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Protection of DNA during Oxidative Stress by the Nonspecific DNA-Binding Protein Dps

ASUNCION MARTINEZ AND ROBERTO KOLTER*

Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts 02115

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Reactive oxygen species can damage most cellular components, but DNA appears to be the most sensitive target of these agents. Here we present the first evidence of DNA protection against the toxic and mutagenic effects of oxidative damage in metabolically active cells: direct protection of DNA by Dps, an inducible nonspecific DNA-binding protein from *Escherichia coli*. We demonstrate that in a *recA*-deficient strain, expression of Dps from an inducible promoter prior to hydrogen peroxide challenge increases survival and reduces the number of chromosomal single-strand breaks. *dps* mutants exhibit increased levels of the G · C→T · A mutations characteristic of oxidative damage after treatment with hydrogen peroxide. In addition, expression of Dps from the inducible plasmid reduces the frequency of spontaneous G · C→T · A and A · T→T · A mutations and can partially suppress the mutator phenotype of *mutM* (*fpg*) and *mutY* alleles. In a purified in vitro system, Dps reduces the number of DNA single-strand breaks and Fpg-sensitive sites introduced by hydrogen peroxide treatment, indicating that the protection observed in vivo is a direct effect of DNA binding by Dps. The widespread conservation of Dps homologs among prokaryotes suggests that this may be a general strategy for coping with oxidative stress.

All organisms living in aerobic environments are exposed to reactive oxygen by-products that can damage most cellular components, including lipids, proteins, and DNA. Active oxygen species are generated during normal aerobic metabolism, as well as by exogenous sources such as near-UV radiation, gamma radiation, and redox cycling drugs. There is evidence that oxidative damage is related to mutagenesis, aging, and a variety of human diseases, including cancer (3). Although all organisms have evolved mechanisms of defense against oxidative damage, those have been best characterized in the bacterium *Escherichia coli*. Some of the mechanisms of coping with oxidative stress appear to be conserved through evolution, since enzymes similar to those first described in the bacterial systems have been recently identified in eukaryotes (9).

When *E. coli* encounters oxidative stress during aerobic growth, it induces the expression of a set of genes encoding enzymes that degrade the reactive oxygen species and repair the damaged macromolecules, such as DNA repair systems (10). These genes are organized in two independent stimulons that are turned on by different oxidative stresses, the superoxide stress response (induced by the superoxide radical O_2^- and regulated by *soxR* and *soxS*) and the peroxide stress response (induced by H_2O_2 and organic peroxides and regulated by *oxyR*). These inducible responses are critical for survival of *E. coli* after oxidative stress, as well as for decreased mutagenesis. When bacteria are starved their ability to respond to environmental stresses by de novo protein synthesis is compromised by the lack of nutrients. Instead, they have evolved systems that prepare them in advance for the possible appearance of a variety of stresses (11). In particular, stationary-phase cells are intrinsically more resistant to H_2O_2 than actively growing cells. The development of the resistance requires the stationary-phase-specific sigma factor σ^S, which directs the expression, among others, of the HPII catalase, encoded by *katE*, and the DNA repair enzyme exonuclease III, a product of the *xthA* gene. *dps* (*pexB*), another σ^S-dependent gene, is also required for the development of starvation-induced H_2O_2 resistance (1). Dps is a 19-kDa protein highly abundant in stationary-phase cells. In vitro Dps binds DNA without apparent specificity, forming extremely stable complexes. Mutants lacking Dps are very sensitive to H_2O_2 in stationary phase and show an altered pattern of protein synthesis. The mechanism of Dps function is not known, although it has been postulated that it may play a role in DNA protection (1). The finding that *dps* is also expressed during exponential growth after treatment with low doses of H_2O_2 as part of the OxyR regulon suggests that Dps is critical for survival during oxidative stress, independently of whether cells are actively growing or not (2, 21).

In this paper, we describe experiments designed to characterize the function of Dps. We show that in the presence of Dps, the number of DNA strand breaks and mutagenic base damage events introduced by H_2O_2 is reduced in vivo. This effect is due to direct protection of DNA by Dps binding since it can be reproduced in a purified in vitro system. Thus, Dps represents the first example of a new level of defense against oxidative damage in actively growing cells: direct protection of DNA by an inducible nonspecific DNA-binding protein.

MATERIALS AND METHODS

Construction of pBAD18-dps. The plasmid pBAD18-dps contains the *dps* gene cloned under the control of the arabinose-inducible P_{BAD} promoter of pBAD18 (12). The *dps* gene was PCR amplified from plasmid pJE106 (1) with the primers 5′dpsORF (CGGAATTCGGGACATAACATCAAGAG) and 3′dpsORF (GCTCTAGAGCGATGGATTTATTCG). These primers introduce an *Eco*RI site 5′ to the *dps* open reading frame (ORF) and an *Xba*I site 3′ to *dps*, respectively. The PCR product was digested with *Eco*RI and *Xba*I and cloned into *Eco*RI/*Xba*I-digested pBAD18. Expression of *dps* from pBAD18-dps is not detectable by Western analysis in the absence of arabinose. To prevent metabolic degradation of the inducer, arabinose, the arabinose operon was deleted from the strains harboring pBAD18-dps by P1 transduction of Δ*ara*714 *leu*::Tn*10* followed by selection of spontaneous Leu^+.

