Photodynamic Inactivation and Mutagenesis by Angelicin (Isopsoralen) or Thiopyronin (Methylene Red) in Wild-Type and Repair-Deficient Strains of Bacteriophage T4

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Photodynamic inactivation of bacteriophage T4 particles, mediated by either angelicin or thiopyronin, is enhanced by defects in the T4 uvsW-uvsX-uvsY postreplication repair system but not by a defect in the denV pyrimidine-dimer-excision system. There was no evidence for functional interactions between the two repair systems. As observed previously with 8-methoxypsoralen, photodynamic mutagenesis with angelicin is abolished by defects in the uvsW-uvsX-uvsY system.

Dye-mediated photodynamic irradiation is both lethal and mutagenic to many organisms. In bacteriophage T4, photodynamic inactivation has been studied with proflavin (10), psoralen (5), 8-methoxypsoralen (8-MOP) (7, 11), 4,5′,8-trimethylpsoralen (TMP) (13), and thiopyronin (5, 6), and mutagenesis has been studied with proflavin (10), psoralen (5), 8-MOP (7), and thiopyronin (5). Studies of the roles of T4 repair systems in the viral response to photodynamic irradiation have revealed that the uvsW-uvsX-uvsY (WXY) system is required for both full survival and mutagenesis with 8-MOP (7, 11), TMP (13), and thiopyronin (6). Although single defects in the denV system do not affect survival with thiopyronin (6), 8-MOP (11), or TMP (13), uvsX-denV double mutants were reported to be substantially less sensitive than the uvsX mutant alone with either thiopyronin (6) or TMP (13). Because the denV system now appears to repair only UV-induced pyrimidine dimers (see reference 1 for review), this interaction appeared puzzling and in need of reinvestigation.

In addition to the wild-type bacteriophage T4D and T4B strains, the following repair-defective mutants were studied: the denV and uvsX mutants and the denV-uvsX recombinant isolated by Harm (8) in a T4D background and the uvsY mutant of Boyle and Symonds (2) backcrossed from a T4D into a T4B background in this laboratory. The denV mutation inactivates pyrimidine-dimer excision repair, whereas the uvsX and uvsY mutations inactivate error-prone postreplication repair (1, 7).

The UV sensitivities of wild-type T4D and its repair-defective mutants were measured to confirm their expected responses (Fig. 1A), and their sensitivities to angelicin-mediated photodynamic inactivation were then determined (Fig. 1B). After UV irradiation, survivals of wild type, uvsX, denV, and uvsX-denV were in the approximate ratios of 1:1.6:2.0:4.0, as estimated from the linear portions of the survival curves, indicating separate action of the denV and uvsX systems in determining survival. After photodynamic irradiation, the uvsX mutants were about 1.8-fold more sensitive than their wild-type counterparts, whereas their sensitivities were unaffected by the allelic state of the denV gene. T4D and T4B were of similar photodynamic sensitivity, as were a uvsX and a uvsY mutant (data not shown).

Mutations in the uvsX or uvsY genes sensitize T4 particles to the lethal action of a number of radiations, but only by about 1.7-fold (see reference 1 for review). Mutations in the umuC gene of Escherichia coli have effects upon cell survival and mutagenesis that resemble those of mutations in uvsX or uvsY upon virus survival and mutagenesis (see reference 12 for review), but umuC effects upon T4 are not very well explored. In contrast to the rather small effects of umuC mutations upon cell survival after, for instance, UV irradiation, a umuC defect is reported to increase killing by angelicin-mediated photodynamic action quite substantially (9). It was therefore appropriate to determine whether uvsX or uvsY mutations increased sensitivity to angelicin-mediated photodynamic irradiation by substantially more than 1.7-fold and whether a host umuC mutation affected virus survival.

A possible involvement of the host umuC function in the repair of T4 damage was tested by assaying survivors after angelicin-mediated photodynamic irradiation with isogenic umuC+ and umuC strains. The umuC strain carried the umuC36 mutation in an E. coli K-12 F′ pro-lac/Δ(pro-lac) thi trpE9777 background and was obtained from Barry W. Glickman (strain NR8002; isogenic umuC+ strain NR3835). In neither the wild type nor uvsX or uvsY mutants were umuC effects discernible, sensitivities being unaffected within about 3% (data not shown).

Mutation rates after angelicin-mediated photodynamic irradiation were determined for the pathway rI → r (mostly rI mutants being recovered after plating on BB cells) in the wild type and in uvsX and uvsY mutants (Table 1). As seen previously with both 8-MOP-mediated photodynamic irradiation and UV irradiation (7), a uvsX or uvsY mutation abolished mutagenesis. The wild-type mutation rate was about 3.3 × 10⁻⁴ r mutations per lethal hit, a value similar to that obtained with UV irradiation and plating on BB cells (3). (White-light or near-UV irradiation alone was generally neither lethal nor mutagenic to T4 in doses even larger than those used here, and the surviving fraction of 0.8 in the near-UV control in Table 1 was not statistically different from 1.)

