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Overexpression and Characterization of an Iron Storage and DNA-Binding Dps Protein from *Trichodesmium erythraeum*

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Although the role of iron in marine productivity has received a great deal of attention, no iron storage protein has been isolated from a marine microorganism previously. We describe an Fe-binding protein belonging to the Dps family (DNA binding protein from starved cells) in the N_2-fixing marine cyanobacterium *Trichodesmium erythraeum*. A *dps* gene encoding a protein with significant levels of identity to members of the Dps family was identified in the genome of *T. erythraeum*. This gene codes for a putative $Dps_{T.\ erythraeum}$ protein (Dps_{tery}) with 69% primary amino acid sequence similarity to *Synechococcus* DpsA. We expressed and purified Dps_{tery}, and we found that Dps_{tery}, like other Dps proteins, is able to bind Fe and DNA and protect DNA from degradation by DNase. We also found that Dps_{tery} binds phosphate, like other ferritin family proteins. Fe K near-edge X-ray absorption of Dps_{tery} indicated that it has an iron core that resembles that of horse spleen ferritin.

Iron is used in numerous metabolic reactions and is essential in most living organisms. However, the low solubility of iron in oxygenated waters limits its bioavailability to aquatic organisms. This is the case in the surface waters of the ocean, where Fe is found primarily as particulate oxyhydroxides and Fe input from the atmosphere and deep seawater can be limited (8, 24). Recent studies have shown that low availability of Fe to phytoplankton directly limits primary production in some regions of the ocean (5, 14, 15, 30). Other studies have suggested that Fe may limit nitrogen fixation by marine cyanobacteria in other oceanic regions and thus control primary production by limiting the input of fixed nitrogen (17, 34).

Trichodesmium spp. are diazotrophic marine cyanobacteria that play a key role in the tropical and subtropical regions of the oceans not only as major primary producers but also as suppliers of new nitrogen through N_2 fixation (9). *Trichodesmium* is responsible for an estimated input of at least 65 Tg fixed N_2 per year. Nitrogenase is a two-component metalloenzyme with MoFe and Fe cofactors. The MoFe and Fe proteins contain metalloclusters (Fe-S cores) that are required for nitrogenase activity (18, 26). Because of the high Fe content of the nitrogenase enzyme, the iron requirement of nitrogen fixers has been estimated to be 2.5 to 5.2 times higher than that of other phytoplankton (40). The mechanisms by which *Trichodesmium* acquires and utilizes Fe are thus of great interest. In this study we focused on the question of Fe storage, which is critical because of the episodic nature of Fe input into many tropical and subtropical regions of the oceans.

Free iron in cells is extremely toxic as it catalyzes the generation of reactive oxygen species, such as the hydroxyl radical (· OH), which causes oxidative damage (Haber-Weiss/Fenton reactions). Thus, iron storage in cells is effected by ferritins, a superfamily of proteins that store iron in a readily available yet nontoxic form (4). Mammalian-type ferritins (i.e., ferritins proper), bacterioferritins, and Dps proteins (DNA binding proteins from starved cells) belong to this family of proteins. Ferritins and bacterioferritins are composed of 24 subunits that form a spherical protein shell with a hollow center in which Fe can be stored (up to 4,500 Fe atoms in ferritins and 2,000 Fe atoms in bacterioferritins) (4, 20). Bacterioferritins differ from mammalian ferritins in that they have up to 12 noncovalently bound heme groups (4, 39). The heme centers have bis-methionine ligation (12, 13). Dps proteins have not been as thoroughly characterized as ferritins or bacterioferritins yet. These proteins are composed of only 12 subunits and can accommodate correspondingly fewer Fe atoms (~500 atoms) (22). Iron is incorporated into the central cavity of ferritins by oxidation of Fe^{2+}, followed by formation of a microcrystalline ferrihydrite-phosphate core. The conversion of Fe^{2+} to Fe^{3+} is catalyzed by a ferroxidase center that is found in the subunits in ferritins and bacterioferritins (4) and between subunits in Dps proteins (22, 35, 38). Studies with various organisms have confirmed that the role of all ferritins is to store Fe and prevent its toxicity (41). In addition, it has been found that Dps protects DNA against oxidative agents by cocrystallization of the nucleic acid with the protein (3, 31, 51). DNA binding appears not to be sequence specific since no DNA binding motifs have been identified in Dps proteins yet. The protection of DNA appears to occur through oxidization and sequestration of Fe^{2+} ions, avoiding Fenton chemistry (31, 51).

Despite the current interest in the use of iron by autotrophs and particularly by nitrogen fixers in the oceans, no protein belonging to the ferritin family has been isolated or characterized from a marine microorganism previously. Here we describe the identification, isolation, and characterization of a Dps protein in *Trichodesmium erythraeum*.

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METHODS AND MATERIALS

Bacterial strains. The DH5 strain of *Escherichia coli* was used for cloning, and the BL21(DE3)pLysS strain (Novagen) was used for protein production. Genomic DNA from *T. erythraeum* IMS101 was used for PCR amplification (kindly provided by Eric Webb, Woods Hole Oceanographic Institution). For DNA damage assays, pUC19 (Sigma-Aldrich) from *E. coli* was used.

Cloning of the *dps* gene. The *T. erythraeum* IMS101 *dps* gene was amplified by PCR with oligonucleotide primers 5′-GAAATAAATCATATGTCTAACGC-3′ (upper primer) and 5′-CATTTTTCTAGATCTTGCAGG-3′ (lower primer). The upper primer was designed to recognize restriction endonuclease NdeI, and the lower primer was designed to recognize BglII. The resulting PCR product was cloned into the pCR 2.1 vectors (Invitrogen) by the TA cloning method and was subcloned into pRSET A (Invitrogen) by utilizing the NdeI and BglII restriction sites, which resulted in pRSET-*dps*. DNA sequencing reactions were performed by using the plasmid template pRSET-*dps* with ABI Prism BigDye 3.1 sequencing kits (Applied Biosystems) and an ABI PRISM 3100 genetic analyzer (Applied Biosystems). For protein production the recombinant plasmid pRSET-*dps* was transferred to the BL21(DE3)/pLysS host, which is inducible with isopropyl-β-D-thiogalactopyranoside (IPTG).

