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Bimodal Protection of DNA by *Mycobacterium smegmatis* DNA-binding Protein from Stationary Phase Cells*

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Some members of the DNA-binding protein from stationary phase cells (Dps) family of proteins have been shown to play an important role in protecting microorganisms from oxidative or nutritional stress. Dps homologs have been identified in various bacteria such as *Escherichia coli*, *Bacillus subtilis*, and *Listeria innocua*. Recently we have reported the presence of a Dps homolog, Ms-Dps, in *Mycobacterium smegmatis*. Ms-Dps was found to have a nonspecific DNA binding ability. Here we have detected two stable oligomeric forms of Ms-Dps *in vitro*, a trimeric and a dodecameric form. Interestingly, the conversion of Dps from a trimeric to a dodecameric form takes place upon incubation at 37 °C for 12 h. These two oligomeric forms differ in their DNA binding properties. The dodecameric form is capable of DNA binding and forming large crystalline arrays with DNA, whereas the trimeric form cannot do so. However, even in the absence of DNA binding, the trimeric form has the capacity to protect the DNA against Fenton's-mediated damage. The protection is afforded by the ferroxidase activity of the trimer. However, the trimeric form cannot protect DNA from DNaseI attack, for which a direct physical shielding of DNA by the dodecamer is required. Thus we suggest that Ms-Dps provides a bimodal protection of DNA by its two different oligomeric forms.

Microorganisms have developed efficient mechanisms to adapt rapidly and to survive a variety of chemical and physical stress conditions (1). Generation of reactive oxygen species (ROS)[1] is one such stressful condition. ROS are potent cellular oxidizing agents that damage proteins, membrane lipids, and DNA (2–3). During aerobic growth, generation of ROS and of hydrogen peroxide (H_2O_2) is unavoidable. Reaction of H_2O_2 with free transition metals like ferrous iron can result in the formation of highly reactive hydroxyl radicals (OH^-) (4). To minimize damages through such ROS, microorganisms have evolved a number of protective ways that help in maintaining the biomolecules in native state. ROS scavenging enzymes such as superoxide dismutases, catalases, and peroxidases, oxidative damage repair enzymes (2, 3), and a nonspecific DNA binding and protecting protein, Dps, (**D**NA binding **p**rotein from **s**tationary phase cells) (5) are a few examples in this category. Almost all the bacteria when exposed to ROS exhibit an adaptive response by switching on the expression of genes coding for these proteins (6). Such strategies are all the more important for pathogenic bacteria because production of reactive oxygen species is a major killing mechanism adopted by many hosts. These schemes also become important during the growth of the organism in stationary phase or during nutrient limiting condition. Thus, the regulation of gene expression upon the induction of starvation and during the stationary phase has been an area of intense research.

In the stationary phase cultures of *Escherichia coli*, the existence of a novel protein Dps was discovered around a decade ago (5). It is a nonspecific DNA binding protein with a structure very similar to ferritins. Because of their structural homology they have been classified under the same superfamily in the SCOP data base (7). Despite being a DNA binding protein, Dps lacks any of the known DNA binding motifs. Even though the crystal structure of *E. coli* Dps has been solved (8), the exact mechanism with which it binds to DNA is not fully understood. Whatever the mechanism, Dps provides a novel way of binding to DNA. Highly crystalline and ordered assemblies of Dps-DNA complexes have been identified both *in vitro* (5) and *in vivo* (9).

Functionally, Dps has been shown to protect the cells against oxidative radicals generated by Fenton's reaction and also against various other DNA damaging agents (10, 11). Because the predominance of such radicals becomes greater during the stationary phase of the bacterial growth cycle, Dps expression has also been shown to be induced in stationary phase and upon nutrient starvation (12). Besides its protective role, Dps also has a global regulatory role in controlling gene expression during prolonged starvation (5).

Dps-like proteins have been identified in distantly related bacteria such as *E. coli*, *Bacillus subtilis*, *Listeria innocua*, and *Synechococcus sp.* (5, 13–15). We have earlier reported the presence of a Dps homolog, Ms-Dps, in *Mycobacterium smegmatis* (16). In this study we throw further light on the DNA binding ability of Ms-Dps. We show that only the dodecameric species of Ms-Dps is capable of complex formation with DNA. We also report a bimodal type of protection of DNA by Ms-Dps, one without physical interaction with DNA and the other by direct binding to DNA. Last, the importance of Ms-Dps as an evolutionary link between ferritins and Dps is also discussed.

MATERIALS AND METHODS

Bacterial Strains and Plasmids—The DH5α strain of *E. coli* was used for cloning purposes and the BL21 DE3 (pLys) strain was used for protein purification. pET-*dps* is a pET21b derivative containing the *M. smegmatis dps* gene (16). Plasmid pUC19 (17) was used for *in vitro* DNA binding and DNA damage assays.