H_2O_2 survival assays. Overnight Luria-Bertani (LB) cultures of the desired strains were diluted 1:500 into 2 ml of fresh medium and incubated at 37°C with shaking for 3 h. The exponentially growing cultures were exposed to the indi-

* Corresponding author. Mailing address: Department of Microbiology and Molecular Genetics, Harvard Medical School, 200 Longwood Ave., Boston, MA 02115. Phone: (617) 432-1776. Fax: (617) 738-7664. E-mail: kolter@mbcrr.harvard.edu.

cated concentration of H_2O_2 for 15 min at room temperature. Treatment was stopped by the addition of catalase (10 μg/ml of culture), and cultures were diluted into M63 buffer and plated on LB plates to determine the number of CFU. Induction of the *dps* gene in pBAD18-dps was accomplished by the addition of the indicated concentration of L-arabinose to the medium at the time of subculture. The number of Dps molecules per cell prior to treatment was determined by Western analysis with purified Dps as a standard.

Alkaline sucrose gradients. A *thyA* mutant of the *dps*::*kan* Δ*recA* Δ*ara* strain obtained as previously described (30) was used in this experiment to increase the incorporation of [^{3}H]thymine into DNA. An overnight culture of the *dps*::*kan* Δ*recA* Δ*ara thyA* strain harboring pBAD18-dps was diluted per duplicate 1:1,000 in fresh LB medium containing 10 μCi of [^{3}H]thymine and 100 μg of ampicillin per ml. Arabinose (0.2%) was added to one of the cultures. After 2.5 h of growth at 37°C, both cultures were divided in two. One-half of the culture was left untreated, and the other half was treated with 2 mM H_2O_2 for 15 min. The treatment was terminated by addition of catalase (to 10 μg/ml of culture). The induction of DNA single-strand breaks in 100 μl of each culture (corresponding to approximately 5,000 trichloroacetic acid-precipitable cpm) was monitored by sedimentation through alkaline sucrose gradients (24) performed with modifications as described previously (5). The number of single-strand breaks per chromosome in each sample was calculated as described in reference 19, with λ DNA as a molecular weight standard. The first 15 fractions (which contain plasmid DNA) and the last 4 were excluded from the calculations.

In vitro DNA damage assays. The in vitro DNA damage assays were performed as described previously (31) with the following modifications. All reaction mixtures (40-μl final volume) contained pUC19 plasmid DNA (310 ng) in 12.5 mM Tris-Cl (pH 8.0)–12.5 mM NaCl. When indicated, $FeCl_3$ (0.1 mM), EDTA (0.4 mM), H_2O_2 (10 or 100 mM), and purified Dps (1.2, 3.0, or 6 μg) were added to the reaction mixtures. After incubation for 1 h at room temperature, the reactions were stopped by the addition of the iron chelator dipyridyl to 5 mM. Dps was removed from half of each reaction mixture by extraction with chloroform-isoamyl alcohol (24:1) after addition of sodium dodecyl sulfate to 2%. Supercoiled DNA and nicked plasmid DNA were resolved by electrophoresis in 0.8% agarose gels and visualized by ethidium bromide staining.

In the experiments including DNA digestion with Fpg, the treatment was performed as described above, except that the reaction was stopped by addition of deferoxamine mesylate to 10 mM. Dps was removed as described above. The residual sodium dodecyl sulfate (which interferes with Fpg activity) was precipitated on ice by addition of NH_4 acetate to 3.5 M. After centrifugation, the supernatant was transferred to a clean tube, and the DNA was precipitated with isopropanol and resuspended in Fpg buffer (70 mM HEPES-KOH [pH 7.6], 100 mM KCl, 2 mM EDTA). Half of each sample was digested with Fpg (10 ng) for 15 min as described previously (8). The fraction of supercoiled DNA in each reaction was measured by Southern blot analysis with ^{32}P-labeled pUC19 as the probe. The average number of nicks per plasmid molecule was calculated assuming a Poisson distribution of the lesions (17). The number of nicks induced directly by H_2O_2 was measured in the samples not treated with Fpg. The total nicks were measured in the samples treated with Fpg. The difference between these two numbers is the number of nicks introduced by Fpg.

H_2O_2-induced mutagenesis assays. The frequency of each of the six possible base substitutions was measured by monitoring the appearance of $LacZ^+$ revertants in a set of strains designed to allow rapid detection of point mutants (7). Cells were grown in M63 glucose medium for 3 days, the time at which the effect of the *dps*::*kan* allele on survival in response to H_2O_2 is maximal (1). H_2O_2 treatment was performed as described previously (16) with the following modifications. A total of 0.1 ml of cells (10^8 cells) was treated with 50 μg of H_2O_2 in 0.5 ml of M63 salts (0.3 mM final H_2O_2 concentration) for 30 min at room temperature. The mixture was poured onto a minimal lactose plate with 2.5 ml of molten top agar. The number of $LacZ^+$ colonies was scored after incubation at 37°C for 3 days. In each independent experiment, the assay was performed at least in duplicate. The results are expressed as the average number of $LacZ^+$ colonies per plate, with the standard deviation indicated in parentheses.

Papillation assay for spontaneous mutations. Papillation assays (26) were performed by streaking liquid cultures grown in minimal glucose medium for 24 h with the appropriate drugs onto M63 plates containing 0.2% glucose, 0.05% lactose, and 40 μg of 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) per ml, with or without 0.1% arabinose as indicated. The appearance of blue papillae was monitored after 5 days of incubation at 37°C.

