Campylobacter jejuni Dps Protein Binds DNA in the Presence of Iron or Hydrogen Peroxide

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Iron is an essential cofactor for many enzymes; however, this metal can lead to the formation of reactive oxygen species. Ferritin proteins bind and oxidize Fe^{2+} to Fe^{3+} , storing this metal in a nonreactive form. In some organisms, a particular subfamily of ferritins, namely, Dps proteins, have the ability to bind DNA. Here we show that the *Campylobacter jejuni* Dps has DNA binding activity that is uniquely activated by Fe^{2+} or H_2O_2 at below neutral pH. The Dps-DNA binding activity correlated with the ability of Dps to self-aggregate. The Dps-DNA interaction was inhibited by NaCl and Mg^{2+} , suggesting the formation of ionic interactions between Dps and DNA. Alkylation of cysteines affected DNA binding in the presence of H_2O_2 but not in the presence of Fe^{2+} . Replacement of all cysteines in *C. jejuni* Dps with serines did not affect DNA binding, excluding the participation of cysteine in H_2O_2 sensing. Dps was able to protect DNA *in vitro* from enzymatic cleavage and damage by hydroxyl radicals. A *C. jejuni* dps mutant was less resistant to H_2O_2 *in vivo*. The concerted activation of Dps-DNA binding in response to low pH, H_2O_2 , and Fe^{2+} may protect *C. jejuni* DNA during host colonization.

Campylobacter jejuni is a commensal organism of chickens and is the most common cause of human gastrointestinal disease in developed countries (1). *C. jejuni* infection is characterized by fever, abdominal cramps, and watery or bloody diarrhea and is typically self-limiting; however, in some cases *C. jejuni* infection can lead to development of the neurologic disease Guillain-Barré syndrome (2, 3).

Iron is an essential cofactor for many enzymes, required for both survival and pathogenicity of most bacteria; however, this metal can also lead to the formation of reactive oxygen species under oxidizing conditions (4). In the ferrous form (Fe^{2+}), iron can react with hydrogen peroxide to produce very reactive and toxic hydroxyl radicals through the Fenton reaction ($\text{H}_2\text{O}_2 + \text{Fe}^{2+} \rightarrow \text{OH}^- + \text{OH}^\cdot + \text{Fe}^{3+}$). In order to avoid the toxic effects of free Fe^{2+} , organisms belonging to all domains of life store iron inside a ferritin family protein (5). Ferritins bind and oxidize Fe^{2+} to Fe^{3+} , storing iron in a nonreactive iron-oxide-form Fe_2O_3 trapped in the protein cage (6).

One particular subfamily of ferritins is Dps (DNA binding protein from starved cells), first characterized in *Escherichia coli* as a DNA binding protein induced in the stationary phase or under stress (7). Since this pioneer study, many different Dps proteins have been characterized. These proteins are structurally conserved and widely distributed in prokaryotes (8, 9). Unlike typical ferritins that have 24 subunits and 432 symmetry, Dps proteins assemble in a quasispherical dodecamer with 23 symmetry and can store ~ 500 iron atoms (4, 10). Furthermore, while in typical ferritins each subunit carries its own ferroxidase center, the ferroxidase centers of Dps are located between the subunits, where two Fe^{2+} ions bind to form a binuclear iron center (4, 11).

A number of *in vivo* studies have demonstrated that Dps is involved in resistance to a variety of stressful conditions, including reactive oxygen species, UV, ionizing radiation, and thermal and acidic stress (7, 12–21). Furthermore, Dps is able to protect DNA from oxidative damage *in vitro* (7, 22–26). In all Dps proteins studied, DNA protection is exerted through the removal of free Fe^{2+} from the solution by reduction of the formation of reactive

oxygen species through Fenton chemistry. Furthermore, some Dps proteins can also physically protect DNA through the formation of nonspecific protein-DNA complexes (7, 22, 27–30).

The mechanism of DNA binding by Dps proteins is not completely understood (9). An initial DNA binding event is followed by condensation into highly ordered DNA-protein structures also known as biocrystals (9, 22, 27). Apparently, the ability of Dps to condensate the DNA is related to its capacity to self-aggregate under certain conditions (24).

The N terminal of the *E. coli* Dps has been implicated in interaction with DNA. This portion of *E. coli* Dps is very flexible and rich in positively charged lysine residues (10, 24). However, different mechanisms for Dps-DNA interaction have been proposed. In *Deinococcus radiodurans* Dps1, the N-terminal region has been implicated in DNA binding through a mechanism involving the occupancy of a metal binding site located within this region (31, 32). In *Mycobacterium smegmatis*, Dps has a positively charged C terminal that has been implicated in DNA binding (30). On the other hand, the *Helicobacter pylori* Dps (NapA) can bind DNA despite the absence of positively charged N- or C-terminal extensions; in this case, it has been postulated that the protein's highly positive surface may be involved in DNA binding (22, 33).

