherichia coli K-12 (polA1, uraB, and recA36) exposed to near-ultraviolet radiation [black light (BL)] in the presence of the DNA cross-linking agent 8-methoxypsoralen (8-MOP) or in the presence of photosensitizers forming primarily monoadducts with DNA [angelicin; 3-carbethoxypsoralen (3-CPs); 5,7-dimethoxyxoumarin (DMC)], and after exposure to blue light (BluL) in the presence of 8-MOP or 3-CPs. An interpretation of these data suggests that DNA polymerase I is required for the major pathway of monoadduct repair, but appears to play little or no role in the repair of 8-MOP cross-links. The uraB and recA strains were very sensitive, both to the cross-linking agent and to the monoadduct formers. The markedly different results for BL plus DMC or 3-CPs compared to angelicin suggests that the DMC and 3-CPs monoadducts are repaired by a different mechanism than are the angelicin monoadducts, or else DMC and 3-CPs undergo photochemical side reactions that produce DNA lesions other than the expected monoadducts. From photochemical evidence, we predicted that fewer 8-MOP monoadducts should be converted to cross-links by BluL vs BL; this appears to be the case. 3-CPs showed dramatically different biological results when irradiated with BL vs BluL, suggesting that 3-CPs may form more types of photoproducts than the expected monoadducts. BluL, however, appears to favor monoadduct formation.

INTRODUCTION

The photosensitization of DNA to near-ultraviolet (near-UV) radiation (320-400 nm) by bifunctional furcocumarins involves a sequence of events initiated by the formation of an equilibrium complex with DNA that is light-independent, the production of two types of monofunctional adducts to DNA in the first photochemical step and the subsequent photochemical conversion of some of these monoadducts to interstrand DNA cross-links (e.g., the review of Song and Tapley, 1979). Since psoralen is not a symmetrical molecule, it forms two types of monoadducts that differ in chemical structure and absorption spectra. They are the covalent cycloaddition products linking either the 4',5'-carbon atoms or the 3,4-carbon atoms of the furcocumarin with the 5,6-carbon atoms of a pyrimidine. The 4',5'-monoadducts of psoralen absorb below ~380 nm, while the 3,4-monoadducts absorb below ~330 nm (Musajo and Rodighiero, 1972). Therefore, wavelengths between 380 and 400 nm should generate only monoadducts, wavelengths between 330 and 400 nm can generate both types of monoadducts but only convert the 4',5'-monoadducts to cross-links, while wavelengths below ~330 nm can generate both types of monoadducts and convert both to cross-links. These conclusions are consistent with the results of Chatterjee and Cantor (1978) who found that wavelengths from 380-400 nm lead to only 4'-aminomethyl-4',5'-8-trimethyl psoralen monoadducts with DNA and that subsequent irradiation at 350 nm induced cross-linking of about half of the monoattached furcocumarin. These photochemical reactions are diagramed in Fig. 1. From these considerations, the BL lamps, whose spectral output is shown in Fig. 2, are expected to convert both types of monoadducts.

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Figure 1. Scheme of the two-step photochemical reactions that lead in the first step to the formation of two types of furcocumarin monoadducts, followed by their photochemical conversion to interstrand DNA cross-links. The symbols designate the furcocumarin (F), complexed with DNA in the dark, the 4',5'-monoadducts (M1,), the 3,4-monoadducts (M2,) and the cross-links (C).
to cross-links, whereas the BluL lamps (Fig. 2) should cause the formation of both types of monoadducts, and convert only the 4',5'-monoadducts to cross-links.

The photochemical pathway also depends on the structure of the furcocoumarin used. 8-Methoxypsoralen (8-MOP) (Fig. 3) forms 4',5'-monoadducts and 3,4-monoadducts, both of which can be converted to DNA cross-links when excited within their respective absorption bands (Song and Tapley, 1979). Angelicin (Fig. 3) and its derivatives only form monoadducts because of the angular shape of the molecule (Bordin et al., 1975, 1978, 1979). 5,7-Dimethoxycoumarin (DMC) can only form 3,4-monoadducts because the furan ring is absent (Fig. 3). Similarly, 3-carbethoxypsoralen (3-CPs) might be expected to form only 4',5'-monoadducts in view of the blocked 3,4-double bond (Fig. 3).

