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Dps confers protection of DNA sequence integrity in UV-irradiated *Escherichia coli*

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Dps is a DNA-binding protein that plays a critical role in protecting *Escherichia coli* cells from many types of environmental stresses, such as UV radiation, gamma radiation, oxidative stress, thermal stress, and acid/base shock. It is the most abundant protein during stationary phase, during which it binds to bacterial DNA in a non-specific manner, forming a biocrystal. It has also been implicated in other important protective processes, such as the genetic regulation of key enzymes involved in DNA repair. In this study, we examined the ability of Dps to protect the sequence integrity of DNA against UV-induced mutations during stationary phase by using *E .coli* K-12 strains ZK126, a wild-type Dps$^+$ control, and ZK1146, an isogenic *dps* mutant. Specific mutations in the quinolone resistance-determining regions (QRDR) of *gyrA* and *gyrB* are known to confer resistance to nalidixic acid. After UV irradiation, a significantly higher level of survival of the ZK1146 strain was observed in the presence of nalidixic acid, compared to the ZK126 strain. This confirms that Dps confers protection against UV-induced mutations. Furthermore, our results suggest that Dps acts to regulate the frequency of mutation in *E. coli* during stationary phase.

In both eukaryotic and prokaryotic cells, UV radiation induces potentially harmful mutations in the DNA sequence that can lead to the malformation of proteins and ultimately the inhibition of growth through the formation of pyrimidine dimers (18). In *E. coli* in stationary phase, Dps (**D**NA-binding **p**rotein from **s**tarved cells) is upregulated, causing it to bind and protect DNA from UV radiation damage (12).

Although *dps* mRNA is constitutively expressed, there are increased levels under stressful conditions, peaking during early stationary phase. When bound to DNA, Dps forms a highly ordered and stable nucleoprotein complex known as the biocrystal (12). This association is maintained through the interaction between the basic amino acid residues of Dps and the positively-charged backbone of the DNA. Dps is also known to play an important role in protecting cells against oxidative stress and nucleases via compaction of the chromosome, exclusion of other macromolecules, and regulation of stress response genes (12).

Repair of DNA mutations in the absence of light plays a role in reversing the damage caused by UV mutagenesis. This process, known as dark repair or excision repair, is an enzyme-catalyzed, light-independent process that eliminates UV-induced pyrimidine dimers in DNA (18). By removing such dimers, a new strand of DNA complementary to the undamaged strand can be synthesized. An endonuclease (encoded by *uvrA*, *uvrB*, and *uvrC*) first detects and nicks the dimers, followed by separation of the two DNA strands by UvrD, a DNA helicase II. Finally, DNA polymerase I fills in the nucleotides in a 5'-3' direction, and afterwards, DNA ligase completes the connection (18).

To enhance our understanding of the level of protection conferred by Dps, UV radiation was used to induce mutations in isogenic *E. coli* strains which differ only in their ability to produce Dps: wild-type *dps*$^+$ *E. coli* K-12 ZK126 and *dps* mutant *E. coli* K-12 ZK1146. Resistance to nalidixic acid (NAL), which is not present in wild-type cells, is interpreted as mutation(s), ultimately reflecting the ability of Dps to protect the DNA sequence integrity in these specific genes. Given the known effect of Dps in protecting cells from UV radiation, the lack of Dps in ZK1146 cells is expected to increase the overall frequency of mutation, and thus contribute to NAL resistance.

Nalidixic acid, the parent compound of all quinolone antibiotics, inhibits DNA gyrase, a type II topoisomerase that relaxes superhelical tension in DNA during replication, transcription, or repair (6). Bacterial growth is deterred when these processes are inhibited by NAL. Resistance is mediated by alteration of the target protein, DNA gyrase, through point mutations in the quinolone resistance-determining regions (QRDR) of *gyrA* and *gyrB* (2, 8, 19). These encode for the dsDNA-binding and ATP-dependent sealing subunits, respectively (6).

