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THE ROLE OF TRANSLESION DNA POLYMERASE(S) IN THE SURVIVAL OF *ESCHERICHIA COLI* EXPOSED TO UVA LIGHT IN THE PRESENCE OF PSORALEN AND ANGELICIN

By

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THESIS QUESTION: When *E. coli* cultures, grown in the presence of psoralen or angelicin, are exposed to UVA ultraviolet light, how does the absence of translesion DNA synthesis affect their ability to survive?

ABSTRACT

The chemical compounds 8-methoxy-psoralen and angelicin are two types of furocoumarins that intercalate into DNA and form mono-adducts when exposed to UVA light. However, 8-methoxy-psoralen, is also capable of forming a DNA interstrand cross-link in addition to mono-adducts. These lesions change the structure of DNA and block the DNA polymerase during replication, leading to lethality, mutagenesis, or rearrangements if not repaired. The repair of monoadducts is known to be carried out by nucleotide excision repair. However, how interstrand DNA crosslinks are repaired is less clear. The repair of crosslinks has been proposed to involve a number of pathways, which include: homologous recombination, base excision repair, nucleotide excision repair, and translesion synthesis. The purpose of this study is to determine whether a bacterial strain lacking all three translesion DNA polymerases is hypersensitive to these photosensitizing chemicals, and whether it is specifically hypersensitive to DNA interstrand crosslinks. Wild type, *polB-dinB-umuDC* (lacking the three polymerases), and *uvr*A mutants were each treated with either 8-methoxy-psoralen or angelicin and irradiated with UVA light to determine their relative survivals. I found that the mutant lacking all three DNA polymerases was more sensitive to 8-methoxy-psoralen than angelicin when compared to the wild type cells; yet, the overall sensitivity of the mutant was far less than that of the *uvr*A mutant. These observations suggest that translesion synthesis plays a role in the repair of

interstrand crosslinks and could be consistent with models suggesting that translesion synthesis operates to fill gaps left following the incision of the initial strand.

INTRODUCTION/BACKGROUND

Psoralens are a chemical compounds that are widely used in PUVA (psoralen and UV-A light) to effectively treat psoriasis, vitiligo, and other skin- diseases. Unfortunately, the use of psoralen is also associated with in increased risk of skin cancer. [2,9] These cancerous conditions come about due to the emergence of adducts within one's DNA, when the psoralen reacts following absorption of UVA light. The repair of these lesions entails the interaction of a number of genome DNA repair pathways and depends upon how much or what particular fraction of the DNA has been damaged [4].

Two predominant lesions are formed in DNA following PUVA treatment. Monoadducts form when one of the two strands of a double helix has become linked to the psoralen. In order to repair this form of damage, nucleotide excision repair makes incisions on the damaged strand surrounding the lesion and DNA Polymerase I resynthesizes the region using the undamaged DNA strand as a template. [4,5,11].

The second type of lesion formed by PUVA treatment is the interstrand DNA crosslink which occurs when both strands of the double helix become linked to the psoralen molecule [3]. These lesions prevent the DNA from unwinding for transcription or replication and are particularly perilous to the cell's ability to survive [12].

Less is known about the mechanism of how interstrand crosslinks are repaired. Previous research has suggested several repair processes may act cooperatively to effect repair of these

complex lesions; including nucleotide excision repair, homologous recombination, and/or translesion synthesis [11,15].

Most models agree that nucleotide excision repair (NER) initiates the repair process, and there is both in vitro and in vivo evidence to support the idea that NER creates incisions on the 5′and 3′sides of one strand at the damaged region [9]. However, since the lesion remains attached to the DNA, repair synthesis using the undamaged template strand cannot occur. Less is known about how subsequent steps deal with the lesions in the second strand and at this point, models become more speculative. In general, models suggest that either homologous recombination and/or translesion DNA synthesis act to replace the incised region with an undamaged template, before nucleotide excision repair can remove the lesion from the second strand [9,10,14].

In homologous recombination, the region opposite to the crosslink is proposed to be filled in via a RecA- -mediated strand invasion of the single-stranded DNA from a homologous chromosome [4,9,11]. The Holliday-junctions resulting from the crossover are then resolved to produce a recombinant product [15].