Overexpression and purification of Dps. *E. coli* BL21(DE3)pLysS containing pRSET-*dps* was grown to an optical density at 600 nm of 0.7 in 1 liter of Luria-Bertani broth containing 50 μg/ml ampicillin and 34 μg/ml chloramphenicol. IPTG (0.4 mM) was added to the cultures, and this was followed by incubation for 2 h for production of recombinant protein. The cells were collected by centrifugation at 4,000 × *g* for 15 min and resuspended in 250 ml of 20 mM Tris-HCl (pH 8.0). The cells were disrupted by using a sonicator (Branson Sonifier) at 50% power for 30 s (repeated three times) and then heat shocked at 70°C for 10 min. The supernatant was separated by centrifugation at 10,000 × *g* for 15 min and dialyzed overnight in 50 mM Tris-HCl (pH 7.9)–50 mM NaCl. Final purification of the protein was performed by using a Source 30-Q anion-exchange column and a Superdex-30 size exclusion column. To concentrate the purified protein, a Centriprep YM-50 MW centrifugal filter was used. The purity of the overexpressed Dps protein was checked by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE), followed by staining with Coomassie brilliant blue R250. The protein concentration was determined by the method of Lowry et al. (29).

Preparation of ^{55}Fe-loaded Dps. To obtain radiolabeled cultures, *E. coli* cells harboring the pRSET-*dps* and pRSET vectors were grown in LB medium containing $^{55}FeCl_3$ and purified as described above. The native PAGE gel containing the Dps protein was then exposed to a phosphor screen and scanned with a STORM 860 Phosphorimager (Molecular Dynamics) to detect ^{55}Fe.

Gel retardation assays. Dps_{tery} was mixed with pUC19 at DNA/protein molar ratios 1:500, 1:800, and $1:10^3$ in 50 mM Tris-HCl (pH 7.9) and incubated for 30 min at 30°C. The complexes obtained were resolved by 1% agarose gel electrophoresis in 0.5% Tris-borate-EDTA (TBE) buffer and were detected by ethidium bromide staining. To determine the k_d, 0.15 nM pUC19 was titrated with Dps_{tery}. The solutions were then incubated for 30 min at 30°C, and the products were resolved by 1% agarose gel electrophoresis in 0.5% TBE buffer and detected by ethidium bromide staining. DNA was quantified by using the IMAGE J program, and the method used for determination of k_d was based on the method of Carey (1, 10).

In vitro DNA damage assay. DNA-Dps complexes were used to assess the ability of the overexpressed protein to protect the DNA from digestion by DNase I. pUC19 and DNA-Dps complexes obtained from incubation of pUC19 and Dps_{tery} were incubated with 1 U of DNase I for 5 min at room temperature, and the reaction was stopped with 50 mM EDTA. The resulting products were then resolved on a 1% agarose gel with 0.5% TBE buffer and stained with ethidium bromide.

In vitro loading of Fe and phosphate. The Dps protein was loaded with iron in the presence of O_2 by using the method of Ishikawa et al. (23). To a 100-μg/ml Dps solution in 20 mM Tris-HCl (pH 7.0)–150 mM NaCl–0.1 mM EDTA, 500 μM freshly prepared iron(II) ammonium sulfate hexahydrate was added, and the preparation was left at room temperature for 3 h. After incubation, the protein-iron solution was dialyzed against 20 mM Tris-HCl (pH 7.0)–150 mM NaCl–0.1 mM EDTA. To test hydrogen peroxide as an oxidizing agent, a 150-μg/ml Dps solution in 20 mM Tris-HCl (pH 7.0)–150 mM NaCl–0.1 mM EDTA under N_2 was added to 250 μM iron(II) ammonium sulfate hexahydrate. Hydrogen peroxide (125 μM) was immediately added, and the solution was left at room temperature for 3 h and dialyzed as described above.

The protein was loaded with both phosphate and iron by using the method described by Aitkin-Rogers et al. for bacterioferritin (2). Solutions containing 1 mM and 5 mM phosphate were prepared using potassium phosphate (monobasic) in 20 mM Tris-HCl (pH 7.0)–150 mM NaCl–0.1 mM EDTA, and to both solutions 300 μg of Dps was added and incubated for 5 min. Then 500 μM iron(II) was added, and the solutions were incubated for 3 h.

After the protein solutions were dialyzed, they were washed with a solution containing 50 mM EDTA and 100 mM sodium oxalate (pH 7) to remove unbound iron. This was done by adding 500 μl of the EDTA-oxalate solution to concentrated protein, followed by 10 min of incubation. The EDTA-oxalate solution was removed by centrifugation at 3,313 × *g* for 30 min in a Centricon 10 tube. Then 1 ml of 50 mM Tris-HCl (pH 7.9) buffer was added and centrifuged for an additional 30 min to remove any remaining EDTA-oxalate solution.

Fe and phosphate analysis. The iron concentration was determined chemically by the method of Stookey, modified for protein use (43). Protein (10 μl) was digested with 1 M HCl (50 μl) for 1 h and neutralized with 1 M NaOH (50 μl). After neutralization, 500 μl of 2 M sodium dithionite was added, and the preparation was incubated for 30 min. Finally, 250 μl of 0.004 M ferrozine and 250 μl of 0.5 M sodium acetate buffer were added, and the solution was left overnight. The absorbance at 562 nm of the samples was determined with a UV-visible spectrophotometer. Phosphate was measured using the malachite green method, as described by Linge and Oldham and modified for protein use (28). Protein (20 μl) was digested with 50 μl of 1 M HCl for 1 h and neutralized with 50 μl of 1 M NaOH. Then 1 ml of the malachite green reagent was added, and the preparation was incubated at room temperature for 30 min. The absorbance at 625 nm was determined with a UV-visible spectrophotometer.

Fe K-edge X-ray absorption. Fe K near-edge X-ray absorption (XANES) spectra were collected at beamline 11-2 at the Stanford Synchrotron Radiation Laboratory, Stanford, CA, using an Si (220) monochromator crystal. The incident photon flux was measured using an ion chamber filled with N_2. The XANES spectra of all samples and standards were collected in fluorescence mode using a 30-element Ge detector. The standards were also examined in transmission mode to minimize saturation effects in the fluorescence yield spectra. All spectra were normalized to the incident photon flux, and their energies were calibrated with respect to the pre-edge peaks in goethite with the high-energy pre-edge transition set to 7,101.1 eV. The slits were set to 6 × 1 mm to obtain high-resolution XANES spectra.

Nucleotide sequence accession number. The nucleotide sequence encoding Dps_{tery} has been deposited in the EMBL nucleotide sequence database under accession number AJ875220. The same sequence (contig 414 gene 1836) has been annotated in the *Trichodesmium* genome as a Dps-like protein gene (http://jgi.doe.gov).