Stationary phase cultures of *E. coli* K-12 ZK126 and ZK1146 strains were plated onto Mueller-Hinton (MH) agar with and without NAL, and with or without UV irradiation at 8 mJ/cm^2. This UV dose yielded an appropriate level of mutation as observed by the

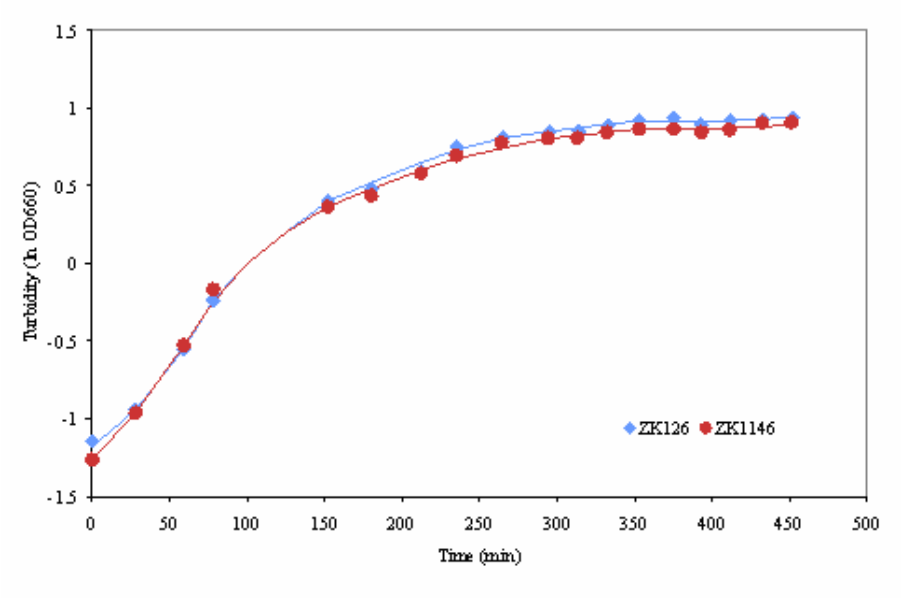

Fig. 1. Growth characteristics of wild-type (ZK126) and *dps* mutant (ZK1146) *E. coli* as measured by optical density readings at 660 nm. Experimental cultures were prepared by conducting a 1-in-10 dilution from overnight cultures grown under identical conditions.

survival rate. Viable plate counts were determined and the differences in the survival of the two strains indicated different sensitivities to UV (in the presence and absence of Dps) and nalidixic acid (through the acquisition of resistance via UV mutagenesis). By comparing such differences, it was observed that the ZK126 strain had a selective advantage over ZK1146 in terms of survival under UV irradiation in the absence of NAL, while the contrary was observed in the presence of NAL. Thus, Dps was shown to protect DNA against UV irradiation at the level of the gene.

MATERIALS AND METHODS

Bacterial strains. *E. coli* K-12 strains ZK126 [W3110Δ*lac*U16tna2], a wild-type Dps^+ control, and ZK1146 [ZK126dps::cam], an isogenic *dps* mutant, were graciously provided from the MICB 421 Culture Collection, Department of Microbiology and Immunology, University of British Columbia, Vancouver.

Culture conditions and media. Both strains were cultured in Luria-Bertani (LB) broth (10 g tryptone (Becton Dickinson, 4311921), 5 g yeast extract (Difco, 0127-01), and 5 g NaCl (EMD, SX0420-1) in 1 L distilled water) and incubated at 37°C in a shaking water bath. Serial dilutions were performed using LB broth and the cultures were plated onto Mueller-Hinton (MH) (Difco 0252-01-4) agar or MH agar supplemented with 4 µg/ml nalidixic acid (NA), which corresponded to a minimum inhibitory concentration (MIC) (13). Additional samples were plated on MH agar supplemented with 1x and 5x of 32 µg/ml rifampicin, 1 µg/ml gentamicin, and 2 µg/ml trimethoprim, corresponding to 1x and 5x of a known MIC for each antibiotic (13).

