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INFLUENCE OF DNA ADENINE METHYLASE ON THE SENSITIVITY OF *ESCHERICHIA COLI* TO NEAR-ULTRAVIOLET RADIATION AND HYDROGEN PEROXIDE

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Near-ultraviolet (NUV) radiation and hydrogen peroxide (H_2O_2) inactivation studies were performed on *Escherichia coli* K-12 DNA adenine methylation (*dam*) mutants and on cells that carry plasmids which overexpress Dam methylase. Lack of methylation resulted in increased sensitivity to NUV and H_2O_2 (a photoproduct of NUV). In a *dam* mutant carrying a *dam* plasmid, the levels of Dam enzyme and resistance to NUV and H_2O_2 were restored. However, using a multicopy *dam*$^+$ plasmid strain, increasing the methylase above wildtype levels resulted in an increase in *sensitivity* of the cells rather than *resistance*.

Currently there is concern about the effects of decreased stratospheric ozone levels and a subsequent increase in the levels of near-ultraviolet radiation (NUV) that might reach the earth's surface (1, 2). Cells have evolved mechanisms for coping with excess NUV (3). We have investigated how altering the DNA by adenine methylation effects the sensitivity of *Escherichia coli* to NUV and its photoproduct, hydrogen peroxide (H_2O_2). Various mutants that are deficient in DNA adenine methylase, and cells that harbor plasmids which overexpress DNA adenine methylase were utilized in this study.

In wildtype *E. coli*, methylation of the adenine, in the sequence 5'-GATC-3', is catalyzed by the *dam* gene product, a methylase. This is referred to as "hemimethylation" (13), since it occurs on only one strand of the DNA. As reviewed by Barras and Marinus (13), the hemimethylation of the 5'-GATC-3' sequence (but not methylation on both strands), provides recognition sites for mismatch repair enzymes, and for attachment of specific proteins to 5'-GATC-3' sequences in promoters (i.e., provides a template for transcription). Thus, the dam gene product may provide the cell with a way to respond to environmental changes, such as exposure to NUV and other oxidative stresses.

MATERIALS AND METHODS

Bacterial Strains: *E. coli* strains are listed in Table 1.

Media and Buffers: Cells were grown in Luria-Bertani (LB) medium (3); platings were on LB agar plates. NUV irradiations and dilutions and H_2O_2 dilutions

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Table 1. Bacterial Strains

Strain Designation	Methylase-related Genotype	Other Relavent Genotypes	Source
GM215	*dam-3*	F- *rns-1 supE44 endA1*	B. Bachmann
GM161	*dam-4*	F- *hsdS1 supE44*	B. Bachmann
GM2199	*dam-13*::Tn9	F- *supE44 rpsL136* (Cm^r)	B. Bachmann
GM2290	*dam*+ pTP166 (overproducer)	*lacI*^{q} *lacPL8* (Ap^r)	M. Marinus
AB1157	W.T.	*rpsL31 tsx-33- supE44*	B. Demple
AE0710	*dam*+ (W.T.)	Same as GM2290 without pTP166	This lab
GM30	*dam*+	Same as GM2199 before transduction with *dam-13*::Tn9	M. Marinus
AE0716	*dam*+	Same as GM2199 but transformed with pTP166 (Ap^r, Cm^r)	This lab
GM3819	*dam-16*	Same as AB1157 but Kan^r	M. Marinus
JC4588	*dam*+	Very similar to AB1157 but *endA gal his322 thi*	P. Modrich
AE0726	*dam*+ pGG503 (overproducer)	Same as JC4588 but transformed with pGG503 (Tet^r)	This lab

were performed with M9 salts (3). Plates were incubated for colony formation at 37 C for 24 hours. Liquid cultures were grown at 37 C in an orbital water shaker.

NUV Irradiation and Inactivation Procedure: An aliquot was removed from an exponentially growing culture and washed in M9 salts. The sample was then resuspended in a 15 X 100 mm Pyrex tube of M9 salts. Air was pumped into the tube for aeration and mixing. The sample was then lowered into an irradiation box that consisted of a bank of eight G. E. BLB-8T germicidal lamps arranged radially around the sample (4). The bulbs had a fluence of 216 Jm^{-2} sec^{-1} as measured by a Spectronic DM-365N ultraviolet meter. Temperature consistency was maintained by a fan positioned in the bottom of the box. Aliquots were removed periodically during the irradiation and diluted in M9 salts for plating. Colony counts were expressed as percentage of killing.