RESULTS AND DISCUSSION

Identification of iron storage proteins. We searched the previously published genome of *T. erythraeum* IM101 (http://www.jgi.doe.gov) and identified two genes, designated genes A and B, that encode amino acid sequences homologous to known bacterioferritin sequences (Fig. 1). Gene A exhibits sequence similarity with bacterioferritin genes from *Azotobacter vinelandii* (13%), *Pseudomonas putida* (17%), *Synechocystis* sp. strain PCC 6803 (14%), and *Desulfovibrio desulfuricans* (18%). Analysis of an alignment of the sequence encoded by *Trichodesmium* gene A with bacterioferritin sequences revealed conservation of the amino acid residues that act as ligands to the ferroxidase center (5). Amino acids Glu-18, Glu-50, His-54, Glu-94, Glu-127, and His-130, which form the ferroxidase center in *A. vinelandii*, are conserved in the gene A product as Glu-23, Glu-55, His-59, Glu-100, Glu-132, and His-135 (5). However, the gene A product lacks the heme-methionine ligand that is conserved in all bacterioferritins, which indicates that it is likely a bacterial ferritin instead.

Gene B codes for a sequence consisting of 180 amino acids (molecular mass, ~20.23 kDa) with sequence identity to known bacterioferritin sequences of *Synechocystis* (19%), *P. putida* (19%), *D. desulfuricans* (15%), and *A. vinelandii* (13%). Although identified with bacterioferritin sequences, the protein encoded by this gene does not contain all the amino acids residues involved in the di-iron ferroxidase coordination site in bacte-

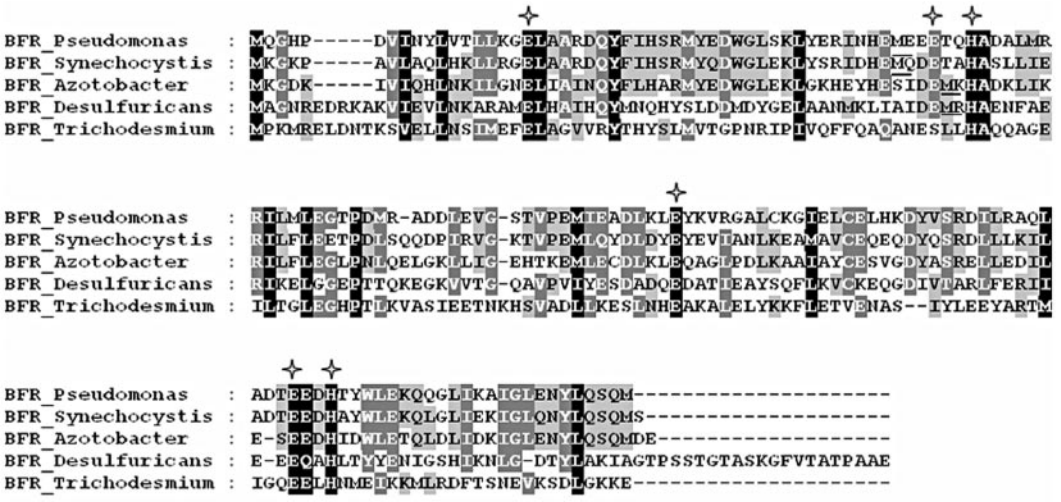

FIG. 1. Sequence alignment for bacterioferritins and a homologous protein in *T. erythraeum* (encoded by gene A). The residues involved in metal binding are indicated by stars, and the methionine heme ligand is underlined. The bacterioferritin sequences of *A. vinelandii* (BFR_Azotobacter), *P. putida* (BFR_Pseudomonas), *Synechocystis* sp. strain PCC 6803 (BFR_Synechocystis), and *D. desulfuricans* (BFR_Desulfuricans) were used to identify the homologous protein in *Trichodesmium* (BFR_Trichodesmium). Residues identical in all sequences are shown with a black background, and conserved residues are indicated by a gray background.

rioferritins. A comparison of the gene B product with known Dps proteins revealed high levels of similarity with members of the Dps family (Fig. 2). Gene B thus codes for a putative Dps protein, which we designated Dps_{tery}; this protein exhibits 69% primary amino acid sequence identity with *Synechococcus* DpsA, 32% primary amino acid sequence identity with *E. coli* Dps, and 30% primary amino acid sequence identity with *Listeria innocua* Flp.

Sequence alignment of Dps_{tery} and other proteins revealed conservation of the amino acid motifs that are thought to be involved in the formation of the intersubunit dinuclear ferritin-like ferroxidase center in Dps proteins (Fig. 2). In the crystal structure of the Flp protein from *L. innocua*, a member of the Dps family, 12 iron atoms have been observed occupying the putative ferroxidase centers. The amino acids involved in the coordination of iron are His-31, His-43, Asp-47, Asp-58, and Glu-62 (22). Equivalent amino acid residues were found in Dps_{tery} (His-53, His-65, Glu-70, Glu-81, and Glu-84). Recent work in vivo has shown that the amino acids involved with the putative ferroxidase center are crucial for the incorporation of iron. Site-directed mutagenesis of the negatively charged amino acids, Asp-74 and Glu-78, with Ala prevented iron incorporation by a Dps homologue in *Streptococcus suis* (37).

Amplification and cloning of Dps_{tery}. After overexpression of the putative Dps_{tery} in *E. coli*, cell extracts were subjected to SDS-PAGE analysis. Figure 3 shows the presence of the expected 20-kDa band after induction of *E. coli* harboring pR-