In *C. jejuni*, the gene encoding the Dps protein is required for resistance to H_2O_2 stress (17), biofilm formation, and colonization of poultry and piglets (34, 35). Previous studies failed to detect *C. jejuni* Dps-DNA binding activity (17). Here we show that *C. jejuni* Dps does have DNA binding activity, that it is stimulated by

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TABLE 1 C. jejuni strains, plasmids, and primers used in this study

Strain, plasmid, or primer	Genotype, phenotype, or sequence ^a	Source or reference
Strains		
11168-O	Wild type (human isolated strain), originally strain 5636/77	51
LFH2	<i>cj1534c::cat</i> Cm ^r	This work
Plasmids		
pCR-BluntII-TOPO	Km ^r cloning vector	Invitrogen
pLHPCRcj1534c	Km ^r (pCR-BluntII-TOPO) <i>C. jejuni</i> Dps coding region NdeI-BamHI	This work
pET19b	Amp ^r expression vector T7 promoter	Novagen
pLHPETcj1534c	Amp ^r (pET19b), expresses N-terminal His-tagged <i>C. jejuni</i> Dps	This work
pLHPETcj1534c-C-S	Amp ^r (pET19b), expresses N-terminal His-tagged <i>C. jejuni</i> Dps C54S C68S C85S	This work
pC46	Cm ^r	This work
pGEM	Amp ^r cloning vector	Promega
pLHPCRcj1543cCAT	Cm ^r pLHPCRcj1534c carrying the <i>cat</i> insertion <i>dps::cat</i>	This work
Primers		
Cj1534c NdeI	<u>CATATGATGT</u> CAGTTACAAAAACAATTATTACAAATG	
Cj1534c BamHI	<u>GGATCCTT</u> ACATTTTGCAAGCACCTTGTAAGTAGC	
Cj1534c InvR	<u>GAAGATCT</u> CCATTAATACTTTTGGCAAGT	
Cj1534c InvF	<u>GAAGATCT</u> GCAAAAAGTCCAAAAGTTGCAA	
CATF	<u>GAAGATCT</u> GATTGAAAAGTGGATAGATTTATG	
CATR	<u>GAAGATCT</u> ATTTTTCGATTGGCGCGCTGAGGG	

^a In the sequences, restriction sites are underlined.

the presence of Fe²⁺ or H₂O₂, and that *C. jejuni* Dps was able to protect DNA from oxidative damage and enzymatic degradation *in vitro* as well as able to enhance cellular resistance to H₂O₂ *in vivo*.

MATERIALS AND METHODS

Bacterial strains, plasmids, and oligonucleotides. The bacterial strains, plasmids, and oligonucleotides used in PCRs are listed in Table 1.

Media and growth conditions. *E. coli* was grown in LB medium at 37°C. *C. jejuni* was grown at 42°C in Columbia agar supplemented with 5% defibrinated horse blood, vancomycin (10 µg ml⁻¹), polymyxin B (0.3 µg ml⁻¹), and trimethoprim (5 µg ml⁻¹) under microaerobic conditions (10% CO₂ and 5% O₂ in N₂).

Cloning and molecular biology methods. Agarose gel electrophoresis, bacterial transformation, and cloning were performed using standard procedures as described previously (36). Isolation of plasmid DNA was performed using the QIApre MidiKit (Qiagen). Enzymes were obtained from New England BioLabs (NEB) and used according to the manufacturers' instructions. DNA sequencing was performed using dye-labeled terminators in an automated DNA sequencer at the Australian Genome Research Facility.

Cloning, expression, and purification of *C. jejuni* Dps. The gene coding for *C. jejuni* Dps (*cj1534c*) was amplified by PCR using Phusion polymerase (NEB) and a boiled culture of *C. jejuni* 11168-O as the template. The primers used were Cj1534c NdeI and Cj1534c BamHI (Table 1). The amplified product was ligated into the vector pCR-BluntII-TOPO (Invitrogen). The resultant plasmid was named pLHPCRcj1534c; the DNA insert was completely sequenced to confirm its integrity. The *dps* gene was then subcloned into the expression vector pET19b using the NdeI-BamHI sites, producing the plasmid pLHPETcj1534c, which expressed *C. jejuni* Dps with an N-terminal His tag.