RESULTS

Dps is not required for the repair of oxidatively damaged DNA. Expression of Dps is induced both during starvation and in response to oxidative stress during exponential phase (1, 2, 21). Even though mutants lacking Dps display sensitivity to H_2O_2 in stationary phase, prior work did not reveal whether Dps protects DNA or is part of the repair system, perhaps binding to the oxidatively damaged DNA and targeting it for the repair enzymes. In order to distinguish between these two possibilities, we analyzed the effect of a *dps* deletion on survival after H_2O_2 treatment in exponentially growing cells. Killing of growing *E. coli* cells by H_2O_2 is bimodal (15). Mode 1 killing has its maximum at low H_2O_2 concentrations (roughly 2.5 mM), requires active metabolism, and is caused by DNA damage, particularly single-strand breaks (4). Mode 2 killing occurs at higher H_2O_2 concentrations (>20 mM), does not require active growth, and is due to uncharacterized cell damage. Mutants defective in genes required for the repair of oxidatively damaged DNA (such as *recA*, *polA*, and *xthA*) are very sensitive to mode 1 killing (15). By analogy, if Dps is required for the repair of oxidatively damaged DNA, we should observe pronounced sensitivity to mode 1 killing in a *dps*-deficient strain. Our results show that was not the case (Fig. 1A). While the *recA56* strain exhibited the characteristic sensitivity to low concentrations of H_2O_2, the killing pattern of the strain containing the *dps*::*kan* allele was indistinguishable from that of the wild-type strain at concentrations of H_2O_2 below 10 mM, demonstrating that Dps is not required for the repair of the DNA lesions responsible for mode 1 killing. At concentrations of peroxide above 20 mM, however, the *dps*::*kan* mutant showed decreased survival after the treatment.

Dps protects DNA from oxidative damage in vivo. Next, we explored whether Dps is involved in directly protecting DNA from oxidative damage. If that is the case, the presence of Dps in the cells prior to the H_2O_2 challenge would be expected to increase survival. This effect should be more noticeable in a DNA repair-deficient background, in which the inability to efficiently remove the DNA damage results in a dramatic decrease in viability after oxidative treatment (Fig. 1A). The *dps* gene can be induced during growth in an OxyR-dependent manner by treatment of exponentially growing cells with low concentrations of H_2O_2 (2), but such treatment would also lead to the induction of other genes of the peroxide regulon. In order to study the effect of the presence of Dps itself prior to treatment, we chose to express *dps* from an inducible promoter on a plasmid. The plasmid pBAD18-dps contains the *dps* gene cloned under the control of the arabinose-inducible P_{BAD} promoter of pBAD18 (12). Induction of the plasmid-borne *dps* by growth in arabinose prior to the H_2O_2 challenge significantly increased the survival of a *dps*::*kan* Δ*recA* Δ*ara* strain (Fig. 1B). Survival was 30- to 100-fold better at the low H_2O_2 concentrations that cause mode 1 killing (<3 mM) and more than 200-fold better at the concentrations at which mode 2 killing occurs (>30 mM). Induction of Dps expression after the challenge, however, did not affect survival (data not shown), as expected if Dps operates at the level of DNA protection. In this experiment, the amount of Dps per cell after induction with 0.2% arabinose was approximately 1.6 million molecules, as estimated from Western analysis with purified Dps as a standard (data not shown). When levels of Dps were manipulated by varying the concentration of the inducer in the growth medium, the survival in response to 2 mM H_2O_2 increased exponentially with linear increases in the intracellular concentration of Dps at the time of the oxidative challenge (Fig. 1C).

In order to test whether the increase in survival was indeed due to DNA protection, we looked at the state of the chromosome after treatment with 2 mM H_2O_2. We analyzed the frequency of alkali-labile bonds (abasic sites) and single-strand breaks introduced into chromosomal DNA by H_2O_2 treatment by sedimentation of radioactively labeled chromosomes through alkaline sucrose gradients (24). Under gradient conditions, the DNA is denatured and the presence of single-strand breaks is revealed by a decrease in the mobility of the chromosomal fragments through the gradient. The presence of Dps before the treatment resulted in a shift in the DNA sed-