H_2O_2 Inactivation Procedure: A 1/100 dilution of an overnight culture was placed on an orbital water shaker at 37 C until an O. D. of 0.1 was first observed. At that time, varying concentrations (5, 10, 15 and 20 mM) of H_2O_2 were added and the sample returned to the shaker for 10 minutes. Aliquots were then removed and

diluted in M9 salts and plated. Plates were incubated for colony formation and inactivation expressed in terms of percentage of killing.

DNA Adenine Methylase Activity Assay: Methylase activity was determined by the procedure of Geier and Modrich (5).

RESULTS

NUV Inactivations: Mutant strains that are deficient in DNA adenine methylase activity (*dam*-3, *dam*-4, *dam*-16 and *dam-13*::Tn*9*) were sensitive to the effects of NUV (Fig.1). There was an increased sensitivity in strain that is deficient due to inactivation of the *dam* gene by Tn*9* (dam-13::Tn*9*). The effect of Tn*9* is to completely inactivate the *dam* gene (6) as can be seen in the DNA adenine methylase activity assay (Table 2).

Since strains deficient in methylase activity were sensitive to the effects of NUV, a logical conclusion would be that Dam methylase may protect the cell from the effects of NUV, or permit efficient repair. This, however, was not a simple case of the presence of the enzyme. The strain that carries the plasmid pTP166 (inducible overproducer of Dam methylase) exhibited increased levels of sensitivity to NUV, compared to *dam* mutants, when the plasmid was expressed (data not shown). This effect was best observed in the strain harboring the plasmid pGG503 (Figure 1). This

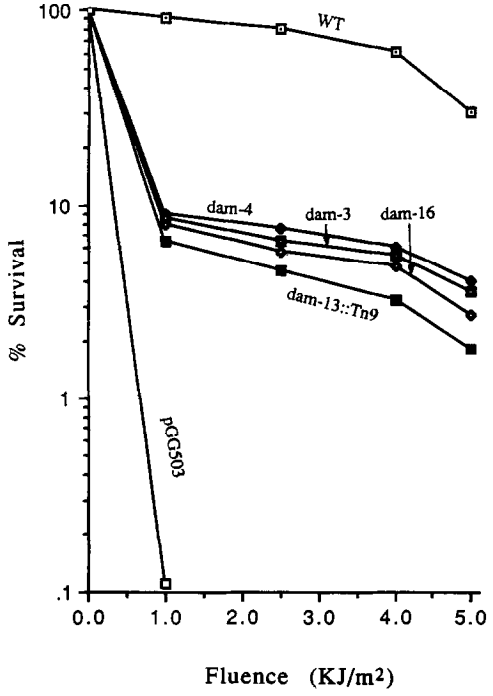

Figure 1. Survival of W.T., *dam-4*, *dam-3*, *dam-16*, *dam-13*::Tn*9* and pGG503 harboring cells after exposure to G.E. BLB-8T lamps with emission in the 300-400 nm range with a peak of 365 nm.

Table 2. Methylase Activity Assay

Strain Designation	Relavent Genotype	Counts/ Minute	Activity relative to Wildtype
AB1157	W.T.	5,012	1.0
GM215	*dam-3*	105	0.2
GM161	*dam-4*	1,486	0.3
GM2199	*dam-13*::Tn*9*	120	0.02
GM2290 (250 mM IPTG)	*dam*+ pTP166	4,995	20
GM2290 (500 mM IPTG)	*dam*+ pTP166	200,390	40
AE0710	*dam*+	4,937	1.0
GM30	*dam*+	5,130	1.0
AE0716	*dam*+	4,810	1.0
GM3819	*dam-16*	1,005	0.2
JC4588	*dam*+	5,120	1.0
AE0726	*dam*+ pGG503	250,547	50