E. coli BL21(ΔDE3) carrying pLHPETcj1534c was grown overnight in 10 ml LB containing 100 µg ml⁻¹ ampicillin. This culture was used to inoculate 500 ml of LB containing ampicillin at 100 µg ml⁻¹. Cells were grown at 37°C with shaking at 180 rpm, and 0.3 mM IPTG (isopropyl-β-D-thiogalactopyranoside) was added after 2 h. Cells were collected after 4 h by centrifugation, the pellet was resuspended in 15 ml of buffer 1 (50 mM Tris-HCl [pH 8], 0.1 M NaCl, 10% glycerol), and the cells were lysed

by sonication on ice. The extract was clarified by centrifugation at 30,000 × *g* for 30 min at 4°C. The supernatant was applied to a 1-ml Talon column (Sigma). The column was equilibrated with 5 ml buffer 1 before sample loading. The Dps protein was eluted using a stepwise gradient of buffer 1 containing 10, 50, 300, and 500 mM imidazole. Fractions containing Dps were pooled and dialyzed in a mixture of 50 mM Tris-HCl (pH 8), 0.1 M NaCl, and 50% glycerol. Dps was stored in aliquots at -80°C until use.

Site-directed mutagenesis. The *C. jejuni* *dps* genes carrying the C54S, C68S, and C85S mutations and flanked by the NdeI and BamHI restriction sites were synthesized and cloned into pIDTsmarKm vector by Integrated DNA Technologies (IDT). The mutant genes were subcloned into the expression vector pET19b using the NdeI-BamHI sites, producing the plasmid pLHPETcj1534c-C-S (C54S C68S C85S). The final construct was verified by DNA sequencing. The expression and purification of the variant protein were performed as described for the wild-type Dps.

Construction of the *C. jejuni* isogenic *dps* mutant strain. The entire pLHPCRcj1534c plasmid was amplified by inverted PCR using the Expand long template PCR system (Roche) and the Cj1534c InvF and Cj1534c InvR primers. The chloramphenicol resistance gene from pC46 was PCR amplified using Phusion polymerase (NEB) and the CATF and CATR primers (Table 1). Both PCR products were purified, digested with BglII, and ligated. One plasmid containing the chloramphenicol cassette, inserted into the *cj1534c* gene, in the same transcriptional orientation (verified by DNA sequencing), was named pLHPCRcj1543cCAT. This plasmid was purified and electrotransformed into *C. jejuni* 11168-O, and colonies resistant to chloramphenicol were selected. One colony, named LFH2, was selected for further characterization. The insertion of the chloramphenicol cassette into the *cj1534c* gene by double-crossover recombination was confirmed by PCR using the Cj1534c NdeI, Cj1534c BamHI, CATF, and CATR primers.

Protein analysis. Protein electrophoresis was carried out by SDS-PAGE (37), and gels were stained with Coomassie blue. For molecular weight determination of the Dps in its native state, samples were loaded on regular SDS-PAGE without prior boiling. Protein concentrations were determined by the Bradford assay (Bio-Rad) using bovine serum albumin (BSA) as a standard (38).

DNA binding analysis using agarose gel electrophoresis. Purified *C. jejuni* Dps was diluted in 50 mM Tris-HCl (pH 6.8) to 0.15 μ M (final dodecamer concentration) containing 2 nM pGEM (Promega). For the experiments in the presence of Fe^{2+} , preweighed FeCl_2 was freshly dissolved (within less than 1 min before the experiment) in 50 mM Tris-HCl (pH 6.8) and added to the final concentration of 50 μ M (unless stated otherwise). For the experiments in the presence of H_2O_2 , a freshly prepared solution of H_2O_2 in 50 mM Tris-HCl (pH 6.8) was added to the final concentration of 10 mM (unless stated otherwise). When indicated, dithiothreitol (DTT; 2 mM final concentration) was incubated with Dps for 30 min before the addition of DNA. The Dps samples treated with iodoacetamide were first reduced in the presence of 2 mM DTT for 10 min followed by addition of 10 mM iodoacetamide for 1 h at room temperature. Iodoacetamide and DTT were removed using a desalting column (Bio-Rad).

DNA binding reactions were performed in a final volume of 20 μ l for 10 min at 25°C, the reaction was mixed (at 10:2 ratio) with 50% glycerol and 0.01% bromophenol blue and loaded into a 1% agarose gel prepared in Tris-acetate-EDTA (TAE) buffer (pH 8) containing 0.1 μ l ml^{-1} ethidium bromide. After electrophoresis for 20 min at 120 V at room temperature, DNA was visualized after UV exposure using a Gel Doc system (Bio-Rad).