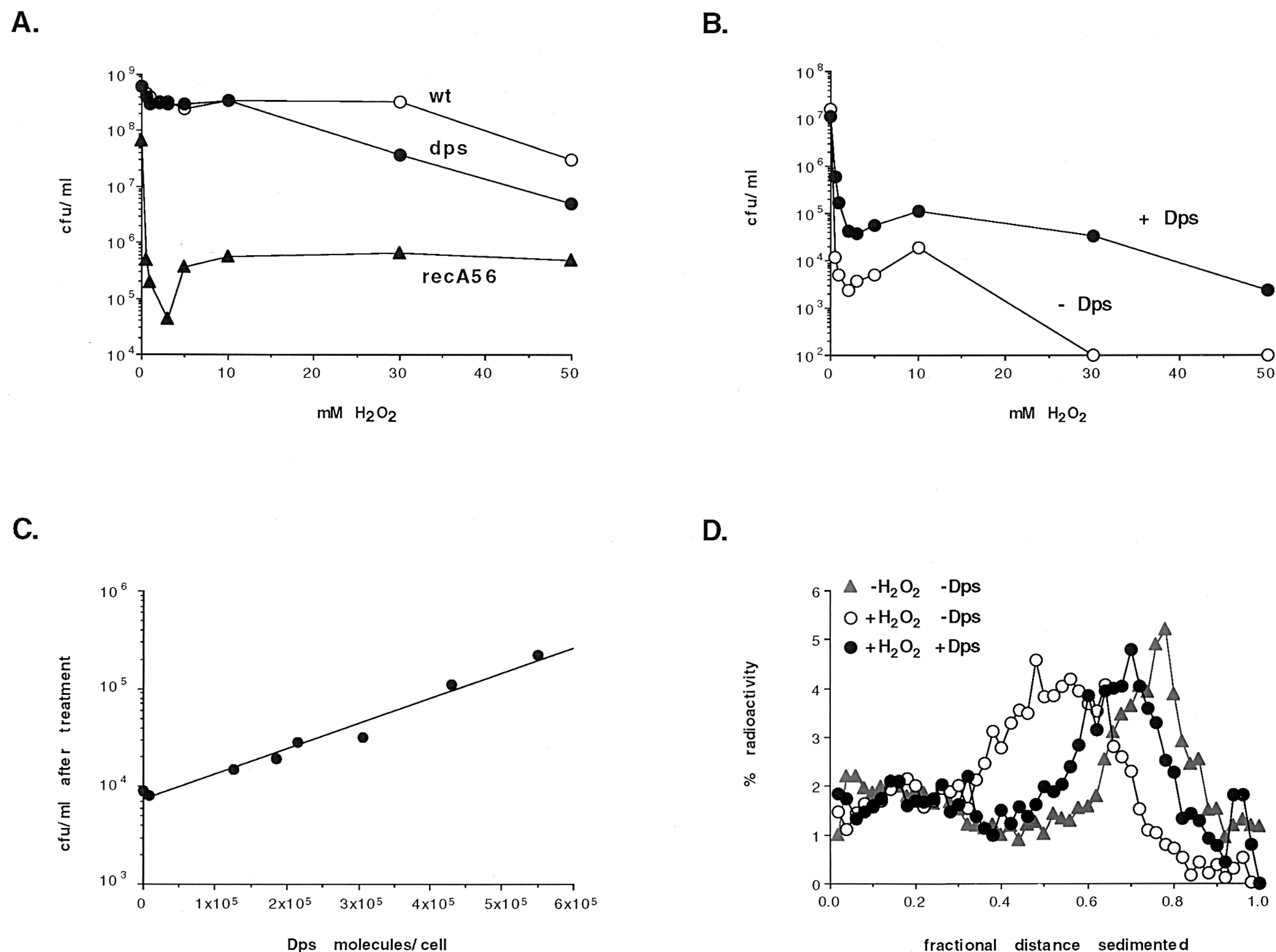

FIG. 1. (A) A Dps mutant is not hypersensitive to mode 1 killing. Exponentially growing cells in LB medium were exposed to the indicated concentrations of H_2O_2 for 15 min at room temperature. Treatment was stopped by the addition of catalase (10 μg/ml of culture). Cultures were diluted in M63 buffer and plated to determine CFU. Open circles, wild type (wt); solid circles, *dps*::*kan*; solid triangles, *recA56*. (B) The presence of Dps before H_2O_2 treatment increases the survival of a repair-deficient strain. A *dps*::*kan* Δ*recA* Δ*ara* strain containing pBAD18-dps was grown in the absence (open circles) or presence (solid circles) of inducer (0.2% arabinose). The presence of pBAD18 vector alone had no effect on survival in either the presence or the absence of inducer (data not shown). (C) The degree of survival of the repair-deficient strain depends on the levels of Dps before H_2O_2 treatment. The *dps*::*kan* Δ*recA* Δ*ara* strain containing pBAD18-dps was grown in the absence or the presence of various concentrations of inducer (0.01 to 0.2% arabinose). Samples of all cultures were removed prior to treatment, and the amount of Dps per cell was determined by Western analysis with purified Dps as the standard. Half of each culture was treated with 2 mM H_2O_2 for 15 min. All of the untreated cultures had approximately 1.7×10^7 CFU/ml. (D) Dps reduces the number of DNA single-strand breaks induced by H_2O_2 in vivo. The induction of single-strand breaks by 2 mM H_2O_2 was followed by sedimentation in alkaline sucrose gradients. Solid triangles, untreated *dps*::*kan* Δ*recA* Δ*ara thyA* strain containing pBAD18-dps grown in the absence of arabinose; open circles, same strain grown in the absence of arabinose treated with 2 mM H_2O_2; solid circles, same strain grown in the presence of 0.2% arabinose treated with 2 mM H_2O_2. The profile of the untreated sample grown in the presence of arabinose is similar to that of the untreated sample grown in its absence (data not shown).

imentation profile of a *recA*-deficient strain towards that of the untreated cells (Fig. 1D). Quantification of the number of single-strand breaks per chromosome (19) revealed that the presence of Dps prior to H_2O_2 treatment decreased the number of single-strand breaks fourfold under our experimental conditions (111 single-strand breaks introduced in the absence of Dps versus 27 in the presence of Dps). These results demonstrate that Dps protects DNA from cytotoxic oxidative damage, but they do not distinguish between protection arising directly from DNA binding and that arising indirectly through an effect of Dps on other cellular functions.

Dps protects DNA from oxidative damage in vitro. We studied the effect of purified Dps in an in vitro DNA damage assay to determine whether Dps can directly protect DNA from oxidative damage. In this system (31), the combination of Fe(III), the iron chelator EDTA, and 10 mM H_2O_2 results in the introduction of single-strand breaks that are visualized by the change in mobility of the substrate plasmid DNA in agarose gels (Fig. 2A, lanes 1 to 3). Addition of increasing amounts of purified Dps to the reaction mixture resulted in a progressive increase in the formation of Dps-DNA complexes that were retained in the wells (lanes 4 to 6), while the unbound plasmid DNA was damaged to the same degree as that in the reaction mixture lacking Dps. Removal of Dps prior to electrophoresis was used to visualize the degree of damage suffered by the plasmid DNA that was bound by Dps during the treatment. The results (lanes 13 to 15) show that the presence of increasing amounts of Dps resulted in a progressive decrease in plasmid nicking, to the point that the treated DNA was indistinguishable from the untreated control at the