By comparing the relative survival of several DNA repair deficient strains of Escherichia coli K-12 after treatment with the monoadduct and cross-link forming furcocoumarins (and using light sources that should alter the extent of cross-link formation), we should gain a better understanding of the relative lethality of DNA monoadducts and cross-links and of the genetic control of the repair of these lesions.

Table 1. E. coli K-12 derivatives used

<table>
<thead>
<tr>
<th>Our stock number</th>
<th>Source number</th>
<th>Relevant genotype</th>
<th>Other markers</th>
<th>Source</th>
</tr>
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<tbody>
<tr>
<td>SR176</td>
<td>MM450</td>
<td>recA36</td>
<td>F^- rha lacZ rpsL deo</td>
<td>M. Monk</td>
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<tr>
<td>SR281</td>
<td>DY178</td>
<td>urrB5</td>
<td>F^- rha-5 lacZ53 rpsL151 leuB19 thyA36 deo(C2?)</td>
<td>D. A. Youngs</td>
</tr>
<tr>
<td>SR385</td>
<td>JG139</td>
<td>wild type</td>
<td>F^- rha-5 lacZ53 rpsL151 thyA36 deo(C2?)</td>
<td>E. C. Friedberg</td>
</tr>
<tr>
<td>SR760*</td>
<td>—</td>
<td>polA1</td>
<td>F^- rha-5 lacZ53 rpsL151 deo(C2?)</td>
<td>M. Tang</td>
</tr>
</tbody>
</table>

* Spontaneous Thy+ revertant of JG138, which is a cotransductant with SR385.
Sensitivity of *E. coli* to furocoumarins and near-UV

Figure 4. Survival of *E. coli* K-12 strains after exposure to black light (5.7 W/m²) in the presence of 10 μg/m² 8-methoxypsoralen.

**MATERIALS AND METHODS**

*Bacterial strains and culture conditions.* The strains of *E. coli* K-12 used are listed in Table 1. A minimal salts glucose medium (Ganesan and Smith, 1968) supplemented with thiamine-HCl (at 0.5 μg/ml), thymine (at 10 μg/ml) and 1 mM l-leucine was used for overnight and log phase cultures. The cells were grown at 37°C for several generations to about 2 × 10⁸ cells/ml, harvested in log phase on membrane filters (0.45 μm pore size, Millipore Corp.) and resuspended in 0.067 M phosphate buffer (pH 7.0) at 2 × 10⁸ cells/ml.

*Irradiation conditions.* The cells were irradiated in phosphate buffer at room temperature in covered Kimax petri dishes on a rotary shaker located 10 cm below two 15-W fluorescent lamps. General Electric (G.E.) F15T8-3BL lamps were used for ‘black light’ (BL) (280-475 nm; peak ~365 nm) irradiations, and G.E. F15T8-B lamps were used for ‘blue light’ (BluL) (340-650 nm; peak ~440 nm) irradiations. The incident radiation fluence was measured with a calibrated Epbley Laboratory thermopile. The average fluence rate transmitted by the Kimax dish top was 5.7 W/m² for BL, and 5.8 W/m² for BluL. The experiments were carried out under G.E. ‘gold’ fluorescent lamps to minimize photosensitization by the ambient light.

*Survival curves.* In the photosensitization experiments, nine parts of the cell suspension in buffer were added to one part of the photosensitizer in absolute ethanol. The initial mixing led to about 10-15% lethality, attributed to the presence of the ethanol, after which all strains remained viable in the dark over the time span of the experiments.

After adding the drug, the cells were held in the dark for 30 min prior to irradiation to allow the drug to bind with the DNA. The irradiated cells were diluted with phosphate buffer and plated on supplemented minimal medium solidified with 1.6% Difco Noble agar to prevent the inhibition of the recA gene-dependent pathway of excision repair by impurities in less pure agar (Van der Schueren et al., 1974). The plates were incubated 48-72 h at 37°C in the dark. The survival curves shown represent the average of two or more experiments.

