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Dps Augments LexA Autocleavage after UV-C-Induced DNA Damage in Stationary Phase *Escherichia coli*

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Ultraviolet radiation is known to induce bacterial DNA damage, resulting in the dimerization of thymine base pairs and the production of single stranded DNA. Several DNA repair mechanisms are currently known to exist in *Escherichia coli*. The genes encoding these repair systems belong to the SOS regulon. Normally the SOS-mediated DNA repair system is repressed by LexA. Upon detection of DNA damage, the active form of RecA facilitates the autocleavage of LexA, thereby inducing the global DNA repair response. The DNA-binding protein from starved cells (Dps) is known to protect DNA during starvation and environmental stress. Apart from structural protection, it has been speculated that Dps may play a regulatory role in DNA repair. To investigate the influence of Dps on the activation of the SOS repair system after UV-induced DNA damage, *E. coli* ZK126 (wild type) and ZK1146 (*dps* mutant) were assayed immunologically for LexA cleavage. In the *dps* mutant, the repressor form of LexA monomers was not cleaved throughout the 3 minute time frame, compared to the observed decline in the wild-type sample, indicating a lack of RecA activation in the absence of Dps. The results of this study support the hypothesis that Dps plays a role in the induction of the SOS response.

The properties of the DNA-binding protein from starved cells (Dps) have been examined extensively in several bacteria, particularly in the paradigm organism, *Escherichia coli* (3). Dps levels are normally low during log phase growth but can be induced, for example, in the case of oxidative stress (13, 11). During stationary phase, *dps* is substantially upregulated (2). Induction of Dps is just one of the strategies used to provide protection for DNA from environmental assault. Evidence suggests that physical protection is mediated by forming a stable, non-sequence specific DNA-Dps complex (1).

In the context of DNA damage, Dps has been speculated to be involved in augmenting repair processes via the recruitment of repair enzymes (7), although such speculation has not been specific in suggesting putative repair factors recruited and mechanisms that may be involved.

In *E. coli*, the SOS response is a prominent regulatory network, induced by the presence of DNA damage, which results in cell cycle arrest and DNA repair (4). Key proteins involved in DNA damage tolerance, translesion DNA synthesis, and nucleotide excision repair are all encoded by genes that are members of the SOS regulon (4). The SOS response genes are repressed under physiologically stress-free conditions by the LexA repressor, a dimeric DNA-binding protein of 50 kDa (4). DNA damage from environmental stress, such as ultraviolet (UV) radiation which causes thymine dimers, eventually manifests as regions of single-stranded DNA (ssDNA). RecA, a protein that is essential for the activation of the SOS response, binds to ssDNA as filaments leading to RecA activation. The activated form of RecA functions as a co-protease for LexA which results in the autocleavage of the individual LexA monomers within the dimeric DNA-bound complex and a global induction of SOS response genes (4). Since there is evidence that the SOS response can be induced in stationary phase *E. coli* cells (12), it is possible that the DNA repair factors regulated by the SOS response during stationary phase may interact with other DNA-binding proteins present, such as Dps, which would be consistent with previous findings that *dps* mutant strains are more sensitive to UV irradiation compared to wild-type strains (7).

This study aimed to identify whether the presence or absence of Dps has an effect on the activation of the SOS response after UV radiation-induced DNA damage in *E. coli*. To achieve this, the activation of the SOS response in a *dps* mutant strain of *E. coli* was compared to a wild-type (WT) strain by using the known mechanism of LexA autocleavage by RecA following UV-induced DNA damage as a tool.

MATERIALS AND METHODS

Strains and media. *E. coli* K-12 strains ZK126 [W3110ΔlacU16tna2], a WT control, and ZK1146 [ZK126dps::cam], an isogenic *dps* mutant, were obtained from the MICB 421 Culture Collection (Department of Microbiology and Immunology, University of British Columbia, Vancouver). Overnight cultures and cultures for growth rate analysis were grown in Luria-Bertani (LB) broth (1% tryptone (w/v), 0.5% yeast extract (w/v), and 1% sodium

chloride (w/v)). Cells were plated on Mueller-Hinton (MH) agar (Difco 0252-01-4) for the UV sensitivity assay.