Ms-Dps Purification—Ms-Dps purification was performed as described earlier (16). In brief, *E. coli* strain BL21 DE3 (pLys) was transformed with pET-*dps*. These cells were grown at 37 °C in Luria Bertani

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[1] The abbreviations used are: ROS, reactive oxygen species; Dps, DNA-binding protein from stationary phase cells; BSA, bovine serum albumin.

medium to an A_{600} of 0.5 and then induced with 1 mM isopropyl-1-thio-β-D-galactopyranoside. Single-step purification was performed using the Qiagen Ni-NTA affinity matrix according to the manufacturer's instructions. After checking the purity of the protein on a 12% SDS-polyacrylamide gel, protein was dialyzed against 50 mM Tris-HCl (pH 7.9), 50 mM NaCl overnight and used for further analysis. Protein concentration was determined by the method of Lowry *et al.* (18). For the formation of the higher oligomer, protein at a concentration of 1 mg/ml was incubated at 37 °C for 12 h in 50 mM Tris-HCl (pH 7.9), 50 mM NaCl.

Native Polyacrylamide Gel Electrophoresis (PAGE)—A 10% native polyacrylamide gel was prepared using discontinuous buffer system according to the method of Laemmli (19). The gel recipe was the same, excluding the presence of sodium dodecyl sulfate (SDS). Samples were loaded with a dye (10% glycerol, 0.002% bromphenol blue), and the electrophoresis was carried out at a constant current of 15 mA. Gels were then stained with Coomassie Brilliant Blue R250. Bovine serum albumin (BSA) and horse spleen ferritin were used as markers.

Gel Retardation Assays—pUC19 DNA was mixed with Ms-Dps at a DNA:protein molar ratio of $1:10^2$ or $1:10^3$ in 50 mM Tris-HCl (pH 7.9), 50 mM NaCl. The incubation was carried out at 30 °C for 30 min. Wherever indicated, Ms-Dps was incubated at 37 °C for 12 h prior to the DNA binding. The complex was then resolved on a 1% agarose gel in 0.5% TBE buffer consisting of 89 mM Tris borate (pH 8.0) and 1 mM EDTA. The electrophoresis was carried out at a constant voltage of 50 V. The unbound free and protein-bound DNA was then detected by ethidium bromide staining.

Staining of Iron-binding Proteins—Purified Ms-Dps was first incubated at 37 °C for 6 h to allow partial oligomerization. 100 μg of this preparation was then incubated with 1 mM ferrous sulfate in 50 mM Tris-HCl (pH 7.9), 50 mM NaCl for 1 h at room temperature. The products were resolved on a 10% native PAGE. The gel was then stained with potassium ferricyanide solution, 100 mM K_3 ($Fe(CN)_6$) in 50 mM Tris-HCl, pH 7.5, 100 mM NaCl, for 10 min in the dark and destained with 10% trichloroacetic acid/methanol solution. After taking an image of the stained gel, it was subjected to Coomassie Blue staining using standard techniques. Horse spleen ferritin and BSA were used as positive and negative controls, respectively.

In Vitro DNA Damage Assay—pUC19 DNA was used to assess the ability of Ms-Dps to protect DNA from oxidative damage. In a total reaction volume of 20 μl containing 50 mM Tris-HCl (pH 7.9), 50 mM NaCl, Ms-Dps was allowed to interact with pUC19 DNA at 30 °C for 30 min. Then to assay for oxidative damage, $FeSO_4$ was added at a concentration of 25 or 50 μM and incubated for 5 min, followed by further addition of 5 mM H_2O_2 and incubation for another 5 min. To check for DNA damage induced by DNaseI, $MgCl_2$ was added to a final concentration of 40 mM followed by treatment with 1 unit of DNaseI (1 unit is defined as the amount of enzyme causing an increase in absorbance at 260 nm by 0.001 per minute) for 5 min at room temperature. The reactions were stopped with 50 mM EDTA. The products of the reactions were resolved on a 1% agarose gel in 0.5% TBE buffer and stained with ethidium bromide. Wherever indicated, Ms-Dps was incubated at 37 °C for 12 h prior to the DNA damage assays.

Electron Microscopic Analysis—Ms-Dps after incubation at 37 °C for 12 h alone and the Ms-Dps-DNA complexes were placed on copper grids. After 2 min of absorption at room temperature, the samples were negatively stained with uranyl acetate for 5 min. Specimens were examined in a Jeol 100 CxII electron microscope at 80 kV. The photographs were taken at ×65,000 magnification. The diameters of the rings were measured from electron microscopic negatives with the aid of a Wild-Heerbrugg MPS12 zoom stereomicroscope. About 40 numbers of differentially placed rings were measured.

Spectroscopic Analysis of Iron Incorporation—The iron oxidation and incorporation kinetics were followed spectrophotometrically at 305 nm on a Jasco V-530 spectrophotometer. The solution of Ms-Dps (6 μM) was initially scanned for 300 s; subsequently, freshly prepared 10 μM ferrous sulfate was added and scanned again for 300 s. As a control the rate of Fe^{2+} auto-oxidation was measured in parallel.