*Chemicals.* 8-Methoxypsoralen was obtained from Sigma Chemical Co.; 3-carbethoxypsoralen was provided by Dr. E. Bisagni of the Fondation Curie, Institut du Radium, Orsay, France; angelicin was provided by Dr. M. J. Ashwood-Smith of the University of Victoria, British Columbia, Canada; 5,7-dimethoxycoumarin was provided by Dr. P-S. Song of Texas Tech University, Lubbock, TX. All stock solutions of furocoumarins were prepared in absolute ethanol. The chemical structure of the photosensitizers are shown in Fig. 3.

**RESULTS**

*Photosensitization by 8-methoxypsoralen.*

Several strains of *E. coli* K-12 were irradiated with BL in the presence of 8-MOP (Fig. 4). The polA strain was only slightly more sensitive than the wild-type strain (see Table 2), while the uvrB and recA strains were much more sensitive. In control experiments (i.e. without 8-MOP) the wild-type and uvrB strains were unaffected by 60 min of BL irradiation alone. A 50% killing of the recA and polA strains was achieved by 60 min and 45 min, respectively, of BL irradiation alone, but there was negligible killing with the 3 and 10 min, respectively, of BL irradiation used for these strains (data not shown).

<table>
<thead>
<tr>
<th>Strain of E. coli K-12</th>
<th>BL*</th>
<th>8-MOP</th>
<th>A</th>
<th>DMC</th>
<th>3-CPs</th>
<th>8-MOP</th>
<th>3-CPs</th>
<th>BluL/BL</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT (3.25)†</td>
<td>(22.0)</td>
<td>(64.5)</td>
<td>(57.0$)</td>
<td>(49.0)</td>
<td>(103.0)</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>polA</td>
<td>0.71</td>
<td>0.23</td>
<td>0.42</td>
<td>0.54</td>
<td>0.61</td>
<td>0.31</td>
<td>0.86</td>
<td>0.57</td>
</tr>
<tr>
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<td>0.23</td>
<td>0.16</td>
<td>0.29</td>
<td>0.41</td>
<td>0.24</td>
<td>0.23</td>
<td>1.04</td>
<td>0.56</td>
</tr>
<tr>
<td>recA</td>
<td>0.17</td>
<td>0.10</td>
<td>0.04</td>
<td>0.04</td>
<td>0.12</td>
<td>0.14</td>
<td>0.71</td>
<td>3.50</td>
</tr>
</tbody>
</table>

* BL, black light; BluL, blue light; 8-MOP, 8-methoxypsoralen; A, angelicin; DMC, 5,7-dimethoxycoumarin; 3-CPs, 3-carbethoxypsoralen; WT, wild type.
† The values in parentheses are the min of irradiation required to produce 50% survival in the wild-type strain. These values were then used to divide the irradiation times required to achieve 50% survival in all the strains under each experimental condition. These ratios are what are listed in this Table.
The same strains were also exposed to BluL irradiation in the presence of 8-MOP (Fig. 5). Although the incident fluence of BluL required to achieve the same lethality with 8-MOP (Fig. 5) was much higher than with BL (Fig. 4), the relative sensitivities of the different strains was qualitatively similar in the two cases, however, the polA and recA strains were relatively more sensitive to 8-MOP in the presence of BluL (Table 2). In control experiments, 120 min of BluL irradiation alone had no effect on the viability of any of the strains (data not shown).

*Photosensitization by monoadduct-forming furacoumarins*

*Angelica.* The wild-type strain was much less sensitive to BL irradiation in the presence of angelicin (Fig. 6) than in the presence of 8-MOP (Fig. 4). However, compared to the wild-type strain, the polA strain was relatively more sensitive to BL plus angelicin than to BL plus 8-MOP, or to BluL plus 8-MOP (Table 2). Similar to the results for BL plus 8-MOP, the uvrB and recA strains were quite sensitive to BL plus angelicin (Table 2). Angelicin does not absorb BluL sufficiently (Fig. 2) to obtain survival data under these conditions.