Growth rate analysis. Two overnight cultures were grown in LB broth at 37 °C at 200 RPM in an air incubator, one of the WT strain and the other of the *dps* mutant strain. One-in-five dilutions of each overnight culture were made and the diluted cultures were incubated in a shaking water bath at 37 °C. OD_{660nm} was measured for each culture every 20 min over 6 hours using a Spectronic 20D spectrophotometer (Thermo Scientific).

UV sensitivity assay. Additional overnight cultures were prepared in LB broth for both the WT and *dps* mutant strains and were confirmed to be in stationary phase the following morning by measuring turbidity (OD_{660}). Turbidities of both cultures were equalized and 5 ml aliquots of each ON culture were transferred to 10 cm diameter Petri dishes. The control samples, which were not UV-irradiated, were set aside. Each control sample was collected and serially diluted and then spread plated onto three replicate MH agar plates (final plated dilutions of 10^{-7}). All other 5 ml dish cultures were exposed to either 4, 8, 16, 32, or 64 mJ/cm^2 UV radiation in the dark, using a UV Stratalinker Model 2400 (Stratagene 40071), and were serially diluted and spread plated onto three replicate MH agar plates (final plated dilutions of 10^{-7}). All plates were incubated overnight at 37 °C in the dark. Plate counts were performed and normalized to control plates to determine mean percent survival after UV exposure at each intensity.

UV-induced DNA damage and lysate preparation. Additional overnight cultures were prepared in LB broth for both the WT and *dps* mutant strains and were confirmed to be in stationary phase the following morning by measuring turbidity (OD_{660}). Turbidities of both cultures were equalized and 5 ml aliquots of each strain were prepared in 10 cm diameter Petri dishes. The non-UV exposed controls of both stains were immediately flash-frozen at -80 °C in a dry ice/acetone bath. 5 ml dish cultures of each strain were exposed to 16 mJ/cm^2 UV radiation separately and were also collected and flash-frozen either 1 min or 3 min after UV irradiation. Thawed samples were centrifuged at 4800 RPM for 20 min at 4°C and the resulting cell pellets were resuspended in 1 ml radioimmunoprecipitation assay (RIPA) buffer (50 mM Tris-HCl, 150 mM NaCl, 1% NP-40 (v/v), 0.5% sodium deoxycholate (w/v), and 0.1% SDS (w/v), pH 8) supplemented with Complete protease inhibitor cocktail (Roche 11873580001) and 0.4 Kunitz units of DNase I. All samples were then homogenized with an equal portion of 0.1mm glass beads using a FastPrep 24 instrument (MP Biomedicals S6005) at a 6.0 m/s speed setting with three 30 second bursts. Lysates were centrifuged and raw extract supernatants were collected and total protein was quantified using a bicinchoninic acid (BCA) assay (10).

Immunoprecipitation of LexA. Raw extract supernatants for all samples were normalized with respect to volume and total protein concentration. LexA was immunoprecipitated from each sample using a rabbit anti-LexA IgG (Sigma-Aldrich L0415)/Protein A-agarose beads (Bio-Rad) system. As an antibody control, rabbit anti-LexA IgG was incubated with the normalized volume of RIPA buffer. LexA from all samples was eluted using 2X non-reducing SDS-PAGE loading buffer (125 mM Tris-HCl, 2% glycerol (v/v), 4% SDS (w/v), and 0.005% bromophenol blue (w/v), pH 6.8).

Western blot analysis. LexA-immunoprecipitated samples were run through a 12% SDS-PAGE gel (5). The proteins were then transferred to a Hybond-P PVDF membrane (Amersham Biosciences RPN2020F) using a cooled Mini Trans-Blot electrophoretic transfer cell (Bio-Rad 1703935) at 80V for 2 hours, in Western transfer buffer (25 mM Tris base, 180 mM glycine, and 25% methanol (v/v)). The membrane was incubated with rabbit anti-LexA antibody (1/2000 dilution) for two hours followed by a horseradish peroxidase-conjugated goat anti-rabbit IgG antibody (1/10000 dilution) for one hour. LexA bands were visualized with a TMB peroxidase substrate system (Kirkegaard & Perry Laboratories, Inc. 50-77-00).