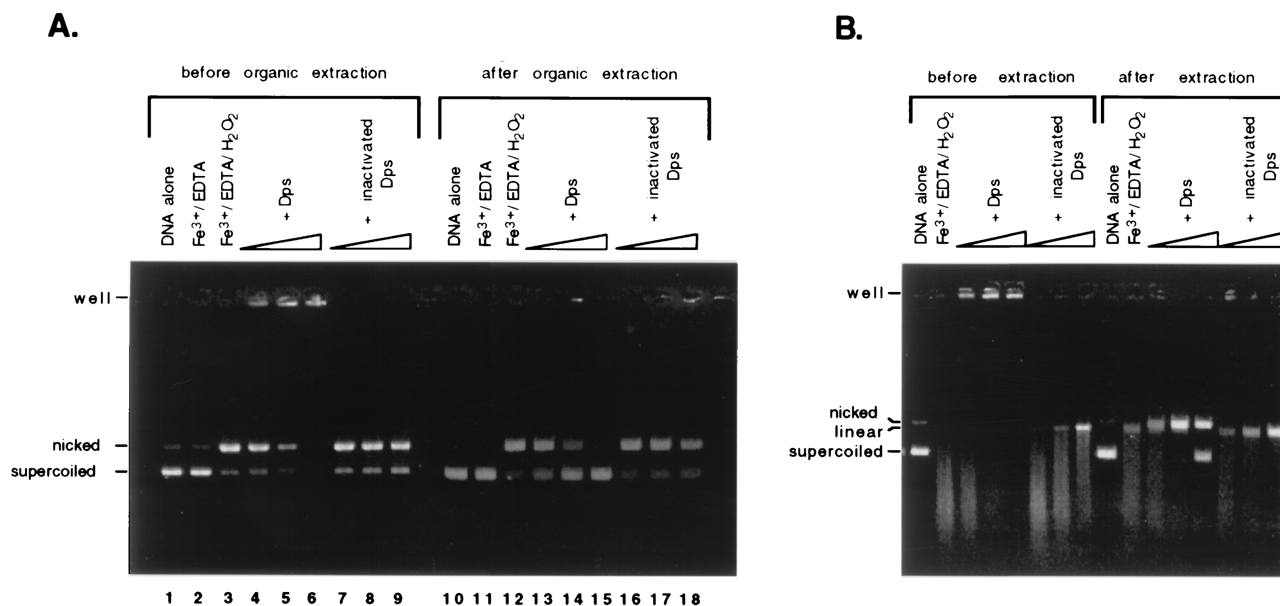

FIG. 2. Dps protects DNA from strand cleavage in vitro. The introduction of a single-strand break in the supercoiled plasmid DNA converts it to a nicked form that is retarded in the agarose gel. pUC19 plasmid DNA was used as the substrate. When indicated, $FeCl_3$ (0.1 mM), EDTA (0.4 mM), H_2O_2 (10 mM [A] or 100 mM [B]), and purified Dps (1.2, 3.0, or 6 μg) were added to the reaction mixtures. After incubation for 1 h at room temperature, the reactions were stopped by the addition of the iron chelator dipyridyl to 5 mM. Dps was removed from half of each reaction mixture, and supercoiled DNA and nicked plasmid DNA were resolved by electrophoresis in 0.8% agarose gels.

Dps concentration that allowed complete binding (lane 15). Heat-denatured Dps, which has lost its DNA-binding activity, had no effect in the degree of plasmid nicking. Dps also provided remarkable protection during treatment with 100 mM H_2O_2, a concentration that caused extensive degradation of naked DNA (Fig. 2B). These results demonstrate that binding of Dps to DNA directly results in efficient protection from single-strand nicks caused by oxidative damage.

The in vitro DNA damage system was also used to analyze the ability of Dps to protect DNA from base modification by H_2O_2. After H_2O_2 treatment and removal of Dps, the plasmid DNA was treated with the DNA repair enzyme Fpg which introduces strand nicks at abasic sites and sites of ruptured or oxidized guanine (8oxoG) (8, 9). The number of strand breaks introduced by Fpg is therefore a reflection of the base damage inflicted in the DNA by H_2O_2. The presence of saturating amounts of Dps during treatment resulted in levels of Fpg-sensitive sites as low as those of the untreated control (Fig. 3), indicating that Dps, in addition to protecting the DNA backbone from breakage, efficiently prevents the types of base damage recognized by Fpg.

Effect of Dps on mutagenesis. The ability of Dps to efficiently protect DNA from H_2O_2-induced base damage in vitro led us to examine the effect of Dps on base damage and mutagenesis in vivo. We used a set of strains containing *lacZ* mutations that allow rapid detection of each of the six possible base substitutions by measuring the appearance of $LacZ^+$ revertants (7). First, we studied the mutation frequency of wild-type and *dps*::*kan* derivatives of such strains after treatment with H_2O_2. The lack of Dps resulted in an approximately 10-fold increase in $G \cdot C \rightarrow T \cdot A$ mutations when stationary-phase cultures were treated with concentrations of H_2O_2 that did not affect a wild-type strain (Table 1). No detectable increase in mutation frequency was observed for any of the other possible base substitutions (data not shown). $G \cdot C \rightarrow T \cdot A$ mutations occur as a result of misincorporation of an A residue across from 8oxoG, one of the damaged bases recognized by Fpg (25). Therefore, these results indicate that Dps can also protect guanine residues from oxidation in vivo. Furthermore, they demonstrate that Dps plays an important role in decreasing mutagenesis resulting from oxidative damage during stationary phase, even in the presence of normal DNA repair systems. Protection appears to be specific for oxidative damage, because no difference in mutagenesis or survival was observed after treatment with the alkylating agent ethyl methanesulfonate (data not shown).