*3,7-Dimethoxy-coumarin.* The survival curves using DMC and BL are shown in Fig. 7. Compared to the wild-type strain, the polA strain was less sensitive to DMC than to angelicin, but was more sensitive to DMC than to 8-MOP plus BL (Table 2). The uvrB strain was quite sensitive to BL plus DMC, but the recA strain was especially sensitive (Table 2). DMC does not absorb BluL sufficiently (Fig. 2) to obtain survival data under these conditions.

*3-Carbethoxy-coumarin.* The survival curves for the photosensitization of *E. coli* by 3-CPs show two unexpected anomalies. The polA and uvrB strains were much more resistant to BL (Fig. 8) than to BluL

![Figure 5. Survival of *E. coli* K-12 strains after exposure to blue light (5.8 W/m²) in the presence of 10 μg/m² 8-methoxypsoralen.](image1)

![Figure 6. Survival of *E. coli* K-12 strains after exposure to black light (5.7 W/m²) in the presence of 20 μg/m² angelicin.](image2)

![Figure 7. Survival of *E. coli* K-12 strains after exposure to black light (5.7 W/m²) in the presence of 50 μg/m² 3,7-dimethoxy-coumarin.](image3)
results are not consistent with the formation of a single type of monoadduct under the two irradiation conditions.

**DISCUSSION**

The lethality of furocumarin DNA cross-links has been demonstrated in *E. coli* with the sensitizer 4,5,8-trimethylpsoralen (TMP) (Cole, 1971, 1973; Sinden and Cole, 1978), in Saccharomyces cerevisiae with 8-MOP (Averbeck et al., 1978), in mammalian cells with 8-MOP (Chandra et al., 1973) and with TMP (Ben-Hur and Elkind, 1973) and may be inferred for many other furocumarin photosensitized cellular systems (see, e.g. the review of Scott et al., 1976). Studies with photosensitizers that produce few if any cross-links are taken as evidence of monoadduct lethality, e.g. angelicin (Bordin et al., 1975; Ashwood-Smith and Grant, 1976; Ashwood-Smith et al., 1977), 4,5-dimethylangelicin (Bordin et al., 1978, 1979), 3-CPs at wavelengths above 340 nm (Averbeck et al., 1978), 5-hydroxypsoralen and 8-hydroxypsoralen (Song et al., 1975), and DMC (Harter et al., 1976). Based on comparisons of relative lethalities for photosensitization by cross-link formers and monoadduct formers, it has generally been assumed that cross-links are the dominant lethal lesions (Scott et al., 1976; Song and Tapley, 1979). One objective of the present work was to test this assumption. To do this we have investigated the photosensitization of several strains of *E. coli* K-12 (having different DNA repair capacities) by 8-MOP and several monoadduct-forming photosensitizers in the presence of 'black light' and 'blue light' (irradiation conditions that should alter the number of 8-MOP DNA cross-links).

**Survival results**

The rationale outlined in the Introduction suggests that BL irradiation should convert both types of 8-MOP monoadducts to cross-links. Therefore, a comparison of the results for the relative survival of the polA and wild-type strains after irradiation with BL plus 8-MOP (Fig. 4) and with BL plus angelicin (Fig. 6), which forms only monoadducts, leads to the conclusion that the polA gene product (i.e. DNA polymerase I) is involved in the repair of DNA monoadducts, but plays little or no role in the repair of 8-MOP cross-links.

Sinden and Cole (1978) reported that a polA strain was quite sensitive to TMP plus near-UV radiation relative to the polA' strain. In view of our results with 8-MOP, these data of Sinden and Cole (1978) suggest to us that a considerable number of TMP monoadducts remained unconverted to cross-links under their irradiation conditions, which excluded wavelengths below about 320 nm, and that the reduced rate of repair of DNA strand breaks that these authors observed for a polA strain may have been more related to the repair of monoadducts than to cross-links.
In the presence of 8-MOP, the polA strain is somewhat more sensitive to irradiation with BluL than with BL (Table 2). This differential sensitivity may result from the accumulation of 3,4-monoadducts of 8-MOP with BluL irradiation, where wavelengths below about 340 nm were not present, as suggested by the scheme in Fig. 1.