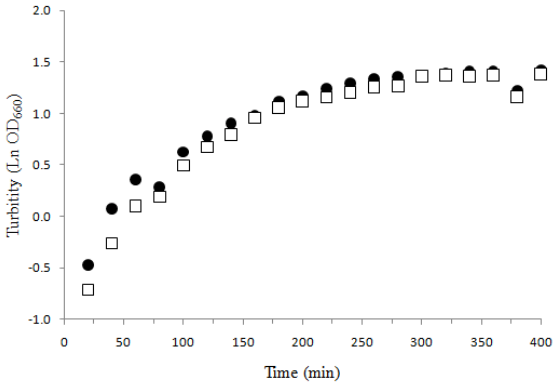

FIG. 1. Growth of WT and *dps* mutant isogenic *E. coli* strains. WT strain indicated by (●) and *dps* mutant strain indicated by (□).

RESULTS

Growth is not Dps-dependent. The growth of WT and *dps* mutant strains in LB was observed periodically over the course of six hours. The purpose of this analysis was to confirm that the two isogenic strains would follow the expectation of comparable growth rates regardless of the *dps* deficiency in the mutant. As seen in Fig. 1, the turbidity (OD_{660}) measurements from the period between 150 and 400 minutes suggested uniformly decreasing rates of growth until stationary phase, for both strains. Therefore, it was reasonable to subject both strains to identical growth conditions to attain comparable growth density at stationary phase, where culture samples were collected for subsequent experiments. It should be noted however, that the mutant strain had consistently lower OD_{660} measurements relative to that of the WT from the period of 20 to 140 minutes. Despite this discrepancy, the rates of growth for both strains appeared to be consistent with each other.

A 16 mJ/cm^2 intensity of UV radiation is optimal for studying DNA damage. A dose-dependent UV sensitivity assay was performed to assess an appropriate intensity of UV irradiation for both strains. Fig. 2 displays the survival of UV exposed samples normalized to percentages of the non-irradiated controls of the respective strains. UV exposures of 8, 16, and 32 mJ/cm^2 generally resulted in a mean percent survival above 90% in the WT strain. 90% and above mean percent survival was considered to represent negligible DNA damage due to the 10% natural variation in biological samples. When exposed to 4 mJ/cm^2, the WT strain showed decreased survival, a surprising result especially since at higher intensities, the WT strain had a higher mean percent survival. In the *dps* mutant samples, UV exposures of 4 and 8 mJ/cm^2 yielded mean percent survivals of 91% and 84.5%, respectively.

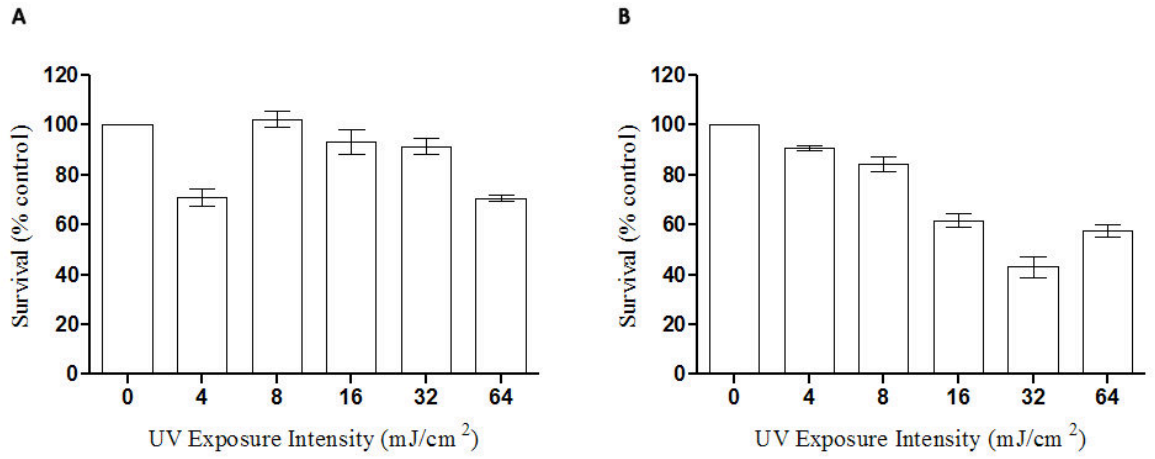

FIG. 2. Mean sensitivity of WT and *dps* mutant isogenic *E. coli* strains after exposure to increasing UV intensities. WT strain sensitivity expressed as a percentage survival relative to the non-UV exposed control (A) and similarly for the *dps* mutant strain (B). Mean ± SEM of measurements are shown as bars.