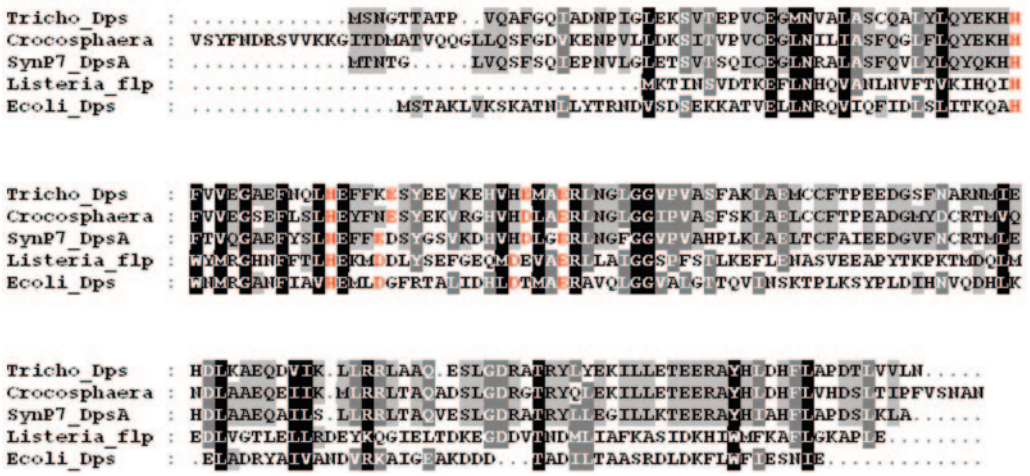

FIG. 2. Alignment of the Dps_{tery} sequence with known and putative Dps sequences. Residues identical in all the sequences are indicated by a black background, and conserved residues are indicated by a gray background. Amino acid residues that are red are the residues known to be involved in metal binding in *Listeria* ferritin (Listeria_flp), and these residues are aligned with homologous positions in Dps_{tery} (Tricho_Dps) *Crocosphaera watsonii* putative Dps (Crocosphaera), *Synechococcus* sp. strain PCC 7942 DpsA (Synp7_DspA), and *E. coli* Dps (Ecoli_Dps).

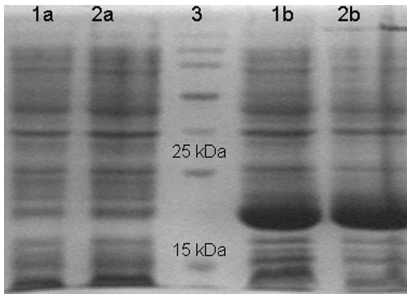

FIG. 3. Production of recombinant protein after induction of *E. coli* harboring pRSET-*dps*. A 12% SDS-PAGE gel was used to determine the protein production by two independent *E. coli* cultures before (lanes 1a and 2a) and after (lanes 1b and 2b) the addition of 0.4 mM IPTG. Lane 3 contained a protein marker. After induction a band at a molecular mass of ~20 kDa was present (lanes 1b and 2b).

SET-*dps* with IPTG. Examination of an 8% native PAGE gel containing the recombinant protein purified by using a ferritin purification protocol revealed a protein whose molecular mass is less than that of horse spleen ferritin (~450 kDa) but more than that of the bovines serum albumin dimer (~132 kDa) (Fig. 4). This result indicates that the native recombinant protein is not composed of 24 subunits and likely consists of 12 subunits (~240 kDa). On the basis of the amino acid sequence, we calculated a molecular mass of 20.23 kDa for one subunit and a molecular mass of ~242.76 kDa for the native protein.

Fe binding. The iron binding ability of Dps proteins (or Dps homologs) has been confirmed with proteins isolated from *E. coli*, *L. innocua*, *Mycobacterium smegmatis*, *Campylobacter jejuni*, and *Streptococcus mutans* (19, 23, 42, 52, 54). Thus, we investigated the iron binding ability of Dps_{tery}. *E. coli* cells with the pRSET (but not Dps) vector were grown in the same medium as a negative control. Upon exposure of the native PAGE gel to a phosphorimager, we observed the radiolabel iron in Dps_{tery}, as expected. No ^{55}Fe was observed in any of the protein bands from the control cells, demonstrating that the result obtained for Dps_{tery} was not due to the presence of Dps from *E. coli* or to nonspecific iron binding (Fig. 5). The intense ^{55}Fe band seen in the phosphorimage reflected the high iron binding capacity of Dps_{tery}.

The iron storage capacity of Dps_{tery} was quantified by incubating the protein with ferrous ammonium sulfate in the presence of O_2 or H_2O_2. We found 260 ± 20 Fe atoms/protein molecule in the presence of O_2 and 270 ± 10 Fe atoms/protein molecule in the presence of H_2O_2. Because the maximum incorporation of Fe by Dps proteins has been reported to be 500 atoms per molecule, a solution of Dps_{tery} that contained 260 Fe atoms per molecule was reincubated with a ferrous ammonium sulfate solution (7, 53). The additional incubation did not increase the incorporation of Fe by the protein (260 ± 40 Fe atoms/protein molecule). In *E. coli* Dps, H_2O_2 has been shown to be a more effective oxidant of Fe(II) than O_2, but in Dps_{tery} the maximum iron capacity remained the same regardless of the oxidant used (21). We do not know if the lower iron capacity that we observed in Dps_{tery} than in Dps from *E. coli* corresponds to an intrinsic difference between the proteins or to a lack of optimization in our Fe loading protocol.

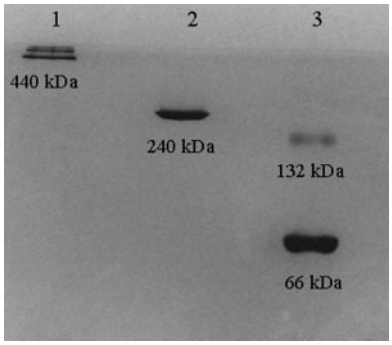

FIG. 4. Nondenaturing gel electrophoresis of Dps_{tery} (lane 2), horse spleen ferritin (lane 1), and bovine serum albumin as a monomer and dimer (lane 3) on a 10% native PAGE gel.

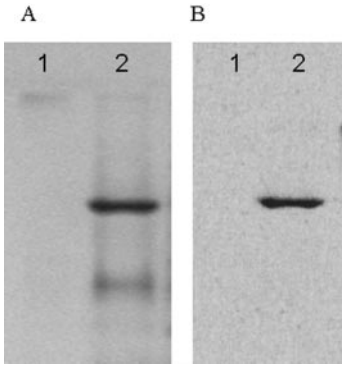

FIG. 5. Iron binding ability of Dps_{tery}. Protein extracts of cells grown in the presence of $^{55}FeCl_3$ were resolved on a 10% native PAGE gel, stained with Coomassie blue (A), and then exposed to a phosphorimager to detect radiolabeled iron (B). The radiolabeled iron was detected in the cells that overexpressed the Dps_{tery} protein (lane 2) and not in cells that lacked the pRSET-*dps* vector (lane 1).