Characterization of growth. The supplied samples of *E. coli* ZK126 and ZK1146 were used to inoculate 25 ml of LB broth. Each culture was incubated for 20 hours at 37°C in a shaking water bath. A 5 ml sample of each culture was then diluted 1:10 (v/v) into fresh LB broth and the optical densities at 660 nm (OD_{660}) of the cultures were measured using a Spectronic 20D+ (ThermoFisher Scientific) spectrophotometer. The cultures were subsequently incubated at 37°C in a shaking water bath and the OD_{660} was measured every 20 minutes for 7.5 hours. The culture continued to grow for an additional 14 hours and another OD_{660} reading was taken the next day to confirm that the cultures had reached stationary phase.

Characterization of UV survival. Overnight cultures of *E. coli* ZK126 and ZK1146, determined to be in stationary phase based on their OD_{660} readings, were serially diluted with LB broth and plated onto MH agar. Duplicate plates were then exposed to 0, 4, 8, 16, 32 and 64 mJ/cm^2 UV light using a UV Stratalinker® Model 2400 (Stratagene, 40071). The plates were subsequently incubated in the dark at 37°C for 20 hours and the viable plate counts were determined.

Characterization of UV mutagenesis. Overnight cultures of *E. coli* ZK126 and ZK1146, determined to be in stationary phase based on their OD_{660} readings, were plated onto MH agar and MH agar supplemented with nalidixic acid. The plates, with their lids removed, were irradiated with 8 mJ/cm^2 of UV light. A second set of control plates did not receive any UV treatment. Both sets were then incubated in the dark at 37°C for 20 hours and the viable plate counts were determined.

RESULTS

The growth characteristics of wild-type *E. coli* (ZK126) and *dps* mutant *E. coli* (ZK1146) are similar (Fig. 1). The ZK126 culture had a slightly higher turbidity than ZK1146 for the duration of growth, however, plate counts of the final sample points indicated that both cultures were relatively equal in cellular concentration (data not shown). Fig. 1 also shows distinct plateaus, indicating that stationary phase was reached by both strains. This was confirmed by turbidity measurements taken 21.5 hours after the final sample points (data not shown).

Moderate doses of UV radiation significantly reduced the survival of both *E. coli* strains (Fig 2). At 8

mJ/cm^2, 18% and 8% survival were detected for ZK126 and ZK1146, respectively, whereas at doses of 16 mJ/cm^2 and higher, no growth was detected. It is clear from Table 1 and Fig. 2 that ZK126 was better able to survive UV irradiation than ZK1146. Further, the rate of decrease in survival is significantly greater in ZK1146, indicated by the steeper slope (Fig. 2).

Table 1. Effect of UV radiation on, and the frequency of mutation conferring resistance to nalidixic acid in wild-type (ZK126) and *dps* mutant (ZK1146) *E. coli* grown to stationary phase. UV-irradiated cultures were exposed to 0 or 8.0 mJ/cm^2 of UV radiation. 95% confidence intervals are shown.

Strain	Relative Viability after UV (%)	Frequency of Mutation (10^{-8})	
		Base-line	UV
ZK126	2.8 ±0.42	4.0 ±0.59	19.0 ±3.5
ZK1146	1.5 ±0.17	1.1 ±0.15	31.6 ±3.6

The plates containing gentamicin and trimethoprim did not yield any growth after treatment with UV light, but confluent lawns were observed after UV treatment in the rifampicin plates. Therefore, only nalidixic acid was used in the later stages of the experiment.