Finally, we analyzed qualitatively the effect of Dps on spontaneous mutagenesis with a papillation assay (26). In this assay, cells containing a mutation that restores the $LacZ^+$ phenotype give rise to blue papillae over a background of white colonies in minimal medium containing glucose, lactose, and X-Gal, a

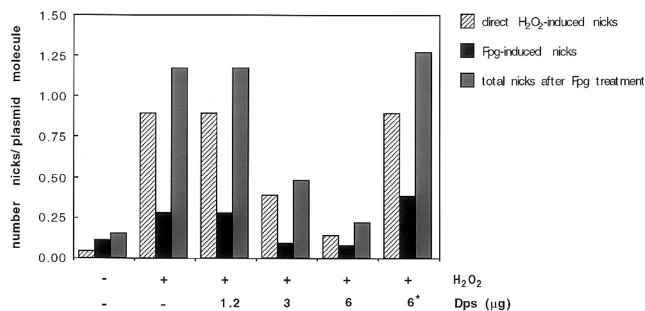

FIG. 3. Dps protects DNA from base modification in vitro. The average number of nicks per plasmid molecule introduced by H_2O_2 and/or by Fpg was measured after treatment with 10 mM H_2O_2 for 45 min in the presence or absence of Dps. *, heat-denatured Dps. Hatched bars, average number of nicks introduced directly by 10 mM H_2O_2; solid bars, average number of nicks introduced by Fpg treatment after Dps removal (indicative of abasic sites and oxidized guanines); shaded bars, total average number of nicks (indicative of strand breaks and base modification induced by treatment with 10 mM H_2O_2).

TABLE 1. Absence of Dps results in increased frequency of G · C→T · A mutations after treatment of stationary-phase cultures with 0.3 mM H_2O_2

Expt	No. of LacZ$^+$ colonies[a]			
	CC104 (G · C→T · A)		CC104 *dps*::*kan* (G · C→T · A)	
	$-H_2O_2$	$+H_2O_2$	$-H_2O_2$	$+H_2O_2$
1	0.2 (±0.4)	0.2 (±0.4)	0.7 (±0.9)	11.3 (±3.8)
2	2.0 (±0.8)	4.3 (±0.5)	2.0 (±1.0)	28.5 (±2.5)
3	2.5 (±0.5)	4.0 (±0.2)	2.0 (±0.0)	20.0 (±8.0)

[a] Values represent the average number of LacZ$^+$ colonies (indicative of G · C→T · A mutations) per 10^8 cells plated.

color indicator of β-galactosidase activity. The introduction of a *dps*::*kan* allele did not significantly alter the number of papillae in any of the six tester strains. The lack of effect of the *dps*::*kan* allele could be explained if the mutations that restore the LacZ$^+$ phenotype arise during growth when *dps* is not expressed. To circumvent this problem, we introduced the plasmid with the inducible *dps* gene into the tester strains. Expression of *dps* from the inducible plasmid significantly reduced the number of spontaneous A · T→T · A and G · C→T · A mutations (Fig. 4). Both mutations can result from oxidative damage: A · T→T · A is the most common base substitution induced by oxidizing mutagens (18), and as mentioned above, G · C→T · A occurs as a result of misincorporation of an A residue across from the oxidized form of G, 8oxoG. Dps expression from the inducible plasmid was also able to partially suppress the mutator phenotype of null alleles of *mutY* and *mutM* (*fpg*), which encode DNA glycosylases that decrease the mutagenic consequences of 8oxoG (Fig. 4) (25). These results demonstrate that under certain conditions, Dps can reduce the rate of some, but not all, spontaneous base substitutions, in particular those likely to be caused by endogenously generated oxidative agents.

DISCUSSION

All previously described mechanisms that allow growing bacteria to cope with the oxidative damage associated with aerobic life can be divided in two groups: those removing active oxygen species (such as catalases, peroxidases, and superoxide dismutases) and those repairing damaged cellular components (such as DNA repair enzymes). Previous work had shown that Dps is required for the resistance to oxidative stress characteristic of starved *E. coli* cells (1) and that it may play a similar