Incorporation of phosphate. Although phosphate incorporation into the iron core of ferritins and bacterioferritins has been reported, until now the possibility that Dps proteins may contain phosphate has not been studied (46, 48). Purified Dps_{tery} obtained from overexpression in *E. coli* was found to contain 10 ± 1 P_i molecules/protein molecule. When Dps_{tery} was incubated with both ferrous ammonium sulfate and 1 mM or 5 mM potassium phosphate loading values of 66.4 ± 0.5 and 50 ± 4 P_i molecules/protein molecule were obtained, showing that the core has a P_i/Fe ratio of about 1:4. In horse spleen ferritin a P_i/Fe ratio of 1:8 has been reported, while in bacterioferritins P_i/Fe ratios between 1 and 2 have been observed (4, 46). Phosphate content is currently thought to affect the structure and size of the iron core in ferritins and bacterioferritins, and there is evidence that phosphate influences the availability of iron; however, the overall role of phosphate in the biochemistry of the protein is poorly understood (25, 45, 46). Nevertheless, the incorporation of phosphate into the iron core in Dps_{tery} provides additional evidence for the ferritin-like properties of the core in Dps proteins.

DNA binding. An unusual feature of Dps compared with other ferritin family proteins is that it binds DNA. This has been shown in *E. coli*, *Porphyromonas gingivalis*, *Synechococcus*, and *M. smegmatis* (3, 19, 36, 47). (Dps-like proteins composed of 12 subunits that do not appear to bind DNA, such as the protein found in *L.*

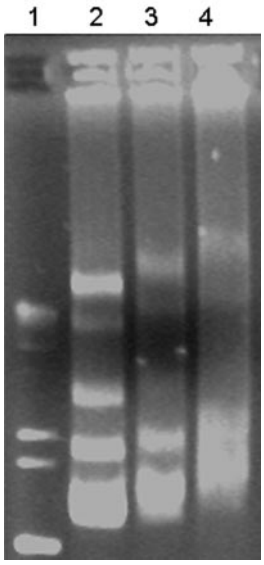

FIG. 6. Binding of DNA by Dps_{tery}. Dps_{tery} was incubated with *E. coli* plasmid pUC19 for 30 min at 30°C in 50 mM Tris-HCl (pH 7.9)–50 mM NaCl. For supercoiled pUC19 DNA alone (lane 1), the different bands represent 100% coiled, 80% coiled, and nicked DNA. Lanes 2, 3, and 4 contained the Dps_{tery}-DNA complex at DNA/protein molar ratios of 1:500, 1:800, and $1:10^3$, respectively. Incubation of DNA with Dps led to the formation of a Dps-DNA complex that did not enter the agarose gel and remained in the well of the gel.

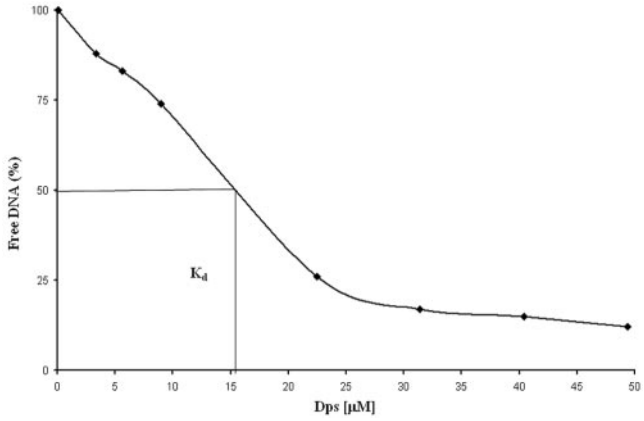

FIG. 7. Dissociation constant of pUC19 for Dps_{tery}. pUC19 (0.15 nM) was incubated with various concentration of Dps_{tery} at 30°C for 30 min, and the products were resolved in a 1% agarose gel. Measurements were determined twice for each combination, and the averages were plotted. The standard deviation was less than 2%. The k_d was determined by the method of Carey (10).

innocua, may logically be classified as a separate type of ferritin.) To determine whether Dps_{tery} binds DNA, we performed a gel mobility shift assay using the *E. coli* pUC19 plasmid as a template. Incubation of pUC19 DNA with Dps_{tery} at 37°C for 30 min decreased the mobility of all the DNA bands on the agarose gel, and this effect was exaggerated at higher concentrations of protein (Fig. 6). Most strikingly, a large fraction of the DNA remained stationary and did not enter the agarose. For determination of the apparent dissociation constant (k_d) of DNA with Dps_{tery}, pUC19 (0.15 nM) was titrated with Dps_{tery}, the products were resolved by agarose gel electrophoresis, and the bands were quantified using IMAGE J (1). The apparent k_d of Dps_{tery} calculated by measuring the protein concentration that resulted in 50% binding of the DNA was about 16 μM (Fig. 7), which is nearly 100-fold higher than the constant reported for the Dps protein from *E. coli* (k_d, ~172 to 178 nM) (6, 10). Until more is known about the affinities of various Dps proteins for DNA, it is difficult to speculate on the meaning of this large apparent difference between the proteins from *T. erythraeum* and *E. coli*.

DNA protection. Dps-DNA complexes have been shown to be extremely stable; in addition, DNA binding stabilizes the Dps structure (3). It has been shown that once a DNA-Dps complex is formed, the DNA is protected from attack by various nucleases, such as DNase I (19). To test the ability of Dps_{tery} to protect DNA, we incubated pUC19 DNA with Dps for 30 min and then added DNase I. Nonincubated pUC19 DNA and pUC19 incubated with DNase I were used as controls. All samples were separated on a 1% agarose gel (Fig. 8). As Fig. 8, lanes 1 and 2, show, incubation of pUC19 DNA with DNase I resulted in complete degradation of the nucleic acid, and there were no visible bands on the gel. In contrast, DNA that was preincubated with Dps_{tery} produced an intense band that remained in the loading well. Thus, the DNA-Dps_{tery} complex appeared to be effectively protected from degradation by DNase I.