In the antibiotic resistance assay, both cultures started with approximately equal concentrations of culturable cells. Namely, 5.70 and 5.62 x10^9 cells/ml for wild-type and *dps* mutant *E. coli*, respectively. However, the relative death due to UV irradiation and nalidixic acid differed between the strains. *E. coli* ZK126 had twice the survival rate of ZK1146 after treatment with 8.0 mJ/cm^2 of UV radiation (Table 1), similar to the trend observed in Fig. 2. The baseline frequencies of resistance to nalidixic acid at 4 μg/ml were 4.0 and 1.1 per million for ZK126 and ZK1146, respectively (Table 1). These results indicate that *E. coli* ZK126 has 3 to 4 times greater base-line resistance than *E. coli* ZK1146, and this difference is statistically significant (Table 1). Furthermore, upon irradiation with UV, the resistance to nalidixic acid of both strains increased – 5x for ZK126 and 28x for ZK1146 (Table 1). Therefore, the relative increase in frequency of resistance in *E. coli* ZK1146 due to UV irradiation is almost 6x that observed for *E. coli* ZK126 (Table 1). Therefore, Dps appears to provide a six-fold decrease in the observable mutational effects of UV radiation at a dose of 8.0 mJ/cm^2. Finally, while the errors observed in Table 1 are considerable, their magnitudes indicate that they are statistically significant. Therefore, a valid comparison can be made, leading to a definite conclusion.

DISCUSSION

The growth characteristics of wild-type and *dps* mutant *E. coli* were similar, although there was a clear difference in the turbidity between cultures of equal concentration (Fig. 1). The turbidity of mutant cell cultures was significantly less, possibly due to a smaller average size of the *dps* mutant cells. The two strains of *E. coli* only differ with respect to a *dps* knock-out mutation in ZK1146; in all other respects, they are isogenic (12). The *dps* gene is flanked by metabolite transporter genes (10), so a polar mutation may have occurred, resulting in metabolic changes that may have decreased the cell size of the *dps* mutants. Furthermore, Dps has a regulatory role for several yet unidentified proteins; lack of such regulation may also result in a smaller cell size. Finally, cellular properties can have an effect on the incident light – the absence of the Dps biocrystal may obstruct less light and result in the relatively lower OD values observed. A previous study conducted using minimal media found the opposite trend in turbidity readings (7), but the exact cause of this discrepancy is unknown.

UV radiation can have numerous harmful effects, most notably the introduction of mutations in vital genes or unrepairable double strand breaks, both of which lead to cell death. The ability to survive various doses of UV radiation was clearly enhanced by the presence of Dps (Fig. 2, Table 1). This confirms the findings of previous studies, which also found decreased survival of *dps* mutant *E. coli* in stationary phase (7, 12). The protective function of wild-type *E. coli* was attributed to the ability of Dps to form biocrystals (3), leading us to question whether Dps mediated protection is limited to structural integrity, or whether it also maintains sequence integrity.

Analysis of the data produced unexpected results for the nalidixic acid control plates. Namely, the base-line resistance to nalidixic acid was significantly greater in wild-type *E. coli* (Table 1). ZK126 and ZK1146 are isogenic, with the exception of *dps,* and therefore equal base-line resistance was expected. Dps is a transcription regulator of several proteins, some of which are likely involved in DNA repair and stress response (12). Further, the frequency of base-line resistance to nalidixic acid was three orders of magnitude higher than the conventionally quoted frequency of spontaneous mutation – one in a billion – which cannot be accounted for by the numerous genes where target modification can occur. It was subsequently found that MICs 10 to 50-fold higher than those used in our study were used in previous experiments cited in the literature (11). Therefore, the difference in base-line resistance would be due to the ability of wild-type cells to better survive the stressful conditions of sub-inhibitory concentrations of nalidixic