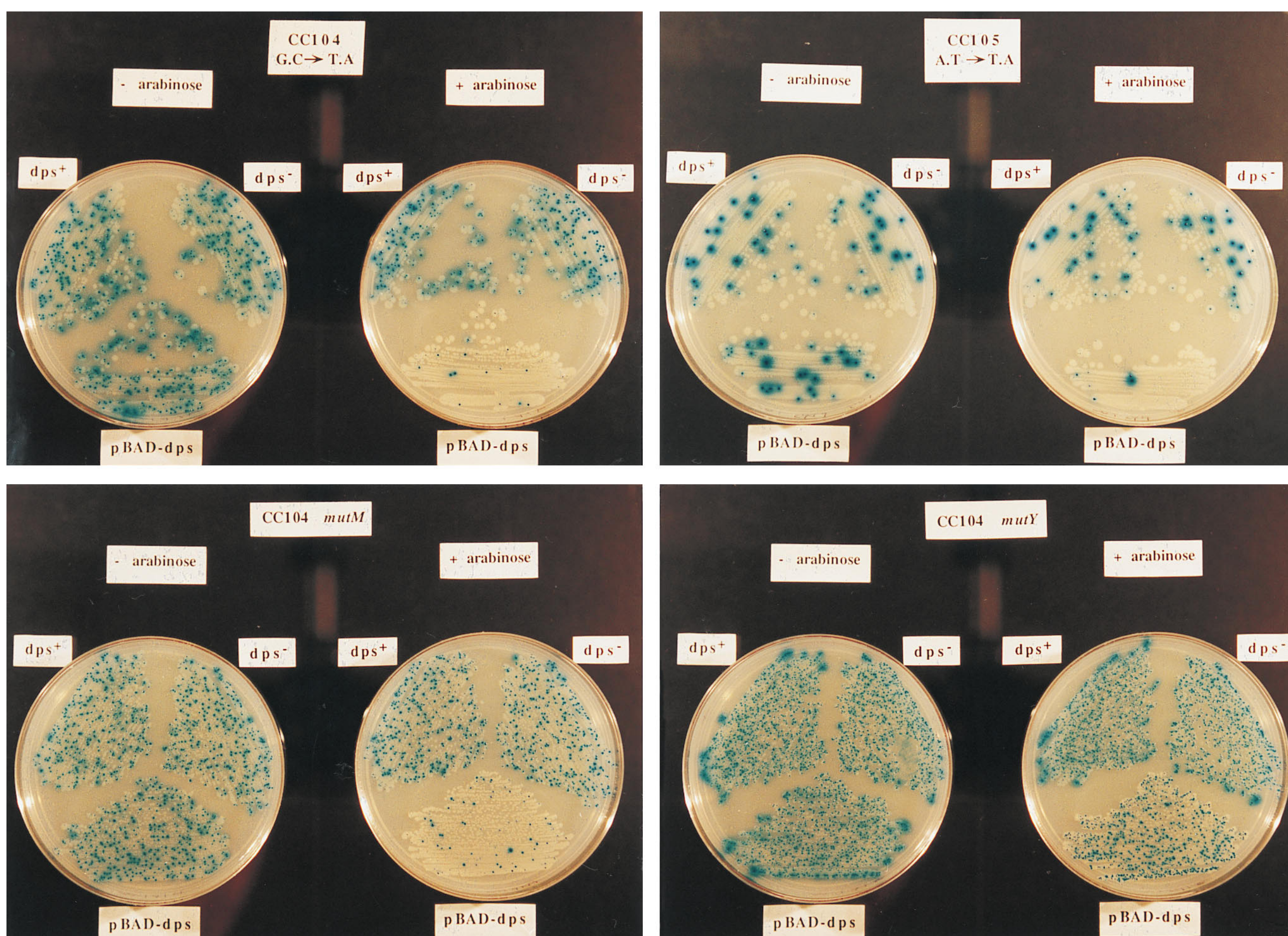

FIG. 4. Effect of Dps in spontaneous base substitutions determined with a papillation assay. Plates (minimal medium containing 0.2% glucose, 0.05% lactose, 40 μg of X-Gal per ml, with or without 0.1% arabinose as indicated) were inoculated from liquid cultures grown in minimal glucose medium for 24 h with the appropriate drugs. The appearance of blue papillae was monitored after 5 days of incubation at 37°C. A · T→T · A and G · C→T · A mutations were detected with Δ*ara* derivatives of CC105 and CC104, respectively, harboring the pBAD18-dps plasmid. dps$^+$, wild-type chromosomal *dps*; dps$^-$, *dps*::*kan* derivative; pBAD-dps, *dps*::*kan* derivative harboring pBAD18-dps. The vector alone (pBAD18) had no effect on the papillation frequency of any strain (data not shown).

role in metabolically active cells, since Dps is also expressed as part of the adaptive oxidative stress response in growing cells (2). Although we have not discounted a possible effect of Dps in the expression of genes encoding known oxidative defense functions, the work presented here demonstrates that Dps binding to DNA can directly prevent oxidative damage, particularly DNA strand breaks and certain types of base damage. Thus Dps represents a novel strategy for survival of growing cells during oxidative stress: direct protection of the damage target by an inducible dedicated protein. This mechanism resembles that of the *Bacillus* small acid-soluble proteins that protect DNA in dormant spores (29).

This new layer of protection conferred by Dps operates in conjunction with other mechanisms of coping with oxidative stress, including DNA repair. Under some circumstances (such as wild-type cells treated with low concentrations of peroxide), the efficiency of the DNA repair process is enough to afford almost complete survival, even in the absence of Dps. The relevance of Dps action, however, is apparent in DNA repair-deficient cells, in which the presence of Dps at the time of exposure to the damaging agent can improve survival by 30- to 200-fold. In addition, Dps is also critical for survival of DNA repair-proficient cells exposed to high doses of H_2O_2 both during growth and during starvation, where the extent of damage caused in the absence of the protective effects of Dps seems to overwhelm the repair systems, resulting in decreased survival and increased mutagenesis.

The ability of Dps to directly protect DNA from oxidative agents was clearly demonstrated in the purified in vitro system in which saturating amounts of Dps completely inhibited the formation of the strand breaks and characteristic base modifications caused by oxidative damage. Two lines of evidence indicate that the DNA protection observed in vitro is due to DNA binding. First, heat-inactivated Dps which is unable to bind to DNA failed to provide protection. Second, when the concentration of Dps is below saturation, only the bound DNA is protected, while the free DNA is damaged to the same extent as that treated in the absence of Dps. Therefore, we conclude that binding of Dps to the DNA is required to provide protection from oxidative damage in vitro.