In translesion synthesis (TLS), which will be the main focus of this paper, the region containing the crosslinks are proposed to be filled in via specialized DNA polymerases. The TLS DNA polymerases are able to insert bases opposite to specific damaged nucleotides that normally block the replicative polymerase [9]. It is important to note, however, that TLS DNA polymerases often have low fidelity (i.e: a high tendency to insert the wrong base) on undamaged templates compared to the accuracy of regular polymerases used during the replication process [9,13].

While evidence exists in cell extracts and biochemical studies to suggest that both recombination and translesion synthesis are capable of carrying out these reactions at a DNA interstrand crosslink [11,12], it is not known whether these processes operate in vivo, and all models remain highly speculative.

Determining whether mutants are defective in interstrand crosslink repair is also complicated by the fact that most all chemical agents inducing crosslinks also form monoadducts. Thus although many mutants are hypersensitive to 8-methoxy-psoralen, it is difficult to determine whether these mutants are required for the repair of monoadducts, crosslinks, or both. Here, I propose to address this question by comparing the sensitivities of strains to 8-methoxy-psoralen and angelicin. Angelicin is a derivative of psoralen that only forms monoadducts [1].

Thus, by comparing the survival rate of various mutant strains to both agents, we will be able to identify mutants that are uniquely sensitive to DNA interstrand crosslinks, based on their increased hypersensitivity to psoralen, relative to angelicin.

METHODS

Psoralen Derivatives:

Angelicin

8-methoxypsoralen (8-MOP; xanthotoxin- a derivative of psoralen) contains two photoactive sites (furan and pyrone) that react opposite thymines at TA:AT sequences after exposure to UV-A light, thus leading to the formation of crosslinks and monoadducts [10]. Angelicin, by contrast, is reported to form only monoadducts.

Psoralen
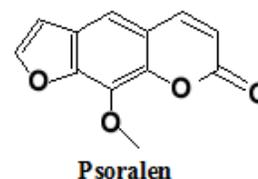
Strains:

Parental SR108 is a thy-deo-derivative of W3110 {Mellon and Hanawalt, 1989, Nature, 342, 95-8}, HL952 (SR108 *uvrA*::Tn10) and CL646 (SR108 *polB*::*omega Sm-Sp*, *dinB*::*kan umuDC595*::*cat*) have been reported previously [6,7].

Media Used:

DGC-thy Medium contains 1X Davis medium supplemented with 0.4% glucose, 0.2% Casamino Acids, and 10 μg/ml thymine [8]. LB-thy contains Luria Broth supplemented with10 μg/ml thymine. Psoralen solution (10ug/mL stock solution), and Angelicin solution (1mg/mL stock solution).

Growth and Psoralen Treament:

First, strains are struck out and grown from a -80ºC freezer. Forceps/tweezers, sterilized in ethanol and flamed dried, are used to pick up a pipet tip and scrap a sample from a frozen vial containing one of the strains, which is then applied to an LB-thy agar plate. The bacteria are spread in three serial sections within the pertri-dish, and incubated overnight to grow at 37ºC.

The next day, overnight cultures for are prepared from the plate colonies. Ethanol flamed, sterile pipet tips are used to pick a single colony from the bacterial plate, and inoculated into 2mL of DGC-thy a 13mm test tube. A tube containing only media and sterile pipet tip is also used to ensure the media and pipet tips were sterile at the time of inoculation. The test tubes are placed in a 37ºC rotating water bath overnight. For the experiment, sub-cultures, are

prepared by dilution the overnight cultures 1:100 in DGC-thy media and growing in a shaking

water bath at 200 rpm 37ºCto an OD_{600} of 0.3(~4 hours).

At this time, 8-methoxypsoralen or angelicin is added to the culture to a final

concentration of 10µg/ml of 20µu/ml, respectively, and incubation continued for 5 minutes prior

to irradiation with UV-A light. Cells were irradiated using two 32-watt UVA bulbs (Sylvania)

with a peak emittance of (320 nm) at an incident dose of 6.9 $J/m_2/s$. Exposure continued until the

appropriate dose was achieved and then 100-µL aliquots were removed from each culture and

serially diluted in 10-fold increments. Triplicate 10-l aliquots of each dilution were spotted onto

Luria-Bertani agar plates supplemented with 10g/ml thymine (LBthy) and incubated at 37°C.