RESULTS

Temperature-induced Change in the Oligomeric Status of Ms-Dps—We have recently reported the identification, purification, and DNA binding ability of Ms-Dps (16). However, in the gel retardation assay, even at a large excess of Ms-Dps over DNA, generation of higher molecular weight species was not observed, unlike in the *E. coli* DNA-Dps complex (5). Because all the Dps family members are known to form multimers and different oligomeric forms might have different DNA binding abilities, it was thought that the oligomeric status of Ms-Dps should first be identified. For this purpose, protein was subjected to native PAGE analysis. The recombinant protein when purified under native conditions shows the presence of two major species on a 10% native PAGE (Fig. 1, *lanes 1–3*), one lower oligomeric form that runs near BSA (MW-63,000) and another higher oligomeric form that runs near ferritin (MW-450,000). Both BSA and ferritin are globular, acidic proteins with isoelectric points similar to that of Ms-Dps (BSA-4.8, ferritin-4.5, Ms-Dps-5.4). Thus they can be used as markers in native PAGE analysis. The mobility of the lower oligomer on the gel indicates that it could be a trimeric species (MW of Ms-Dps monomer-216,000). As is evident in Fig. 1, *lane 3*, the trimeric form is predominant over the higher oligomeric form. This preparation of protein was then checked for its DNA binding ability. As shown in Fig. 2, the protein did not retard the pUC19 DNA on a 1% agarose gel even at $1:10^3$ DNA: protein molar ratio.

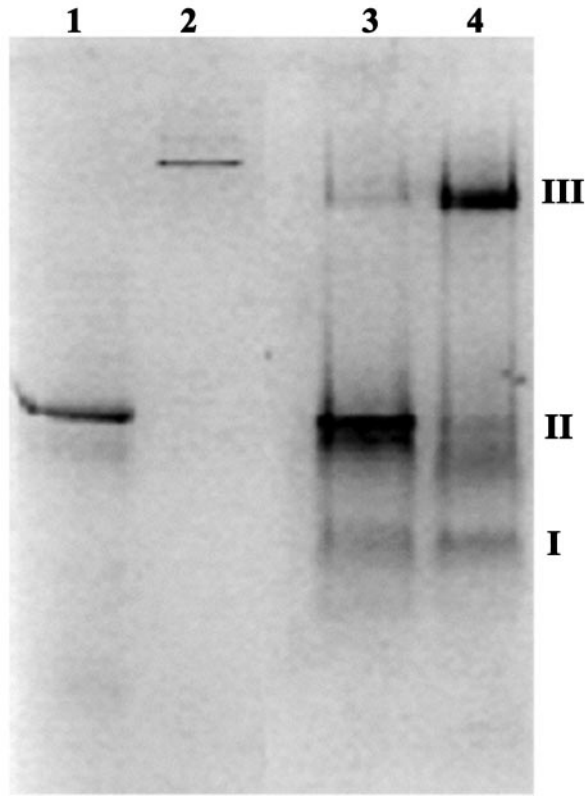

FIG. 1. **Temperature-induced change in oligomeric status of Ms-Dps.** BSA (MW 63, pI 4.8), *lane 1*; horse spleen ferritin (MW 450, pI 4.5), *lane 2*; Ms-Dps after purification at 4 °C, *lane 3*; Ms-Dps after 37 °C-incubation for 12 h, *lane 4*. *Species I*, probable monomer. *Species II*, lower oligomer. *Species III*, higher oligomer.

When this protein preparation, the purification of which had been carried out at 4 °C, was incubated at 37 °C for 12 h and checked on 10% native PAGE, the higher oligomeric form became predominant over the trimeric form (Fig. 1, *lane 4*). The switch in relative ratio of trimer to higher oligomer upon 37 °C incubation was consistently observed with different protein preparations. The formation of the higher oligomer was found to be an irreversible process, *i.e.* when incubated back at 4 °C or at room temperature the higher oligomer did not dissociate into the trimer. It is possible that the formation of the higher oligomer is an energy-requiring process. The probability of temperature-induced structural changes in the monomers, which favor higher oligomer formation, also cannot be ruled

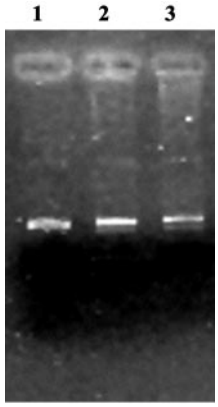

FIG. 2. **Absence of binding of lower oligomeric form of Ms-Dps to DNA.** Free pUC19 DNA (*lane 1*). Incubated with Ms-Dps at 30 °C for 30 min in 50 mM Tris-HCl (pH 7.9), 50 mM NaCl at DNA:protein molar ratio of $1:10^2$ (*lane 2*) and $1:10^3$ (*lane 3*).

out. However, the secondary structures of Ms-Dps both at 4 °C and after incubation at 37 °C were the same, as was seen by circular dichroism studies (data not shown).

A gel retardation assay was then performed after the incubation of Ms-Dps at 37 °C for 12 h. As shown in Fig. 3, this treatment enabled the protein to form a complex with pUC19 DNA, and the complex did not enter 1% agarose gel. This property is similar to that observed with *E. coli* Dps (5, 10). It should be mentioned here that some batches of the purified Ms-Dps showed only a slight mobility shift of DNA (16), but upon incubation at 37 °C, the high molecular weight complex with DNA forms instantaneously. Upon correlating the native PAGE analysis with the gel retardation assays, it is apparent that the presence of the higher oligomeric form of the protein is mandatory for complex formation with DNA.