DNA protection assays. (i) Oxidative stress. DNA protection assays against hydroxyl radicals were performed using similar conditions as described for the DNA binding assay. A fresh solution of FeCl_2 was added to final concentration of 50 μ M to the solution containing Dps and pGEM (Promega). After 10 min at 25°C, the reaction mixtures received H_2O_2 to a final concentration of 10 mM in order to generate hydroxyl radicals through the Fenton chemistry. Reactions were quenched after 30 min with the addition of the iron chelator bipyridyl to 5 mM. The integrity of the DNA was analyzed by 1% agarose gel electrophoresis.

(ii) Nuclease cleavage. Purified *C. jejuni* Dps (0.15 μ M final dodecamer concentration) and 100 ng of pGEM (Promega) were incubated in 50 mM Tris-HCl (pH 6.8), 0.25 mM MgCl_2 , and 0.01 mM CaCl_2 in 20- μ l reaction mixtures. When indicated, FeCl_2 or H_2O_2 was present at final concentrations of 50 μ M and 10 mM, respectively. After 10 min at 25°C, 0.01 U of DNase I was added, reactions were quenched after 15 min at 25°C with 10 mM EDTA, and the integrity of the DNA was analyzed by 1% agarose gel electrophoresis.

***C. jejuni* and *E. coli* resistance to H_2O_2 .** *C. jejuni* cells, cultivated overnight at 42°C in Columbia agar, were collected and suspended in LB to an optical density at 600 nm (OD_{600}) of 0.25. *E. coli* BL21 cells carrying the control plasmid pET19b or the plasmid expressing Dps (pLHPETcj1534c) were cultivated in LB to an OD_{600} of 0.5. IPTG (1 mM) was added to the cultures, and after 4 h of shaking at 37°C, cells were collected and diluted in LB to an OD_{600} of 0.35. Both *C. jejuni* and *E. coli* received 5 mM H_2O_2 , and the cells were incubated in LB under air at 37°C for 30 min. Viable cells were determined by serial dilution CFU counting in LB agar for *E. coli* or Columbia agar for *C. jejuni* strains.

Iron uptake experiments. Purified Dps was diluted to 0.15 μ M or 0.3 μ M (final dodecamer concentration) in 400 μ l 50 mM Tris-HCl (pH 6.8). Freshly prepared FeCl_2 was added to a final concentration of 100 μ M, and the formation of the iron core was monitored at 305 nm under air in a 0.5-cm quartz cuvette. When indicated, 1 mM H_2O_2 was added to the final concentration just before the addition of iron. Control experiments without the addition of Dps showed negligible changes in absorbance during the assay time course.

SPR detection. Surface plasmon resonance (SPR) experiments were performed using a Biacore T100 biosensor system (GE Healthcare) at 25°C in 50 mM Tris-HCl (pH 6.8) at a flow rate of 30 μ l/min. Purified His-Dps was diluted to 0.15 μ M (final dodecamer concentration) in 50 mM Tris-HCl (pH 6.8) and loaded on flow cell 2 (FC2) of a Ni^{2+} -nitrilotriacetic acid (NTA) sensor chip with 5 min of contact time. FC3 and FC4 received His-Dps proteins that were preincubated for 10 min at 25°C with

50 μ M FeCl_2 or 10 mM H_2O_2 , respectively, at the same final concentration and immobilized for the same contact time as the untreated Dps. FC1 had no protein loaded and was used as a reference.

Serial dilutions of purified pGEM (Promega) or a PCR product (the *cat* gene PCR amplified using the CATF and CATR primers and pC46 as the template [756 bp]) were prepared in 50 mM Tris-HCl (pH 6.8) to 50, 25, 12.5, and 6.25 ng/ml. The DNA dilutions were loaded onto the sensor chip using a multicycle kinetics (i.e., after the injection of each dilution, the chip was regenerated with EDTA). Subsequently, the chip was reloaded with Ni^{2+} and His-Dps before the injection of the next DNA dilution. To validate the reproducibility of the SPR response, two cycles of the 12.5-ng/ml dilution were recorded in each experiment. The specificity of the DNA binding was recorded as the response signal difference between each Dps loaded FC and the reference FC1. A 10-min dissociation time was allowed after the addition of each concentration of analyte. SPR signals were analyzed using the Biacore Evaluation software to determine the dissociation constant (K_d).