Viable colonies were counted the next day to determine the surviving fraction.

The number of colony forming units after each treatment is recorded using an Excel

spreadsheet and graphs of the survival trend of for each strain was created using Kaleidograph

software. From the results of the bacterial subjects' ability to survive (denoted by their percent

survival), we will be able to infer the effectiveness of their DNA repair mechanism(s).

RESULTS

In order to test how the absence of translesion DNA synthesis affects survival in *E. coli*

cultures of wild type, *polB-dinB-umuDC*, and *uvr*A mutants were grown and exposed to UVA

ultraviolet light in the presence of psoralen or angelicin. The percent survival of each strain at

various doses was then plotted as shown in Figures 1A and 1B. Angelicin is structurally similar

to 8-methoxypsoralen but forms predominantly monoadducts, whereas 8-methoxy-psoralen

forms both interstrand crosslinks and monoadducts [1]. Thus, we would expect that a mutant that

is specifically defective in the repair of DNA crosslinks would exhibit more sensitivity to 8-methoxypsoralen than angelicin when compared to the sensitivity of wild type cells.
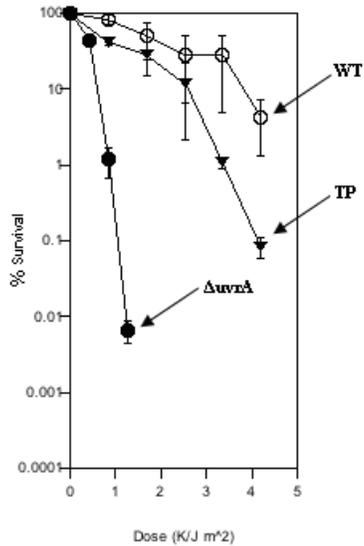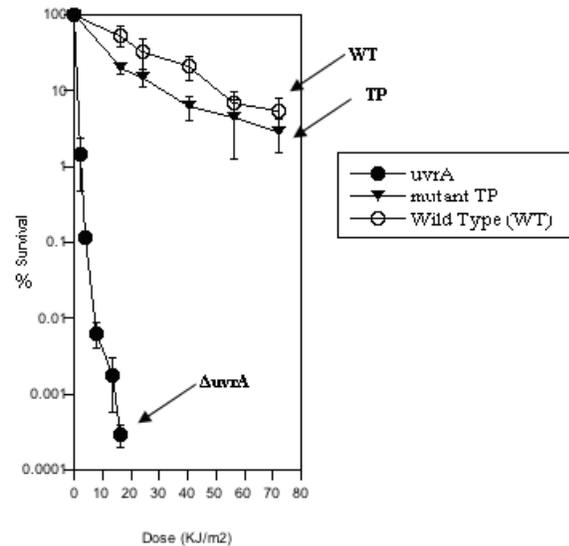
FIGURE 1A   **FIGURE 1B**

Figure 1. When compared to wild type cells, mutants lacking translesion DNA synthesis are more sensitive to interstrand DNA crosslinks than monoadducts. Figure 1A of WT (open circles), *uvrA* (closed circles), and *polB-dinB-umuDC* (mutant TP) mutant (closed triangle) are plotted following irradiation with UV-A light (340nm) in the presence of 10 μg/ml 8-methoxy-psoralen (Figure 1B). They were treated with 20 μg/ml angelicin and irradiated as the dose indicated as in (A) .The graphs represent the average of three independent experiments. The error bars present represent one standard deviation.

The results in both Figure 1A and 1B show that psoralen had a more detrimental effect on the survival of the *E. coli* DNA when compared with the effects of angelicin. In wild type cultures, survival decreased with increasing doses of UVA in the presence of 8methoxypsoralen, with approximately 10% of cells surviving 4KJ/m2 under these conditions (Fig 1A). A similar trend was observed in the presence of angelicin; although a UVA dose approximately 20 times higher than that of 8methoxypsoralenwas required to achieve the same level of killing (Fig1B).