Fe K near-edge X-ray absorption spectra. Despite identification of Dps proteins in numerous microorganisms, little is known about the structure of the Fe core. Figure 9 shows the X-ray absorption spectra of horse spleen ferritin and Dps_{tery} iron cores. The spectra of ferritin and Dps_{tery} are nearly identical and have similar pre-edge features. The weak pre-edge observed at ~7,100 eV corresponds to the 1s → 3d quadrupole-allowed, dipole-for-

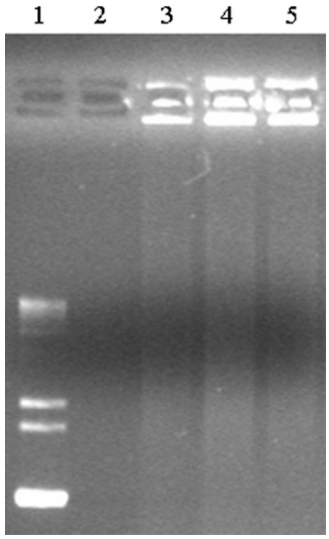

FIG. 8. Dps_{tery}-DNA complex protects DNA from DNase digestion. Lane 1 contained pUC19 plasmid DNA alone, and lane 2 contained pUC19 treated with 1 U of DNase I. The products obtained from incubation of DNA with Dps_{tery} at DNA/protein molar ratios of 1:500 (lane 3), 1:800 (lane 4), and $1:10^3$ (lane 5) were treated with DNase I. The DNA-Dps_{tery} complexes remained intact after incubation with DNase and were visible in the wells of the 1% agarose gel.

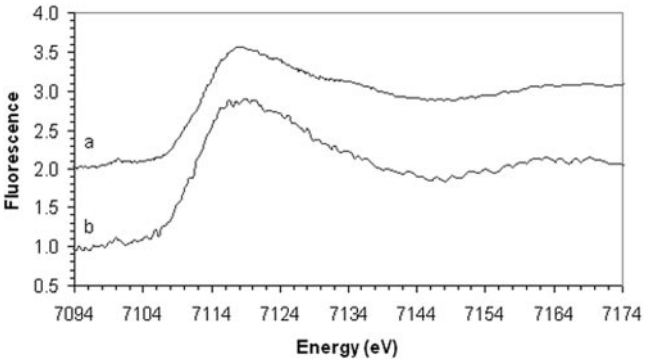

FIG. 9. Near-edge spectra of the iron K-edge of horse spleen ferritin (spectrum a) and Dps_{tery} (spectrum b). The pre-edge feature of ferritin at ~7,100 eV is characteristic of ferric iron octahedrally coordinated to oxygen, and a similar pre-edge feature was observed for Dps_{tery}.

bidden metal electronic transitions characteristic of octahedrally coordinated iron (49, 50). The energy and intensity of the 1s → 3d transition are sensitive to the coordination environment and oxidation state of iron (49). Previous work has shown that the Fe K-edge 1s → 3d pre-edge feature in the ferritin iron core observed at ~7,100 eV corresponds to high-spin ferric iron coordinated with six oxygen atoms (33). As the pre-edge feature of Dps_{tery} is similar to that of horse spleen ferritin, we concluded that the iron core in Dps_{tery} is composed of ferric iron that is octahedrally coordinated. Prior work has demonstrated that the chemistry of the Dps core from *E. coli* is similar to that of ferritin, and our XANES data also demonstrated that the core compositions of Dps_{tery} and ferritin are similar (21).

Potential role of Dps proteins in the marine environment. The Dps_{tery} that we isolated is homologous to previously described Dps proteins and has a similar molecular mass. Dps_{tery} also appears to have all the properties ascribed to such proteins; it binds iron, binds DNA, and protects DNA from degradation. Genomic analysis revealed that genes encoding Dps homologues are present in the genomes of *Prochlorococcus* sp. strain MIT 9313 and *Crocosphaera watsonii*, two of the few marine microorganisms that have been sequenced so far. All three of the functions that we demonstrated for Dps_{tery} may be useful to photorophs that live in the surface ocean. Such organisms must survive in environments where Fe inputs are low or episodic and they are subjected to oxidative stress because of the presence of molecular oxygen and intense sunlight. These organisms may also need to protect their genetic material when they survive in some dormant form during low-nutrient periods or when they are advected out of the photic zone.

The problem of photoxidative damage may be particularly severe for *Trichodesmium*, which accumulates at the surface of the ocean during blooms. Along with carotenoids and other UV-absorbing compounds, the Dps protein identified in *Trichodesmium* may be part of an effective protective mechanism (11, 44). Mutants of *Synechocystis*, *Synechococcus*, and *E. coli* that lack the *dps* genes are extremely sensitive to photooxidative stress and peroxide (16, 27, 32). The protection provided by Dps against degradation by reactive oxygen species probably contributes to the survival of *Trichodesmium*, which must carry out oxygenic photosynthesis in the presence of intense sunlight.