Complex Network Formation of Ms-Dps with DNA—Electron microscopic studies were then performed on the 37 °C-incubated Ms-Dps and Ms-Dps-DNA complexes. Preparations of the protein alone when visualized under electron microscope showed discrete ring-like structures of ~9 nm diameter (Fig. 4*a*). This size correlates well with the diameter of the modeled Ms-Dps dodecameric molecule, which is 8.8 nm (16) and also with that of the crystal structure of *E. coli* Dps, which is 9 nm (8). When the same protein was incubated with DNA and then visualized in the electron microscope, large and highly ordered two-dimensional arrays of the rings were seen (Fig. 4*b*). This honeycomb-like arrangement is very similar to that observed with *E. coli* Dps-DNA complexes (5).

Electron microscopic analysis suggests that the higher oligomer seen on the native gels is a dodecamer. Various different multimeric forms of *E. coli* Dps were detected earlier (8), but it is not known which one of these is the actual DNA binding species. Our results presented here indicate that it is the dodecameric form of Ms-Dps, which is capable of forming high molecular weight complexes with DNA. Other lower oligomeric forms and the monomers do not have this ability.

Iron-binding Ability of Ms-Dps—Some Dps family proteins, like Dps of *E. coli*, the ferritin of *L. innocua*, and Dpr from *Streptococcus mutans* have been shown to bind iron (5, 14, 20). Therefore, in this study the iron-binding ability of Ms-Dps was examined. First the oligomerization was allowed to proceed by incubating purified Ms-Dps at 37 °C. The incubation was carried out only for 6 h so as to attain a population of both the oligomeric forms in the reaction mixture. Both oligomeric forms were then allowed to incorporate iron by incubating with 1 mM ferrous sulfate. The two forms were then separated on a native PAGE. Upon staining with $K_3(Fe(CN)_6$, as is seen in Fig. 5*A*,

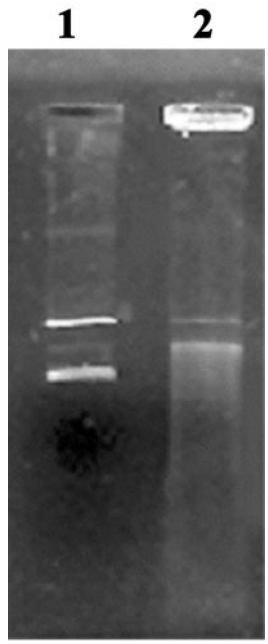

FIG. 3. **Binding of higher oligomeric form of Ms-Dps to DNA.** Ms-Dps in 50 mM Tris-HCl (pH 7.9), 50 mM NaCl at 1 mg/ml concentration was incubated at 37 °C for 12 h prior to DNA binding assay. Free pUC19 DNA (*lane 1*). Ms-Dps DNA complex at $1:10^3$ DNA: protein molar ratio (*lane 2*).

the higher oligomeric form was stained, along with ferritin which was used as a positive control. However, no band was visible that corresponded to the lower oligomer. The same gel when stained with Coomassie Blue showed the presence of the lower oligomer as well as of BSA (Fig. 5*B*).

The following is a structurally interesting observation. The dodecameric structure of modeled Ms-Dps (16) has a hollow core in the center in which ferrous ions can be incorporated. The formation of a trimer, on the other hand, would give rise to an open structure with no such hollow core (Fig. 6). The trimeric species, therefore, offers no place for iron incorporation to occur. The structures of the trimeric and dodecameric forms of *E. coli* Dps as adopted from Ref. 8 are shown in Fig. 6.

Recently it has been shown that in *E. coli* Dps, iron oxidation and hydrolysis can lead to incorporation of about 500 ferric ions inside the dodecameric protein shell (21). Our experiments here suggest that the dodecameric Ms-Dps is also capable of incorporating iron ions inside its protein shell, whereas the probable trimeric species cannot accumulate iron because of structural constraints.

Functional Aspects of Ms-Dps—One important function of Dps *in vivo* is to protect the DNA from oxidative radicals. To investigate whether Ms-Dps also has this protective ability, an *in vitro* DNA damage assay was performed. H_2O_2 in the presence of ferrous ions generates $OH^{\cdot}$ radicals through the Fenton reaction: $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^{\cdot}$.

The $OH^{\cdot}$ radicals thus generated have a DNA-nicking ability. Various concentrations of ferrous ions and H_2O_2 were used to bring about DNA damage. As seen in Fig. 7, in the presence of 25 μM $FeSO_4$ and 5 mM H_2O_2 all the supercoiled pUC19 DNA was nicked, resulting in relaxed DNA, whereas in the presence of Ms-Dps, DNA was protected against this nicking. At 50 μM ferrous sulfate and 5 mM H_2O_2 the DNA was totally degraded, whereas it remained intact in the presence of Ms-Dps. This experiment shows that Ms-Dps has the ability to protect DNA against Fenton's-mediated damage.