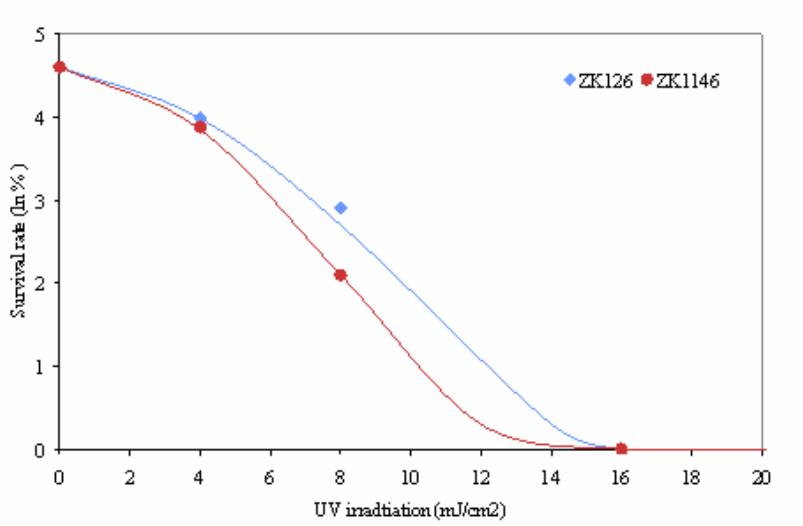

Fig. 2. The impact of UV irradiation on the survival of wild-type (ZK126) and *dps* mutant (ZK1146) *E. coli* in stationary phase. Experimental cultures were grown to reach stationary phase, confirmed by OD_{660} readings and Fig. 1. Experimental cultures were plated on MH agar plates and subsequently subjected to UV radiation at doses of 0, 4, 8, 16, 32 or 64 mJ/cm^2.

acid. Furthermore, this would indicate that Dps enables 4-fold greater survival of stressful conditions in stationary phase. However, this is not a valid explanation since the magnitude of death observed is several orders of magnitude greater than would be expected if the concentration of plated nalidixic acid were sub-inhibitory. An alternate explanation is that a nalidixic acid-efflux pump has been knocked out during the creation of the *dps* mutation. As previously mentioned, there are several metabolite transporters flanking *dps* (10). However, in current literature, none of these genes have been found to act as efflux pumps for nalidixic acid. Although this explanation might account for the presence of a higher base-line resistance in wild-type *E. coli*, it is not supported by any published research concerning nalidixic acid resistance. It is also possible that the difference observed is due solely to experimenter error coupled with natural variation. On the other hand, the magnitude of the difference and the size of 95% confidence intervals for error, according to Poisson distribution, strongly indicate that this is not the case (Table 1). One final explanation involves the transcription regulatory properties of Dps. It is possible that Dps acts to upregulate the DNA repair mechanisms and directly or indirectly regulates genes involved in the SOS response. During the SOS response in *E. coli*, the base-line mutation rate drastically increases in what is known as the SOS mutator effect. This is mainly due to the repression of proofreading and the increase of forced DNA repair (12). Furthermore, a recent publication has shown that *E. coli* regulates mutation rate in accordance with nutrient availability and general stress – more specifically, mutation rate increases during the nutrient limiting conditions of stationary phase (16). Dps is the most abundant protein during stationary phase (1), and it is during this phase that the selective pressures are high and the ability to develop novel mutations is of great advantage. Further, Dps is known to be regulated, at least in part, by RpoS, which is responsible for this increased mutation frequency during stationary phase (12). Therefore, it would not be unexpected to see a relationship between Dps and an SOS-like response. Thus, Dps may be involved in a similar, but less aggressive, SOS-like response. This suggests that the 4-fold increase in base-line nalidixic acid resistance of wild-type *E. coli* (Table 1) can be attributed to a comparable increase in natural mutation frequency. An important clarification of this proposed model is that it suggests that Dps promotes an increase in natural mutations while inhibiting the harmful effects of mutagens, thereby preferentially regulating the frequency of mutation.