As the drop in survival became more readily apparent after exposure to 16 mJ/cm^2 in the *dps* mutant, this level of intensity was selected for the UV-induced DNA repair activation assay. The tested intensities beyond 16 mJ/cm^2 were not chosen as suitable irradiation intensities due to the excessive inhibition of growth for the *dps* mutant strain.

Dps augments LexA cleavage. The cleavage of LexA by RecA is indicative of an activated SOS DNA-repair response and was therefore monitored to assess whether Dps-mediated DNA sequestration affects the activation of the SOS response after UV irradiation. In the absence of DNA damage caused by UV irradiation, there is no cleavage of LexA. The bands identified by the white arrow in Fig. 3 corresponding to 25 kDa represent the dissociated form of the LexA dimers, hence monomers. This 25 kDa band was absent in the antibody control lane, indicating that immunoprecipitation was able to capture LexA. Of the WT samples, the 1 minute sample showed comparable levels of LexA monomers to the control while the 3 minute sample showed relatively diminished levels of the LexA monomers and therefore, possibly, a high level of RecA activation after 3 min of UV radiation exposure. In the *dps* mutant samples, a constant level of the repressor form of LexA monomers was detected suggesting a lack of RecA activation throughout the 3 min time period.

DISCUSSION

Dps should not affect *E. coli* growth under normal physiological conditions since Dps is not expressed during log phase unless an extraneous environmental assault such as oxidative stress exists (13, 11). Indeed, the growth assay demonstrated that there was no difference in growth rates between WT and *dps* mutant strains. Differences in initial turbidities (OD_{660}) between both strains (Fig. 1) can be attributed to the fact that the initial cell concentrations were not equalized prior to the start of the growth experiment. Growth of the *dps* mutant strain culture had initiated with a lower turbidity than the WT and therefore exhibited the observed smaller initial measurements. Turbidity measurements of both strains were not recorded immediately at the start of incubation, and were instead taken after 20 minutes as the first time point. However, the overall goal of this experiment was to confirm comparable stationary phase growth densities so initial variation in turbidity would not have been a concern.

The heightened sensitivity of the *dps* mutant to UV irradiation compared to WT (7) was confirmed overall by the results of the post-UV sensitivity assay (Fig. 2). A negligible decline in survivability at intensities of 8, 16, and 32 mJ/cm^2 UV radiation was observed in the WT. Despite this, there was a significant drop in survival of the WT (71%) at an intensity of 4 mJ/cm^2 which may be indicative of a technical error in the irradiation step. Independent replicates beginning with exposure to UV radiation should have been done to confirm this discrepancy, as opposed to replicate plating of single samples irradiated at each UV intensity. After exposure to 64 mJ/cm^2 of UV radiation, the WT strain declined to 70% survivability which could represent the approach to a threshold UV exposure where the WT strain begins to be killed or inhibited by higher intensities of UV radiation; however, higher intensities of UV radiation should be tested to confirm this. The *dps* mutant strain was observed to be more susceptible to death by UV-

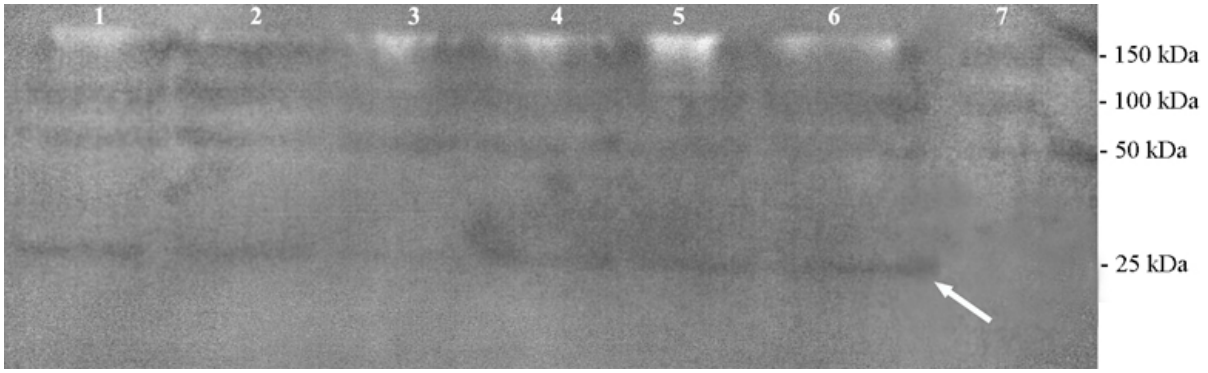

FIG. 3. Western blot of immunoprecipitated LexA protein from lysates of WT and *dps* mutant isogenic *E. coli* strains. The white arrow indicates the dissociated LexA dimers. Lanes 1-3, WT: control, 1 min, and 3 min after UV exposure, respectively. Lanes 4-6, *dps* mutant: control, 1 min, and 3 min after UV exposure, respectively. Lane 7: antibody control.