The protein used in this assay had not undergone 37 °C incubation. Because trimeric Ms-Dps does not bind DNA, it was

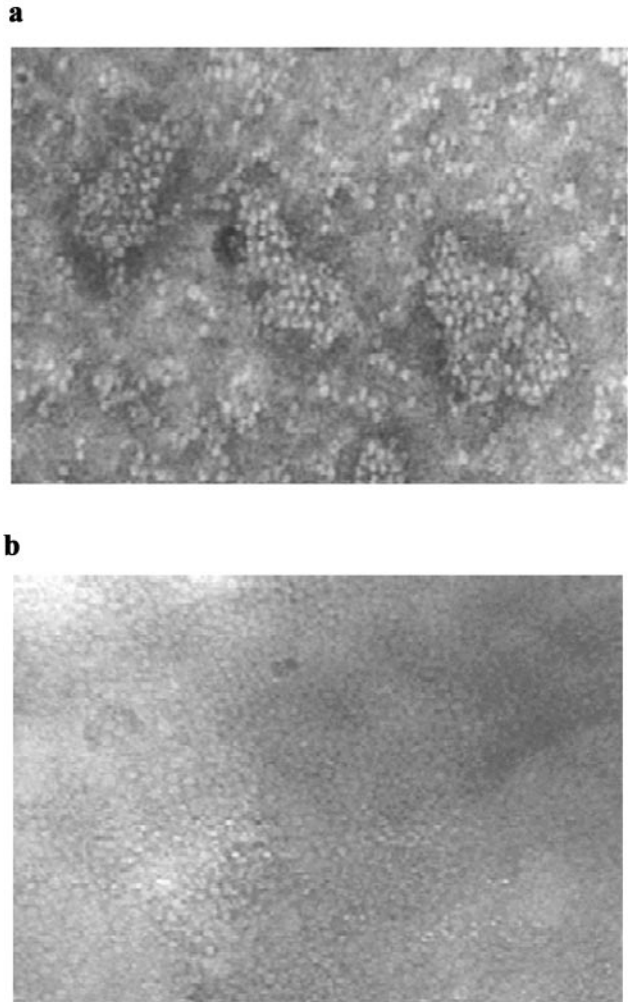

FIG. 4. **Electron microscopic analysis of Ms-Dps and Ms-Dps-DNA complexes.** *a* and *b* are at the same magnification. *a*, Ms-Dps alone. *b*, Ms-Dps-DNA complex.

quite interesting to note its DNA protection ability from the free radical onslaught. As expected, Ms-Dps after incubation at 37 °C also showed DNA protection in the same assay in the presence of $FeSO_4$ and H_2O_2 (Fig. 8).

These observations point toward an important characteristic of the protein. Ms-Dps seems to provide a bimodal type of protection to DNA. When the protein is predominantly in trimeric form, it does not bind DNA. Even in the absence of direct physical interaction with the DNA, the trimeric form is capable of protecting the DNA from Fenton's-mediated damage. Under the conditions where it forms the dodecamer, it generates a complex network with the DNA and thus protects it from damage. This type of protection might also be important against various other DNA-damaging agents such as nucleases, alkylating agents, and chemical mutagens. The capability of these two oligomeric forms to protect DNA against DNaseI was then tested. As shown in Fig. 9, DNA incubated with the trimeric form of Ms-Dps was totally degraded by DNaseI, whereas upon incubation with the dodecameric form, DNA remained intact. It can be inferred from this experiment that because the trimeric form does not bind DNA, DNA was free to be digested by the endonuclease DNaseI. Direct physical interaction of Ms-Dps with DNA is required to protect it from an enzyme like DNaseI, which can be afforded only by the dodecameric form. Thus the mechanism of protection provided by the two oligomeric forms of the same protein is not the same. The higher oligomer protects by physically shielding the DNA, whereas the lower oligomer protects without even directly interacting with DNA.

The latter mode of protection can be explained mechanistically if the protein has an iron-chelating activity. Although we have not seen any iron incorporation ability in the trimeric species (Fig. 5), it does not rule out the possibility of the trimer having iron-binding and ferroxidase activity. As has been reported recently (22), *E. coli* Dps has a ferroxidase activity, *i.e.* conversion of Fe^{2+} to Fe^{3+} using H_2O_2 as an oxidant. The protein has been shown to form coordinate complexes with Fe^{2+}, which is then oxidized to Fe^{3+} using H_2O_2 via a mechanism that does not allow Fenton's reaction to proceed. The generation of $OH^{\cdot}$ radicals is thereby inhibited. We thus speculated that the lower oligomeric form of Ms-Dps could also be protecting the DNA through such a ferroxidase activity. To check this hypothesis spectral analysis at 305 nm was employed, because Fe^{3+} species absorbs at 305 nm where Fe^{2+} does not. As shown in Fig. 10*A*, the buffer alone did not absorb at 305 nm. However, upon addition of $FeSO_4$, the absorbance gradually increased with time, which implies that Fe^{2+} was converted to Fe^{3+} by utilizing molecular oxygen of air. Because the spectrum was taken only until 300 s, all of the ferrous ions did not get oxidized to ferric ions in this short span of time, and thus the saturation was not reached. In the presence of protein, when $FeSO_4$ was added there was a sudden exponential increase in the absorbance, which later reached saturation (Fig. 10*B*). The presence of protein allowed the conversion of total ferrous ions to ferric ions within 300 s. This clearly shows that the trimeric Ms-Dps has a ferroxidase center that can rapidly oxidize Fe^{2+} to Fe^{3+} using molecular oxygen of air. Addition of H_2O_2 after 300 s did not bring about any further change in the absorbance (data not shown).