The focus of our research was to study the ability of Dps to protect the sequence integrity of DNA irradiated with UV light. By analyzing the frequency at which antibiotic resistance was conferred, we could directly determine the frequency of mutations in the genes encoding the targets of these antibiotics, and thus the frequency at which sequence integrity is compromised. Four antibiotics were chosen for this analysis – trimethoprim, gentamicin, nalidixic acid, and rifampicin – for their wide range of frequency of conferring resistance by mutation, with trimethoprim being the lowest and rifampicin the highest. Resistant

mutants to trimethoprim were not detected, likely because conferred resistance requires a specific point mutation in a single gene, which is a rare event (15). Further, no gentamicin-resistant cells were detected, possibly due to the rarity of the mutations required for target modification (4). Mutations conferring rifampicin resistance are very well-characterized, limited to 20 specific point mutations (9). However, the confluent growth that was obtained on plates diluted two orders of magnitude indicates that either the frequency of mutation was too high, or improper experimental conditions were used, such as an incorrect supplied inhibitory concentration (data not shown). On the other hand, mutations conferring resistance to nalidixic acid were both detectable and measurable.

The results show that Dps dramatically reduced the occurrence of nalidixic acid resistance in cultures irradiated with doses of 8 mJ/cm^2 of UV light. Moreover, the *dps* mutant had an increase between base-line and UV-irradiated frequencies of mutation that was 6-fold larger than in the wild-type strain (Table 1). This provides direct evidence for the ability of Dps to conserve the sequence integrity of DNA in stationary phase *E. coli.* Dps likely exerts its protective function in several ways. First, it acts as a non-specific nucleoprotein, compacting DNA and forming a biocrystal (3), thereby acting as a shield to interfere with incident UV radiation. This compacted form would also leave less DNA exposed to the UV radiation, further reducing its damaging effects. Secondly, Dps is a regulatory protein (12), and, as previously mentioned, its regulatory functions may involve DNA repair mechanisms. This could lead to increased expression of enzymes involved in DNA repair, increased efficiency of this repair, as well as increased repair integrity. Finally, Dps is known to protect against oxidative stresses by acting as a replacement substrate for DNA against any free radicals that result from UV irradiation (12).

In conclusion, it was observed that wild type *E. coli* had a four-fold greater survival rate when compared to the *dps* mutant strain, in the presence of nalidixic acid. This indicates that Dps may be involved in a SOS-like response aimed to regulate the frequency of mutation under stressful conditions, specifically increasing this frequency during the nutrient-limiting stationary phase. In addition, under exposure to 8 mJ/cm^2 of UV radiation, the *dps* mutant had a six-fold greater increase in the frequency of mutation relative to the baseline frequency, deduced by a greater observed resistance to nalidixic acid. This is consistent with the theory that Dps protects DNA sequence integrity from UV radiation via biocrystal formation.

FUTURE EXPERIMENTS

First, the potential role of Dps as a mutation rate regulator should be an area of further study. By studying other indicator genes, one could confirm the results of a higher base-line mutation rate in wild-type *E. coli* compared to the *dps* mutant. Moreover, the role of Dps as a gene regulator should be further elucidated. This could be achieved by placing Dps under strict regulation by cloning it into a vector that has a regulatable promoter. One could then examining the mRNA profiles of each strain under Dps induced and repressed conditions. Alternatively, one could compare the mRNA profiles of the wild-type and *dps* mutant strain under Dps-induced conditions, ie. stationary phase. This could be done by hybridizing cDNA from each sample to a microarray chip for standard *E. coli* K-12 mRNA. The analysis should focus on changes in expression of genes involved in DNA repair and the SOS response.

Further, since the ability of Dps to protect sequence integrity was indicated solely by conferred resistance in genes affected by nalidixic acid, a potential bias of indicator choice may exist. It was assumed that Dps conferred equal and random protection of all genes, however, this requires further confirmation, as there could have been specific influence by nalidixic acid on the amount of protection. Therefore, using other indicator genes will potentially reinforce our conclusions.

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