The involvement of DNA polymerase I (Pol I) in the repair of DNA monoadducts is also suggested by the sensitivity of the polA strain to BL plus DMC (Fig. 7). However, 3-CPs appears to be anomalous: there is a lower sensitivity of polA to BL plus 3-CPs (Fig. 8) than to BluL plus 3-CPs (Fig. 9). A possible explanation, consistent with the higher sensitivity of the polA strain to angelicin (Table 2), is that 3-CPs forms monoadducts when irradiated with BluL [which is consistent with the report by Averbeck et al. (1978) for wavelengths above 340 nm], but cross-links (or some other lesion that does not require Pol I for its repair) are generated by BL (i.e., containing wavelengths shorter than 340 nm). 3-CPs has been reported to be photochemically labile (Vigny et al., 1979).

The uralA gene has been implicated in the repair of TMP cross-links (Cole, 1973) and our results (Fig. 4) suggest that the uralB gene may also play a role in the repair of 8-MOP cross-links. Similarly, the high sensitivity of the uralB strain to BL plus angelicin (Fig. 6) implicates the uralB gene in the repair of monoadducts. However, the somewhat reduced importance of the uralB gene product in the repair of DMC monoadducts and its greatly reduced importance in the repair of the putative 3-CPs monoadducts relative to angelicin (Table 2) suggest that either the DMC, 3-CPs and angelicin monoadducts may not be repaired by identical mechanisms, or that DMC and 3-CPs may cause more complicated photochemistry than the formation of simple monoadducts to DNA.

Cole (1973) demonstrated that recA strains of E. coli K-12 are deficient in the repair of TMP cross-links. The sensitivity of the recA strain to all of the photosensitizers that we have studied (Table 2) indicates that the recA gene product plays a key role in the repair of DNA monoadducts as well as cross-links. The fact that the recA strain was more sensitive to BL plus DMC or 3-CPs than to any of the other experimental conditions (Table 2) also suggests a greater complexity of the photochemistry or of the repair of the lesions produced by DMC and 3-CPs relative to angelicin.

GENERAL CONCLUSIONS

(1) As discussed above, the polA gene product (DNA polymerase I) appears to play a major role in the repair of furcocoumarin monoadducts to DNA, but appears to play little or no role in the repair of DNA cross-links.

(2) Compared to the wild-type strain, the polA strain was much more resistant to BL plus DMC or 3-CPs than to angelicin, yet all these compounds are supposed to form only monoadducts. This suggests either that the DMC and 3-CPs monoadducts are repaired by a different mechanism than are angelicin monoadducts, or that BL causes photochemical side reactions with DMC and 3-CPs that produces DNA lesions other than the expected monoadducts. Consistent with this conclusion, the recA strain was much more sensitive to BL plus DMC or 3-CPs than to 8-MOP or angelicin.

(3) Irradiation with wavelengths longer than 340 nm (i.e., with BluL) did not produce as large an effect on the survival of the polA strain (relative to the wild-type strain) as we predicted from the scheme in Fig. 1, but was consistent with this scheme. This may suggest that the 4',5'- and 3,4-monoadducts of 8-MOP are not formed in equal amounts. BluL plus 8-MOP also had a large sensitizing effect on the recA strain, but not on the uralB strain (relative to the wild-type strain).

(4) For cells in the presence of 3-CPs, irradiation with BluL yielded markedly different results than when BL was used: the polA and uralB strains were much more sensitive to BluL, while the recA strain was more resistant. The original studies on 3-CPs were performed at wavelengths longer than 340 nm (Averbeck et al., 1978), which is similar to our BluL irradiation conditions. However, when 3-CPs was employed in the phototherapy of psoriasis (Dubertret et al., 1979), lamps (described in Parrish et al., 1974) were used that were more equivalent to our BL irradiation conditions. Since our results with 3-CPs were dramatically different depending upon which lamp we used, it cautions against carrying laboratory experiments with furcocoumarins over to the clinic when the laboratory experiments are performed under different irradiation conditions than those used in the clinic.

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Sensitivity of *E. coli* to furcocoumarins and near-UV