We would like to speculate here that the trimeric form of Ms-Dps is capable of protecting DNA without physically interacting with it, because of its ability to chelate out Fe^{2+} ions in the vicinity of DNA and then oxidize them to Fe^{3+} using molecular oxygen. This observation is in contrast to that of a recent report (22), in which the authors found that the *E. coli* Dps cannot utilize oxygen to convert Fe^{2+} to Fe^{3+} effectively. H_2O_2 was shown to be a better oxidant than oxygen. However, this property is unlike those of ferritins, because ferritins utilize oxygen as an oxidant. Therefore it appears that Ms-Dps is exhibiting ferroxidase property similar to that of ferritins and not like that of *E. coli* Dps. This protein is thus a unique member of the Dps family with a DNA binding ability like that of Dps and a ferroxidase activity like that of ferritins. One of the members of the family, Dps of *L. innocua*, has been identified as a true dodecameric ferritin functioning in iron storage (14), but this protein does not have DNA binding ability. Recently Dlp1 and Dlp2, the two Dps-like proteins from *B. anthracis*, have been designated as mini-ferritins (23) because they are ferritins that are dodecamers rather than the usual 24-mers. They also do not bind DNA. Although Dps A of *Synechococcus sp.* is a DNA-binding hemoprotein possessing a weak catalase activity, it is not known to have a ferroxidase activity (15). To date, no other single member of this family is known to possess both DNA-binding and ferritin-like ferroxidase activities. As had been discussed elsewhere (15), Dps proteins might have evolved as metal-binding proteins that later acquired DNA binding ability. The Ms-Dps, thus, could be a link between the two extremes, having a DNA binding property while still retaining the ferroxidase activity of ferritins.

DISCUSSION

During the evolution of life, the appearance of atmospheric oxygen offered the opportunity to utilize molecular oxygen as the oxidant in respiration. This provided energetic advantages

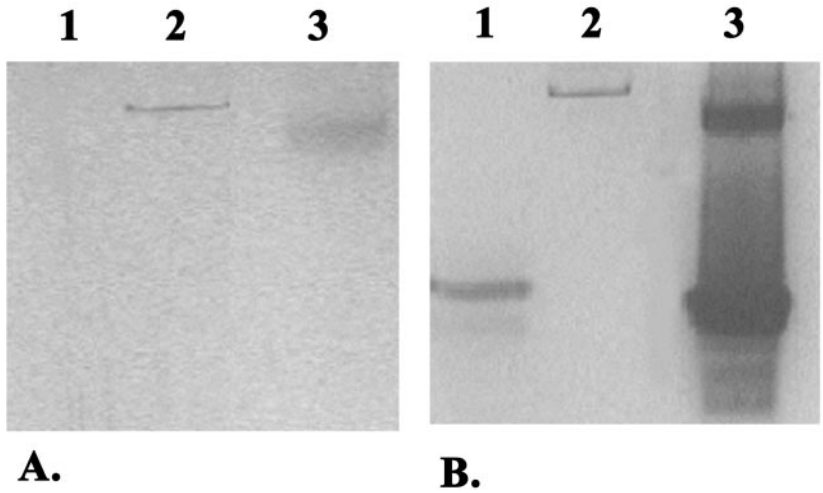

FIG. 5. **Iron binding ability of Ms-Dps.** BSA (*lane 1*), horse spleen ferritin (*lane 2*), and MS-Dps (*lane 3*) were resolved on a 10% native PAGE and stained with potassium ferricyanide (*A*) and then with Coomassie Brilliant Blue R250 (*B*).

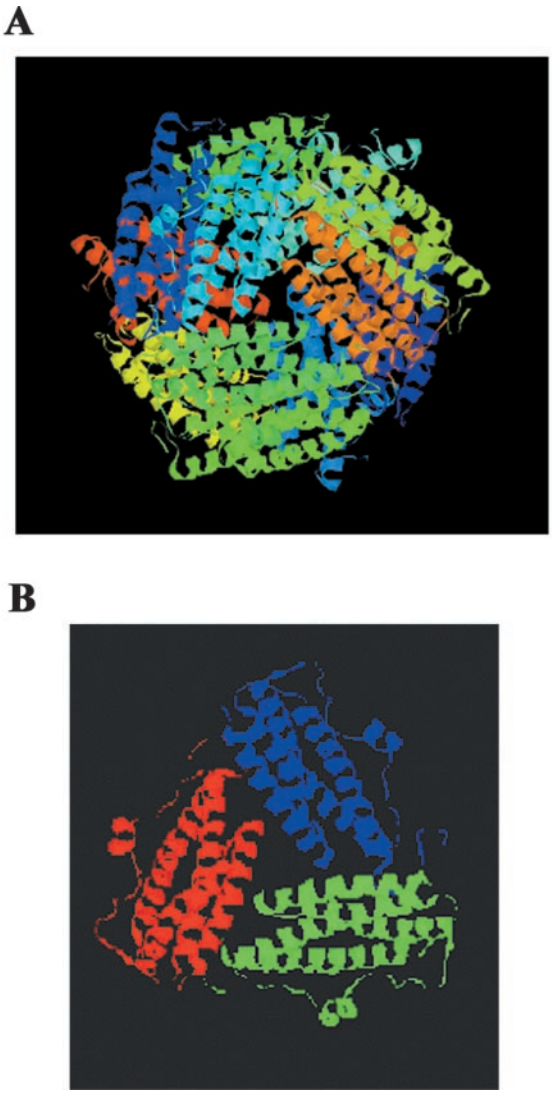

FIG. 6. **Structural comparison between *E. coli* Dps dodecamer and trimer (adopted from Ref. 8).** *A*, dodecameric molecule showing the hollow core. *B*, open trimeric structure.