induced DNA damage compared to the WT strain at UV exposure intensities above 8 mJ/cm^2 (Fig. 2B).

LexA exists as a stable homodimeric protein of approximately 50kDa under physiological conditions, acting as the repressor of the SOS regulon (4). Immunoprecipitation was a requirement to reliably enrich for and detect LexA since there are merely ~1000 LexA molecules per *E. coli* cell which is relatively low in abundance (4). The autocleavage of LexA by RecA occurs at the peptide bond between Ala-84 and Gly-85 within one single monomer, distinct from the site of dimerization (8). Individual monomers form dimers with their entire C-terminal domains via hydrophobic and non-covalent interactions (6). In the presence of SDS in our assay system, we expected to see a disruption in these interactions leading to the dissociation of the LexA homodimers into two equivalent monomers. Therefore, instead of detecting dimeric LexA of ~50 kDa, the repressor form of LexA monomers was observed on the Western blot (Fig. 3) represented by ~25kDa bands. The levels of uncleaved, repressor LexA monomers post-UV irradiation diminished after 3 min in the WT strain whereas the *dps* mutant strain remained relatively constant over 3 min. The reduction of detectable uncleaved LexA monomers in the WT strain were expected given the previous speculations of Dps being involved in the recruitment of DNA repair proteins (7). After UV exposure, RecA becomes active when ssDNA is detected. Active RecA facilitates the autocleavage of LexA. Upon RecA activation, the induction of LexA cleavage begins after 1 minute, and by 5 minutes virtually all monomers of LexA are cleaved (4). After the cleavage of LexA, the resulting 118 residue and 84 residue fragments of LexA are targeted for rapid degradation by ClpXP within 1-5 minutes after cleavage (8), so it was unlikely that these cleavage fragments were to be observed on the Western blot. A control that should have been included to show complete SOS activation (complete LexA cleavage) would be a culture treated with nalidixic acid (9).

The only difference between the isogenic strains was the presence or absence of Dps. Thus, the results from this study suggest that the presence of Dps may positively regulate the SOS repair system through a mechanism involving the activation of RecA and the subsequent inactivation of LexA during stationary phase. Specifically, Dps appears to enhance LexA cleavage after UV-induced DNA damage at least within 3 min after UV irradiation, consistent with the previously described kinetics of LexA cleavage by activated RecA (4). Because the Dps protein complexes with DNA, it may interact directly with RecA in a cooperative fashion or through the recruitment of other factors that increase the efficiency of RecA binding to ssDNA resulting from UV damage. It would be more informative to investigate an extended time frame after UV irradiation to accurately determine if there is indeed a difference in the kinetics of the SOS response between the WT and *dps* mutant. Nevertheless, the loss of structural protection and impaired induction of the SOS response observed may contribute to the reduced survival of the *dps* mutant strain at UV radiation intensities that are tolerated by the WT strain.

FUTURE DIRECTIONS

To further confirm that Dps augments the SOS response, the transcriptional activation of *recA* can be assessed. A *lux* or *lacZ* reporter fused to the promoter of the *recA* gene (12), would have to be constructed. The reporter construct could then be inserted into both the WT and *dps* mutant which would be exposed to the same UV intensity. If Dps plays a role in increasing the SOS response, the reporter construct should reveal a more robust level of transcription for SOS response genes in the WT following UV irradiation.

As our results implicate Dps in the regulation of the SOS response system, it would be interesting to investigate possible interactions between components of this system with other proteins. In this study, it has been speculated that Dps and RecA may physically cooperate to enhance the activation of SOS response. Proteins interacting with Dps could be co-immunoprecipitated followed by blotting of RecA or other SOS system components of interest to directly confirm the speculative recruitment of repair-related proteins by Dps.

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