Are the protective effects observed in vivo due directly to the binding of Dps to DNA? The fact that the molar ratios of Dps to DNA that gave protection in vitro were similar to those that conferred protection in vivo suggests that this is indeed the case. The ratio of Dps to DNA that elicits complete binding and maximum protection under our in vitro conditions is 1 molecule of Dps per 1.4 DNA bp. Assuming that Dps binds to chromosomal DNA in vivo with similar affinity, 2.7 million Dps molecules per cell would be required to provide maximum protection to a completely naked chromosome. This theoretical concentration is similar (only twofold higher) to that obtained by expressing Dps from an inducible promoter in the experiment shown in Fig. 1B, and therefore, it is likely that the decreased DNA damage observed in the DNA repair-deficient strain is a direct effect of binding of Dps to the DNA. The Dps concentration in an overnight culture of a wild-type strain (150,000 to 200,000 Dps molecules per cell) is not as high as that required to saturate the chromosome according to the rough stoichiometry derived from our in vitro system. However, other nonspecific DNA-binding proteins, such as Hns and Hu, although not known to protect DNA from oxidative damage, are present at high concentrations in vivo, and it is conceivable that Dps only needs to bind to exposed or particularly sensitive DNA regions to confer protection from oxidative damage. Indeed, concentrations of Dps similar to those found in overnight cultures of wild-type *E. coli* gave a two- to threefold increase in survival in the *recA* deletion strain during growth. The similarity in the patterns of protection conferred by Dps in vivo and in vitro (decrease in strand cleavage and oxidized guanosine residues) validates the use of the purified in vitro system as a model for Dps function within cells and strengthens the argument that the protection observed in vivo is indeed due to DNA binding.

The mechanism by which DNA binding by Dps elicits protection from oxidative agents is not clear. In addition to protecting the DNA backbone from cleavage, Dps is able to decrease the formation of 8oxoG, as indicated by the partial suppression of the mutator phenotypes of *mutM*(*fpg*) and *mutY* strains, as well as the decrease in Fpg-sensitive sites after oxidative treatment in vitro. These results indicate that Dps can protect groups in the major (DNA backbone) and minor (O8 of G) grooves of the DNA. Dps, however, had no effect on the rate of mutations caused by the alkylating agent ethyl methanesulfonate, which is known to alkylate the O6 of G, also present in the minor groove. Therefore, it is unlikely that the protective effect of Dps is due to mere shielding of DNA from small diffusible molecules, since that mechanism would have been expected to allow protection from alkylating agents as well. Instead, it is possible that Dps specifically prevents oxidative DNA damage and that it does so by interfering with some characteristic step of this process.

The DNA damage resulting from treatment with low concentrations of H_2O_2 (mode 1 killing) is mediated in part by a Fenton reaction in which $HO^{\bullet}$ radicals are formed by monovalent reduction of H_2O_2 by a reduced metal such as Fe(II) (13, 14). Because of its high reactivity, it is believed that the $HO^{\bullet}$ radicals that attack DNA are formed directly on the DNA surface by ferrous atoms complexed with the phosphodiester backbone rather than free in solution. Binding of Dps to the DNA could prevent the coordination of Fe(II) atoms with the DNA and hence the in situ formation of the reactive radicals. The effect of Dps on survival from mode 2 killing (Fig. 1) (1) suggests that DNA damage might be one of the sites of toxic lesions caused by high concentrations of H_2O_2. Indeed, DNA damage is known to occur in mode 2 killing, and it is believed to be caused by ferrous atoms tightly bound to the DNA bases and phosphodiester backbone (14, 15, 22). Thus, prevention of coordination of Fe(II) atoms with the DNA by Dps binding could explain protection from both mode 1 and mode 2 killing. Alternatively Dps could act by scavenging hydroxyl radicals in the vicinity of DNA. Such a mechanism has been proposed to explain the decreased sensitivity to oxidative DNA damage of chromatin (20).

Whatever the mechanism, Dps constitutes a novel type of defense against oxygen radicals in bacteria in that it efficiently prevents DNA base modification and strand cleavage while not interfering with normal DNA metabolism, as judged by the normal growth rates of strains in which *dps* expression during logarithmic growth resulted in the accumulation of more than a million Dps molecules per cell. This is in marked contrast to the small acid-soluble proteins (SASPs) of *Bacillus*, a family of nonspecific DNA-binding proteins that confer protection of DNA against oxidative agents, UV radiation, desiccation, and heat in the spore (29). These proteins lead to nucleoid condensation, a rapid cessation of DNA synthesis and loss of viability when expressed at high levels in growing *E. coli* cells (28), and are detrimental to normal spore outgrowth in *Bacillus* mutants that fail to degrade them during germination (27). Dps could also be involved in chromosome condensation during starvation in *E. coli*, since preliminary studies with electron microscopy indicate that in a *dps*::*kan* mutant, the DNA appears fully dispersed, in sharp contrast to the condensed, chro-

matin-like appearance of the nucleoid in a wild-type strain (25a).

Dps homologs have been identified in very diverse bacteria (1, 6, 23). The *Bacillus subtilis* homolog MgrA also forms stable protein-DNA complexes, and *mgrA* mutants are hypersensitive to H_2O_2, suggesting a role in protection from oxidative damage (6). In addition to being part of the inducible oxidative stress response, *mgrA* expression is metalloregulated, indicating that different bacteria have adapted to use the same defensive tool under different conditions. The cyanobacterial *Synechococcus dps* homolog *dpsA* is thought to be involved in protection from the oxidative damage associated with oxygenic photosynthesis (23). The DpsA protein has weak catalase activity, suggesting additional DNA protection by physical tether of a peroxide-consuming mechanism to the DNA. Recently, the *dps* homolog from *Salmonella typhimurium* has been shown to be upregulated in macrophages, suggesting that DNA protection by Dps might be important for survival and replication of this intracellular pathogen within macrophages (32). The widespread presence of Dps homologs among bacteria suggests that this novel defense mechanism may play a general role in survival of bacteria during oxidative stress both during active growth and under starvation conditions.

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