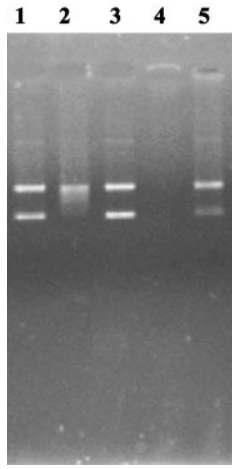

FIG. 7. **The protection of DNA from H_2O_2-mediated damage by trimeric Ms-Dps.** pUC19 DNA alone (*lane 1*): the *lower* band is the supercoiled form and the *upper* band is the relaxed DNA. pUC19 DNA treated with 25 μM $FeSO_4$ (*lane 2*) or 50 μM $FeSO_4$ (*lane 4*) for 5 min followed by 5 mM H_2O_2 for 5 min. pUC19 DNA incubated with the trimeric form of Ms-Dps (DNA: protein molar ratio 1:10^3) before treatment with 25 μM $FeSO_4$ (*lane 3*) or 50 μM $FeSO_4$ (*lane 5*) for 5 min followed by 5 mM H_2O_2 for 5 min.

over fermentation and respiratory pathways, which rely on other oxidants. However, the presence of intracellular oxygen also allowed unavoidable production of reactive oxygen species, which damage critical biomolecules. In most cases toxicity is exerted because of their direct damaging effects on DNA. A number of preventive mechanisms evolved since then to take care of such ROS-mediated toxicity.

One important question that has been quite frequently addressed is the protection of DNA from ROS in a bacterial cell under stationary phase or under some kind of nutritional stress. Because of constraints of resources under these conditions, many energetic expensive mechanisms of DNA protection cannot be employed. This question attains further importance in mycobacterial species because the latent pathogenic mycobacteria can survive within the host for a very long time devoid of all necessary nutrients and later can resume growth at an appropriate moment (24). Naturally, the organism should be able to adopt efficient mechanisms to protect its genetic material under such nutritionally stressful conditions. Recently we have analyzed the proteome of *M. smegmatis* under carbon starvation with the aim of identifying some such mechanisms (16). Although *M. smegmatis* is a non-pathogenic species, we thought it would be worthwhile to identify the proteins that are overexpressed under carbon starvation, because then attempts could be made to correlate the expression of such proteins in its pathogenic counterpart. Comparatively faster growth kinetics of *M. smegmatis* under carbon starvation (25) and the known genome sequence of *Mycobacterium tuberculo-*

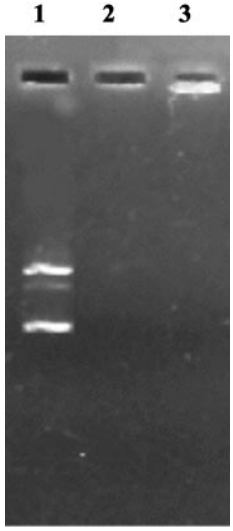

FIG. 8. **The protection of DNA from H_2O_2-mediated damage by dodecameric Ms-Dps.** pUC19 DNA alone (*lane 1*), treated with 50 μM $FeSO_4$ for 5 min followed by 5 mM H_2O_2 for 5 min (*lane 2*), pUC19 DNA first incubated with dodecamer at 30 °C for 30 min in 50 mM Tris-HCl (pH 7.9), 50 mM NaCl (DNA:protein molar ratio 1:10^3) followed by 50 μM $FeSO_4$ for 5 min and 5 mM H_2O_2 for 5 min (*lane 3*). The complex of DNA and Ms-Dps can be seen at the well.

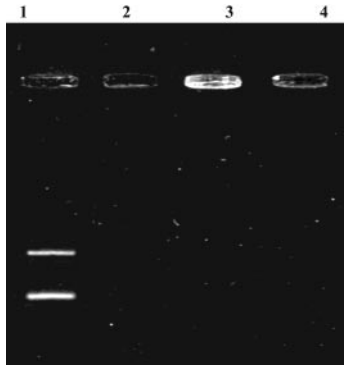

FIG. 9. **The digestion of DNA by DNaseI in the presence of lower and higher oligomers of Ms-Dps.** pUC19 DNA untreated (*lane 1*) and treated with 1U DNaseI for 5 min (*lane 2*). Prior to the DNaseI treatment, DNA was either incubated with the dodecameric form (*lane 3*) or the trimeric form (*lane 4*) of Ms-Dps.