When thiopyronin replaced angelicin as the photodynamic mediator, the various T4 genotypes retained their same relative survivals. Although the survival curves with thiopyronin were slightly concave (Fig. 2), the uvsX mutants...
were about 1.5-fold more sensitive than their \(uv\) counterparts, and the \(den\) mutation had no effect upon survival in either the \(uv\) or \(uv\) background. There were no significant differences in survivals upon plating on the isogenic \(um\) strains (data not shown).

These studies were motivated by three questions concerning the nature of DNA repair in phage T4. The first was whether the efficacy of \(XY\) repair was similar for different kinds of DNA damage, in either the fraction of lethal damages circumvented or the ratio between mutational and lethal events. The second was whether \(XY\) repair was totally independent of host \(um\) repair. The third was whether functional interactions occurred between the \(XY\) and \(den\) systems.

The enhanced sensitivity to photodynamic killing seen in \(uv\) and \(uv\) mutants is no greater with than with thiopyronin or, for that matter, with UV irradiation. Furthermore, the ratio of mutational to lethal events with \(v\) mutants \(X\) than \(uv\) mutants per lethal hit, scored on BB cells) is within less than twofold of the values observed with agents as diverse as UV light, methyl methanesulfonate, 8-MOP, and psoralen (7) when adjustments are made for different scoring systems (especially plating on BB cells, which do not reveal the \(H\) mutants that can be scored on B cells). Only ionizing radiations appear to be more mutagenic (3), perhaps because of their unique ability to induce a large proportion of deletions in addition to point mutations (4). This general similarity of ratios may suggest that a similar fraction of DNA damages, perhaps DNA polymerase-blocking lesions, is converted into mutations by the \(XY\) system without regard to the specific chemical nature of the damages.

The \(uv\) and \(uv\) mutations have similar effects on T4 survival after either photodynamic or UV irradiation. This is in contrast to the dissimilar effects on \(E.\ coli\) survival after photodynamic versus UV irradiation brought about by a bacterial \(um\) defect (9). The \(um\) gene is part of the bacterial SOS system (12), which is similar in many ways to the viral \(XY\) system (1); each is required to be functionally intact for radiation mutagenesis to occur in the respective organisms. However, in view of the general observation that host DNA repair systems rarely act upon T4 DNA damage.

![FIG. 1. Sensitivities of wild-type and repair-defective T4 strains to UV irradiation (A) and Angelicin-mediated photodynamic irradiation (B). Media, plating procedures, UV irradiation, and assays for survival have been described elsewhere (3). Phage stocks were grown in M9CA medium and platedings were with Drake agars. E. coli BB cells were used to grow phage stocks and for survival assays. Angelicin (isosoralen) was obtained from Stephan S. Miller and Eric Eisenstadt (Harvard School of Public Health, Boston, Mass.). Phage particles were suspended in M9 buffer (0.1% NaCl, 0.3% KH\(2\)PO\(_4\), 0.6% Na\(2\)HPO\(_4\), 0.5% NaCl, 1 \(\mu\)M FeCl\(_3\)) with or without Angelicin (molecular weight, 186.16) at 10 \(\mu\)g/ml (53.7 \(\mu\)M) and held for 10 to 20 min at 44\(^\circ\)C to facilitate dye penetration. Irradiation was at room temperature through 1 cm of pyrex glass from two fully warmed Sylvania 15T8 Blacklite Blue bulbs at a flux of about 7.3 J m\(^{-2}\) s\(^{-1}\) at 365 nm, the absorption peak for Angelicin being about 355 nm. Subsequent manipulations before plating were conducted with illumination from a Westinghouse F40G0 Gold bulb, which produced no detectable 365-nm radiation.](http://jb.asm.org/)

![TABLE 1. Photodynamic mutagenesis*](http://jb.asm.org/)
gelicin was used as the dye. Indeed, the allelic state of denV was without effect upon survival. The contradiction between these sets of results is not obviously explicable. However, the present results seem to remove for the moment any need to consider complex models of interaction between the denV and WXY systems.

LITERATURE CITED


FIG. 2. Thiopyronin-mediated photodynamic inactivation of wild-type and repair-defective T4 strains. Symbols: ●, denV+ phage; ○, denV mutant. Most procedures were carried out as described in the legend to Fig. 1. Thiopyronin (methylene red) was obtained from A. Wacker, Institut für Therapeutische Biochemie, Universität Frankfurt, Federal Republic of Germany. Phage particles were suspended in M9 buffer with or without thiopyronin at 7.5 µM and held for 10 min at 44°C to facilitate dye penetration. Irradiation was at room temperature, 21 in. (53.34 cm) below a Sylvania F40CW Cool White bulb masked to an 18-in. (45.72-cm) source. Subsequent manipulations before plating were conducted with weak illumination (e.g., semidarkness) from stray hall light at a flux determined experimentally not to further affect survival; the F40GO bulb used with gelicin produced too much additional flux in the vicinity of 564 nm, where thiopyronin absorbs strongly.

(1), it was not surprising to observe that the functional state of the umuC gene failed to affect T4 survival after either photodynamic or UV irradiation.

In contrast to previous reports concerning the photodynamic inactivation of T4 with thiopyronin (6) or TMP (13), no interaction was observed here between WXY-mediated denV-mediated repair with either thiopyronin or an