This difference is likely due to the of lethal effects of interstrand crosslinks, relative to monoadducts, in the DNA [15]. By comparison, in cultures of *uvrA* mutants, which is known to be required for the repair of both interstrand crosslinks and monoadducts, survival was reduced at much lower doses of UVA (Fig 1A and B).

When we examined the survival of the triple polymerase mutants, we observed that the cultures were more sensitive to the presence of 8methoxypsoralen during irradiation than were wild type cultures, especially at higher UVA doses (Fig 1A). A probable hypothesis for this occurrence suggests that the primary contribution of translesion synthesis takes place when the level of DNA damage in the cell surpasses the capacity of the other repair pathways to deal with the lesions. The hypersensitivity of the polymerase mutant was far less severe than that of the *uvrA* mutant (which lacked the nucleotide excision repair component). This led us to believe that translesion synthesis does indeed contribute to the survival of strain exposed to the effects crosslinks. This occurrence allows us to infer that the polymerase mutants are not acting in a similar method or pathway as nucleotide excision repair in removing these lesions and are indeed a key factor in removal these cross-linking lesions. [15] As a result, we hypothesized that the absence of all three translesion DNA polymerases does somewhat impair the survival of UV-irradiated *E. coli,* exposed to crosslinks, to some extent.

By comparison, when we examined, the survival of the triple polymerase mutant in the presence of angelicin, we observed that the survival resembled that of wild type cultures. In contrast to 8-methoxypsoralen, there was no observed hypersensitivity of the polymerase mutant at high doses in the presence of angelicin relative to wild type cells. However, similar to 8 methoxypsoralen, the triple polymerase mutant was far less sensitive than *uvrA* mutant to angelicin. These results indicate that when in the presence of PUVA, the absence of translesion

synthesis decreases the ability of the *E. coli* to survive and when in the presence of angelicin and UVA light, the absence of translesion synthesis has rarely any effect on the

survival of the *E. coli*. These results indicate that when in the presence of PUVA, the absence of translesion synthesis decreases the ability of the *E. coli* to survive and when in the presence of angelicin and UVA light, the absence of translesion synthesis has rarely any effect on the survival of the *E. coli*.

DISCUSSION

Comparing Figures 1A and 1B, it is evident that 8methoxypsoralen has a more detrimental effect on the survival of *E. coli* than angelicin. Assuming that monoadducts form at similar frequencies with these two chemicals, the most likely reason for this is that interstrand crosslinks are more lethal than monoadducts for the cell.

Also clear from these figures is that translesion synthesis and nucleotide excision repair pathways do not contribute equally to the survival of psoralen-induced damage. Also clear from these figures is I have found that translesion synthesis and nucleotide excision repair pathways do not contribute equally to the survival of psoralen-induced damage. Whereas the lack of nucleotide excision repair renders cells severely hypersensitivite to even low levels of monoadducts and interstrand crosslinks, translesion synthesis contributes to survival only at high levels of damage, and then more so only in the presence of interstrand crosslinks. One possible reason for the hypersensitivity at high doses would be that translesion synthesis contributes to survival only when the level of DNA damage in the cell surpasses the capacity of the other repair pathways (i.e: NER) to deal with the lesions.

These observations that translesion synthesis exhibited elevated hypersensitivity to 8methoxypsoralen (which produced interstrand crosslinks and monoadducts) but not angelicin (that generates only monoadducts) would be consistent with the idea that translesion synthesis can contribute to the survival of interstrand crosslinks in *E. coli*.

To further strengthen my findings, it would be ideal to compare the survival of each individual polymerase mutant, singly, to the effects of angelicin and 8-methoxy-psoralen. If one specific polymerase were responsible for the comparative effect observed in Fig 1A and B, then I would expect to see two of the polymerase mutants exhibit survival curves similar to wild type cells while the polymerase that participates in the repair of crosslinks, would be specifically hypersensitive to 8methoxypsoralen, but not angelicin.

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