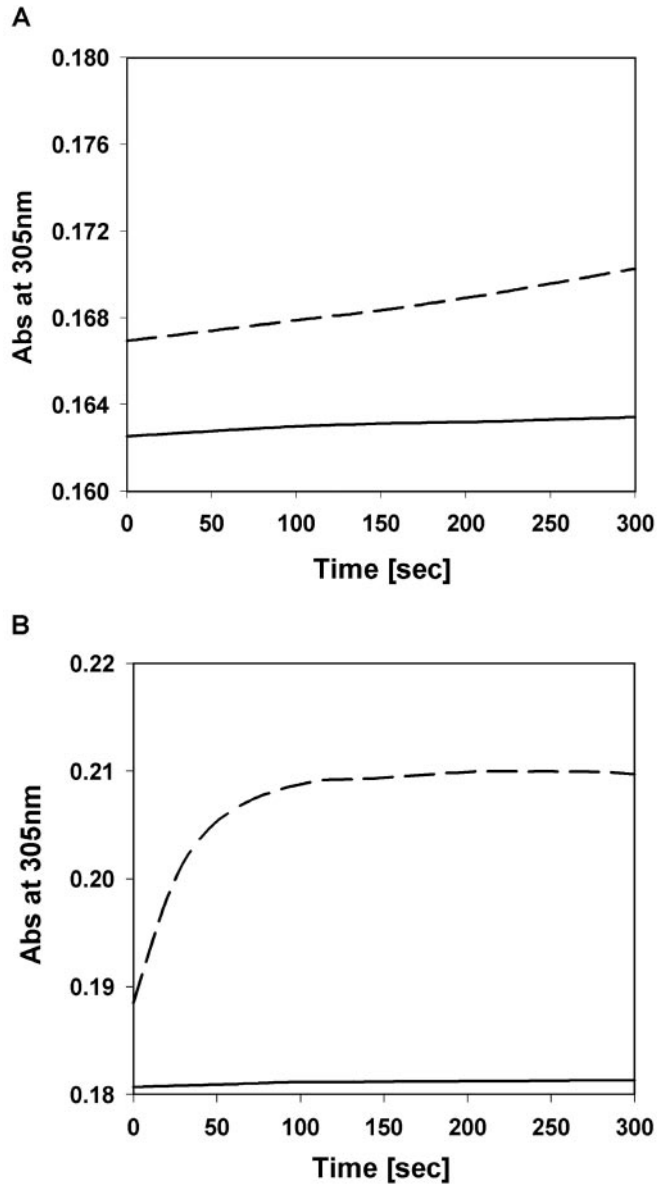

FIG. 10. **Ferroxidase activity of Ms-Dps.** *A*, absorbance of buffer alone (*solid line*) and after addition of 10 μM $FeSO_4$ (*dashed line*). *B*, absorbance of 6 μM trimeric Ms-Dps alone (*solid line*) and after addition of 10 μM $FeSO_4$ (*dashed line*).

sis (26) help in such an endeavor. Through such an exercise we have identified a Dps-like protein in *M. smegmatis* (16).

Although the role of Dps has been worked out in *E. coli* and some other organisms as mentioned earlier, its presence was not reported in mycobacteria before. However, the most interesting aspect was the absence of a sequence homolog of Dps in the *M. tuberculosis* genome, naturally raising questions regarding its ubiquitous function in protecting DNA under starvation conditions. As was mentioned earlier, ferritins and Dps are members of the same superfamily of proteins and are known to have evolved as divergent homologs from a common ancestor (15). However, the functional complementation of these proteins is not reported in literature. Still it is tempting to speculate here that one of the ferritins of *M. tuberculosis* might be performing the function performed by Dps-like proteins in other mycobacteria, such as *M. smegmatis* and *Mycobacterium avium*.

Our results presented in this manuscript suggest that Ms-Dps has a bimodal way of protecting DNA under free radical onslaught. Dps has earlier been shown to cause considerable compaction of DNA upon binding (8). Such a nonspecific complex network formation with DNA can make the DNA inaccessible to polymerases and other important DNA-modifying enzymes during the growth of the bacteria. At the same time, protection of DNA against oxidative stress also has to be taken care of. Our findings here show that Ms-Dps has a unique capability of protecting the DNA from such oxidative damage without physically interacting with it. This protection appears to be carried out by the ability of Ms-Dps to chelate out Fe^{2+} ions in the vicinity of DNA and to oxidize them to Fe^{3+} using molecular oxygen. This reaction bypasses the Fenton's reaction by which DNA-damaging $OH^{\cdot}$ radicals are generated. Thus by oxidizing Fe^{2+} to Fe^{3+}, Ms-Dps is capable of protecting the most important molecule of the bacterial cell in critical situations.

The temperature incubation step involved in the conversion of Ms-Dps from non-DNA binding to DNA binding form is interesting. This indicates that the trimeric species, the non-DNA binding form of Ms-Dps, is more stable and naturally occurring in *M. smegmatis*. It needs to take care of only the oxidative radicals generated during normal growth. Under

stress conditions such as entry into stationary phase or nutrient deprivation, where other DNA damaging agents also come into the picture, oligomerization of Dps into a dodecamer becomes a necessity to provide more efficient DNA protection by physically shielding the DNA. It should be mentioned here that for protein purification we have grown the cells at 37 °C, and then all the further purification steps were performed at 4 °C. The trimeric form of Ms-Dps thus obtained could be irreversibly converted to dodecameric form upon incubation at 37 °C, and this is intriguing. We have no simple answer to this apparent discrepancy. It appears that *in vivo* Ms-Dps maintains equilibrium between trimer and dodecamer as a function of growth or by the participation of other factors. Perhaps this conversion is not irreversible as we have noticed *in vitro*; we are currently pursuing a detailed analysis of the same.

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