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REFERENCES

1. **Abramoff, M. D., P. J. Magelhaes, and S. J. Ram.** 2004. Image processing with ImageJ. Biophotonics Int. **11:**36–42.
2. **Aitken-Rogers, H., C. Singleton, A. Lewin, A. Taylor-Gee, G. R. Moore, and N. E. Le Brun.** 2004. Effect of phosphate on bacterioferritin-catalysed iron(II) oxidation. J. Biol. Inorg. Chem. **9:**161–170.
3. **Almiron, M., A. J. Link, D. Furlong, and R. Kolter.** 1992. A novel DNA-binding protein with regulatory and protective roles in starved *Escherichia coli*. Genes Dev. **6:**2646–2654.
4. **Andrews, S. C.** 1998. Iron storage in bacteria. Adv. Microb. Physiol. **40:**281–351.
5. **Andrews, S. C., J. B. C. Findlay, J. R. Guest, P. M. Harrison, J. N. Keen, and J. M. A. Smith.** 1991. Physical, chemical and immunological properties of the bacterioferritins of *Escherichia coli*, *Pseudomonas aeruginosa* and *Azotobacter vinelandii*. Biochim. Biophys. Acta **1078:**111–116.
6. **Azam, T. A., and A. Ishihama.** 1999. Twelve species of the nucleoid-associated protein from *Escherichia coli*—sequence recognition specificity and DNA binding affinity. J. Biol. Chem. **274:**33105–33113.
7. **Bozzi, M., G. Mignogna, S. Stefanini, D. Barra, C. Longhi, P. Valenti, and E. Chiancone.** 1997. A novel non-heme iron-binding ferritin related to the DNA-binding proteins of the Dps family in *Listeria innocua*. J. Biol. Chem. **272:**3259–3265.
8. **Bruland, K. W., K. J. Orians, and J. P. Cowen.** 1994. Reactive trace-metals in the stratified central North Pacific. Geochim. Cosmochim. Acta **58:**3171–3182.
9. **Capone, D. G., J. P. Zehr, H. W. Paerl, B. Bergman, and E. J. Carpenter.** 1997. *Trichodesmium*, a globally significant marine cyanobacterium. Science **276:**1221–1229.
10. **Carey, J.** 1991. Gel retardation. Methods Enzymol. **208:**103–117.
11. **Carpenter, E. J.** 1983. Physiology and ecology of marine planktonic *Oscillatoria* (*Trichodesmium*). Mar. Biol. Lett. **4:**69–85.
12. **Cheesman, M. R., F. H. A. Kadir, J. Albasseet, F. Almassad, J. Farrar, C. Greenwood, A. J. Thomson, and G. R. Moore.** 1992. EPR and magnetic circular-dichroism spectroscopic characterization of bacterioferritin from *Pseudomonas aeruginosa* and *Azotobacter vinelandii*. Biochem. J. **286:**361–367.
13. **Cheesman, M. R., N. E. Lebrun, F. H. A. Kadir, A. J. Thomson, G. R. Moore, S. C. Andrews, J. R. Guest, P. M. Harrison, J. M. A. Smith, and S. J. Yewdall.** 1993. Heme and nonheme iron sites in *Escherichia coli* bacterioferritin: spectroscopic and model-building studies. Biochem. J. **292:**47–56.
14. **Coale, K. H., K. S. Johnson, F. P. Chavez, K. O. Buesseler, R. T. Barber, M. A. Brzezinski, W. P. Cochlan, F. J. Millero, P. G. Falkowski, J. E. Bauer, R. H. Wanninkhof, R. M. Kudela, M. A. Altabet, B. E. Hales, T. Takahashi, M. R. Landry, R. R. Bidigare, X. J. Wang, Z. Chase, P. G. Strutton, G. E. Friederich, M. Y. Gorbunov, V. P. Lance, A. K. Hilting, M. R. Hiscock, M. Demarest, W. T. Hiscock, K. F. Sullivan, S. J. Tanner, R. M. Gordon, C. N. Hunter, V. A. Elrod, S. E. Fitzwater, J. L. Jones, S. Tozzi, M. Koblizek, A. E. Roberts, J. Herndon, J. Brewster, N. Ladizinsky, G. Smith, D. Cooper, D. Timothy, S. L. Brown, K. E. Selph, C. C. Sheridan, B. S. Twining, and Z. I. Johnson.** 2004. Southern ocean iron enrichment experiment: carbon cycling in high- and low-Si waters. Science **304:**408–414.
15. **Coale, K. H., K. S. Johnson, S. E. Fitzwater, R. M. Gordon, S. Tanner, F. P. Chavez, L. Ferioli, C. Sakamoto, P. Rogers, F. Millero, P. Steinberg, P. Nightingale, D. Cooper, W. P. Cochlan, M. R. Landry, J. Constantinou, G. Rollwagen, A. Trasvina, and R. Kudela.** 1996. A massive phytoplankton bloom induced by an ecosystem-scale iron fertilization experiment in the equatorial Pacific Ocean. Nature **383:**495–501.
16. **Dwivedi, K., A. Sen, and G. S. Bullerjahn.** 1997. Expression and mutagenesis of the *dps*A gene of *Synechococcus* sp. PCC7942, encoding a DNA-binding protein involved in oxidative stress protection. FEMS Microbiol. Lett. **155:**85–91.
17. **Fu, F. X., and P. R. F. Bell.** 2003. Growth, N_2 fixation and photosynthesis in a cyanobacterium, *Trichodesmium* sp., under Fe stress. Biotechnol. Lett. **25:**645–649.
18. **Georgiadis, M. M., H. Komiya, P. Chakrabarti, D. Woo, J. J. Kornuc, and D. C. Rees.** 1992. Crystallographic structure of the nitrogenase iron protein from *Azotobacter vinelandii*. Science **257:**1653–1659.
19. **Gupta, S., and D. Chatterji.** 2003. Bimodal protection of DNA by *Mycobacterium smegmatis* DNA-binding protein from stationary phase cells. J. Biol. Chem. **278:**5235–5241.
20. **Harrison, P. M., and P. Arosio.** 1996. Ferritins: molecular properties, iron storage function and cellular regulation. Biochim. Biophys. Acta Bioenerg. **1275:**161–203.