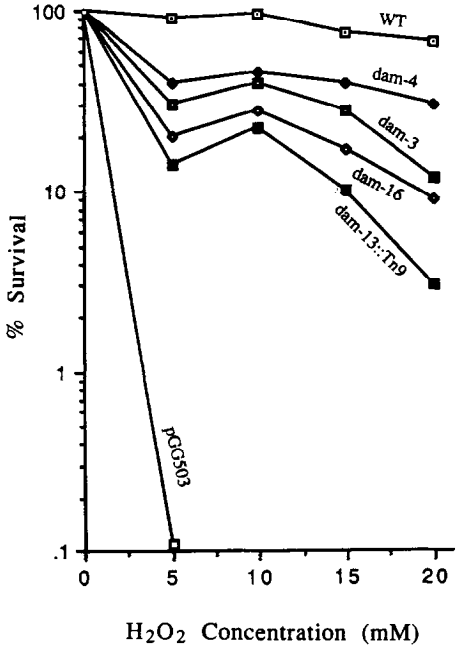

Figure 2. Survival of W.T., *dam-4*, *dam-3*, *dam-16*, *dam-13*::Tn*9* and pGG503 harboring cells after exposure to varying concentrations of H_2O_2 for 10 minutes.

plasmid constitutively overproduced Dam methylase (8) as was seen in the DNA adenine methylase activity assay (Table 2).

H_2O_2 Inactivations and Spot-Test: *dam* mutants (*dam-3*, *dam-4*, *dam-16* and *dam-13*::Tn*9*) exhibited sensitivity to the effects of high concentrations of H_2O_2 (Figure 2). Again, as in the case of exposure to NUV, increased levels of DNA adenine methylase afforded no increase in protection to the effects of H_2O_2. This is best evidenced by the strain carrying the plasmid pGG503 (Figure 2). Strains harboring the plasmid pTP166 (inducible) exhibited a similar effect (data not shown). Many of the strains exposed to H_2O_2 showed evidence of shoulders in their inactivation curves (Figure 2). An explanation of this effect could be seen in the mode-one and mode-two killing by low and high concentrations of H_2O_2 described by Imlay and Linn (9). The mode-one and mode-two killing was also observed in H_2O_2 spot-tests (initial screening of sensitivities to H_2O_2; data not shown) as rings of growing cells in zones of killed cells. This effect was presumably due to a concentration gradient of H_2O_2 being set up as diffusion took place in the agar so that mode-one killing by low concentrations and mode-two killing by high concentrations could be distinguished.

DNA Adenine Methylase Activity Assay: Results are summarized in Table 2.

DISCUSSION

Varying proposals have been made such as involvement of Dam methylation in mismatch repair (10) or gene regulation (11). However, even its necessity can be questioned since mutants which have no detectable methylase activity are still viable (12). Nevertheless, DNA adenine methylation mutants are sensitive to the effects of NUV and its photoproduct H_2O_2. Strains that carry overexpressing plasmids exhibit levels of sensitivity even greater than those that have little or no methylase activity. There is no clear explanation for the increased sensitivity of *dam* mutants to the effects of NUV and H_2O_2. Lesion repair in *dam* mutants could possibly be reduced due to the lack of methylation exhibited in these mutants. Lack of methylation can increase the size of the segments of DNA involved in repair (14), increasing the possibility of incorrect repair. Some repair enzymes do not function with high fidelity in a *dam* background (15). Dam methylase deficient mutants also have an increased number of single-strand chromosome breaks (16), when they are challenged by NUV or H_2O_2, there may already be a saturation of repair enzymes, thus not allowing efficient repair of damage.

The observation that strains that hypermethylate their DNA are more sensitive to the effects of NUV and H_2O_2 than *dam* mutants is an enigma. The model of methyl directed mismatch repair could offer an explanation. Methylation acts as a key to distinguish between newly synthesized and parental DNA strands (10, 17). In strains where there is overexpression of methylase, the DNA is methylated almost

immediately upon replication. Presumably, any mismatches incurred during replication are not repaired. The combination of accumulation of replicative errors along with damage caused by NUV and H_2O_2 may be overwhelming to the cell and contribute to lethality. Another contributing factor may actually be the high concentrations of methylase in the cell. If they are high enough, it is possible that there may be some kind of interaction between the methylase and repair enzymes which would inhibit their function.

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