21. **Ilari, A., P. Ceci, D. Ferrari, G. L. Rossi, and E. Chiancone.** 2002. Iron incorporation into *Escherichia coli* Dps gives rise to a ferritin-like microcrystalline core. J. Biol. Chem. **277:**37619–37623.
22. **Ilari, A., S. Stefanini, E. Chiancone, and D. Tsernoglou.** 2000. The dodecameric ferritin from *Listeria innocua* contains a novel intersubunit iron-binding site. Nat. Struct. Biol. **7:**38–43.
23. **Ishikawa, T., Y. Mizunoe, S. Kawabata, A. Takade, M. Harada, S. N. Wai, and S. Yoshida.** 2003. The iron-binding protein Dps confers hydrogen peroxide stress resistance to *Campylobacter jejuni*. J. Bacteriol. **185:**1010–1017.
24. **Jickells, T. D.** 1999. The inputs of dust derived elements to the Sargasso Sea: a synthesis. Mar. Chem. **68:**5–14.
25. **Johnson, J. L., M. Cannon, R. K. Watt, R. B. Frankel, and G. D. Watt.** 1999. Forming the phosphate layer in reconstituted horse spleen ferritin and the role of phosphate in promoting core surface redox reactions. Biochemistry **38:**6706–6713.
26. **Kim, J. S., and D. C. Rees.** 1992. Structural models for the metal centers in the nitrogenase molybdenum-iron protein. Science **257:**1677–1682.
27. **Li, H., A. K. Singh, L. M. McIntyre, and L. A. Sherman.** 2004. Differential gene expression in response to hydrogen peroxide and the putative PerR regulon of *Synechocystis* sp strain PCC 6803. J. Bacteriol. **186:**3331–3345.
28. **Linge, K. L., and C. E. Oldham.** 2001. Interference from arsenate when determining phosphate by the malachite green spectrophotometric method. Anal. Chim. Acta **450:**247–252.
29. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193:**265–275.
30. **Martin, J. H., K. H. Coale, K. S. Johnson, S. E. Fitzwater, R. M. Gordon, S. J. Tanner, C. N. Hunter, V. A. Elrod, J. L. Nowicki, T. L. Coley, R. T. Barber, S. Lindley, A. J. Watson, K. Vanscoy, C. S. Law, M. I. Liddicoat, R. Ling, T. Stanton, J. Stockel, C. Collins, A. Anderson, R. Bidigare, M. Ondrusek, M. Latasa, F. J. Millero, K. Lee, W. Yao, J. Z. Zhang, G. Friederich, C. Sakamoto, F. Chavez, K. Buck, Z. Kolber, R. Greene, P. Falkowski, S. W. Chisholm, F. Hoge, R. Swift, J. Yungel, S. Turner, P. Nightingale, A. Hatton, P. Liss, and N. W. Tindale.** 1994. Testing the iron hypothesis in ecosystems of the equatorial Pacific Ocean. Nature **371:**123–129.
31. **Martinez, A., and R. Kolter.** 1997. Protection of DNA during oxidative stress by the nonspecific DNA-binding protein Dps. J. Bacteriol. **179:**5188–5194.
32. **Nair, S., and S. E. Finkel.** 2004. Dps protects cells against multiple stresses during stationary phase. J. Bacteriol. **186:**4192–4198.
33. **Nichol, H., O. Gakh, H. A. O'Neill, I. J. Pickering, G. Isaya, and G. N. George.** 2003. Structure of frataxin iron cores: an X-ray absorption spectroscopic study. Biochemistry **42:**5971–5976.
34. **Paerl, H. W., L. E. Prufertbebout, and C. Z. Guo.** 1994. Iron-stimulated N_2 fixation and growth in natural and cultured populations of the planktonic marine cyanobacteria *Trichodesmium* spp. Appl. Environ. Microbiol. **60:** 1044–1047.
35. **Papinutto, E., W. G. Dundon, N. Pitulis, R. Battistutta, C. Montecucco, and G. Zanotti.** 2002. Structure of two iron-binding proteins from *Bacillus anthracis*. J. Biol. Chem. **277:**15093–15098.
36. **Pena, M. M. O., Burkhart, W., and G. S. Bullerjahn.** 1995. Purification and characterization of a *Synechococcus* sp. strain PCC 7942 polypeptide structurally similar to the stress-induced Dps/PexB protein of *Escherichia coli.* Arch. Microbiol. **163:**337–344.
37. **Pulliainen, A. T., A. Kauko, S. Haataja, A. C. Papageorgiou, and J. Finne.** 2003. The role of streptococcal Dpr in H_2O_2 resistance: functional and structural insights. Free Radic. Res. **37:**36.
38. **Ren, B., G. Tibbelin, T. Kajino, O. Asami, and R. Ladenstein.** 2003. The multi-layered structure of Dps with a novel di-nuclear ferroxidase center. J. Mol. Biol. **329:**467–477.
39. **Romao, C. V., M. Regalla, A. V. Xavier, M. Teixeira, M. Y. Liu, and J. Le Gall.** 2000. A bacterioferritin from the strict anaerobe *Desulfovibrio desulfuricans* ATCC 27774. Biochem. **39:**6841–6849.
40. **Sanudo-Wilhelmy, S. A., A. B. Kustka, C. J. Gobler, D. A. Hutchins, M. Yang, K. Lwiza, J. Burns, D. G. Capone, J. A. Raven, and E. J. Carpenter.** 2001. Phosphorus limitation of nitrogen fixation by *Trichodesmium* in the central Atlantic Ocean. Nature **411:**66–69.
41. **Smith, J. L.** 2004. The physiological role of ferritin-like compounds in bacteria. Crit. Rev. Microbiol. **30:**173–185.
42. **Stefanini, S., S. Cavallo, B. Montagnini, and E. Chiancone.** 1999. Incorporation of iron by the unusual dodecameric ferritin from *Listeria innocua*. Biochem. J. **338:**71–75.
43. **Stookey, L. L.** 1970. Ferrozine: a new spectrophotometric reagent for iron. Anal. Chem. **42:**779-&.
44. **Subramaniam, A., E. J. Carpenter, D. Karentz, and P. G. Falkowski.** 1999. Bio-optical properties of the marine diazotrophic cyanobacteria *Trichodesmium* spp. I. Absorption and photosynthetic action spectra. Limnol. Oceanogr. **44:**608–617.
45. **Treffry, A., and P. M. Harrison.** 1978. Incorporation and release of inorganic-phosphate in horse spleen ferritin. Biochem. J. **171:**313–320.
46. **Treffry, A., P. M. Harrison, M. I. Cleton, W. C. Debruijn, and S. Mann.** 1987. A note on the composition and properties of ferritin iron cores. J. Inorg. Biochem. **31:**1–6.
47. **Ueshima, J., M. Shoji, D. B. Ratnayake, K. Abe, S. Yoshida, K. Yamamoto, and K. Nakayama.** 2003. Purification, gene cloning, gene expression, and mutants of Dps from the obligate anaerobe *Porphyromonas gingivalis*. Infect. Immun. **71:**1170–1178.
48. **Watt, G. D., R. B. Frankel, G. C. Papaefthymiou, K. Spartalian, and E. I. Stiefel.** 1986. Redox properties and Mossbauer-spectroscopy of *Azotobacter vinelandii* bacterioferritin. Biochemistry **25:**4330–4336.
49. **Westre, T. E., P. Kennepohl, J. G. DeWitt, B. Hedman, K. O. Hodgson, and E. I. Solomon.** 1997. A multiplet analysis of Fe K-edge 1s→3d pre-edge features of iron complexes. J. Am. Chem. Soc. **119:**6297–6314.
50. **Wilke, M., F. Farges, P. E. Petit, G. E. Brown, and F. Martin.** 2001. Oxidation state and coordination of Fe in minerals: an FeK-XANES spectroscopic study. Am. Mineral. **86:**714–730.
51. **Wolf, S. G., D. Frenkiel, T. Arad, S. E. Finkel, R. Kolter, and A. Minsky.** 1999. DNA protection by stress-induced biocrystallization. Nature **400:**83–85.
52. **Yamamoto, Y., M. Higuchi, L. B. Poole, and Y. Kamio.** 2000. Role of the *dpr* product in oxygen tolerance in *Streptococcus mutans*. J. Bacteriol. **182:**3740–3747.
53. **Yamamoto, Y., L. B. Poole, R. R. Hantgan, and Y. Kamio.** 2002. An iron-binding protein, *dpr*, from *Streptococcus mutans* prevents iron-dependent hydroxyl radical formation in vitro. J. Bacteriol. **184:**2931–2939.
54. **Zhao, G. H., P. Ceci, A. Ilari, L. Giangiacomo, T. M. Laue, E. Chiancone, and N. D. Chasteen.** 2002. Iron and hydrogen peroxide detoxification properties of DNA-binding protein from starved cells: a ferritin-like DNA-binding protein of *Escherichia coli*. J. Biol. Chem. **277:**27689–27696.