RESULTS

The *C. jejuni* Dps protein is a dodecamer and has ferroxidase activity under atmospheric oxygen conditions. The *C. jejuni* Dps protein was purified and analyzed using SDS-PAGE. Dps prepared under standard conditions migrated through the polyacrylamide matrix with the expected molecular mass for a monomer of ~ 20 kDa (Fig. 1A, lane 2). However, when Dps was loaded on SDS-PAGE without prior denaturation by boiling, the protein migrated at ~ 250 kDa, suggesting the formation of dodecamers (Fig. 1A, lane 1). Analysis of the crystal structure of *C. jejuni* Dps (Protein Data Bank [PDB] no. 3KWO) confirmed the dodecameric assembly (see Fig. S1A in the supplemental material). Other characteristic Dps features, such as the presence of acidic residues at the iron pore entrance (see Fig. S1B) and conserved residues at the intersubunit ferroxidase center (see Fig. S2A and S2C in the supplemental material), are conserved in the *C. jejuni* Dps.

The formation of the iron oxide core in *C. jejuni* Dps proteins was investigated by monitoring the absorbance at 305 nm after the addition of Fe^{2+} under atmospheric oxygen conditions. Control experiments showed negligible changes in absorbance of an FeCl_2 solution in the absence of Dps. However, when Dps was incubated with FeCl_2 , there was a rapid increase in the absorbance at 305 nm and this increase was Dps concentration dependent (Fig. 1B). Addition of 1 mM H_2O_2 did not change the initial velocity of iron core formation (Fig. 1B). It is worth mentioning that purified *C. jejuni* Dps did not show an absorbance peak in the region between 290 to 320 nm, suggesting that the purified protein does not carry significant amounts of oxidized iron. These data support that *C. jejuni* Dps acts as an iron storage protein that can use O_2 as an iron oxidant. Furthermore, the Dps ferroxidase activity is not affected by H_2O_2 under atmospheric oxygen conditions.

***C. jejuni* Dps protein binds DNA in the presence of Fe^{2+} or H_2O_2 .** The ability of Dps to interact with supercoiled plasmid DNA was analyzed by electrophoretic mobility shift assays. When *C. jejuni* Dps was incubated with DNA, there were no changes in DNA mobility through agarose gel (Fig. 2A, lane 3). However, when Fe^{2+} or H_2O_2 were present, the DNA mobility shifted with the appearance of two DNA bands: a minor band with decreased mobility (Fig. 2A, indicated by arrow II) and a major band that was too large to enter the gel (Fig. 2A, indicated by arrow I). The appearance of these slower-migrating bands was directly proportional to the amount of Dps added (data not shown), indicating

FIG 1 Analysis of the *C. jejuni* Dps protein. (A) One microgram of purified Dps was loaded onto 12% SDS-PAGE gel before (lane 1) or after (lane 2) incubation at 99°C for 10 min. The gel was stained with Coomassie blue. Lane M contains the molecular mass marker (kDa). (B) Spectrophotometric kinetic curve of Fe^{2+} oxidation by *C. jejuni* Dps. Reactions were performed aerobically in 50 mM Tris-HCl (pH 6.8) at 25°C. Reactions started by the addition of 100 μM FeCl_2 ; the formation of iron core was monitored by measuring absorbance (Abs) at 305 nm in a 0.5-cm cuvette. Symbols for conditions are as follows: squares, 0.30 μM Dps; circles, 0.15 μM Dps; triangles, 0.15 μM Dps in the presence of 1 mM H_2O_2 .

that they represent formation of Dps-DNA complexes in a concentration-dependent manner.

Samples containing 15 μg of purified Dps were incubated under the same conditions and resolved using the same gel shown in Fig. 2A; a part of the gel was separated and stained with Coomassie blue to demonstrate the presence and mobility of Dps (Fig. 2B). The addition of either Fe^{2+} or H_2O_2 shifted the migration behavior of purified Dps so dramatically that part of the protein could not enter the gel, suggesting the formation of protein self-aggregates. The formation Dps self-aggregates is well documented in *E. coli* and *H. pylori*, and it correlates with the ability of these proteins to interact with DNA *in vitro* (22–24).

As *C. jejuni* Dps carries two surface-exposed cysteine residues per subunit (see Fig. S2B and S2C in the supplemental material), we suspected that Dps self-aggregation in the presence of Fe^{2+} or H_2O_2 could be caused by the formation of disulfide bonds between different Dps dodecamers. However, the SDS-PAGE profiles of Dps treated with Fe^{2+} or H_2O_2 were identical to those of the untreated Dps in both the presence and absence of β -mercaptoethanol (data not shown). Hence, Dps self-aggregation does not occur by disulfide bond formation and is readily reversed in the presence of SDS.

The capacity of both Fe^{2+} and H_2O_2 to activate Dps-DNA

FIG 2 The DNA binding activity of *C. jejuni* Dps is promoted by either Fe^{2+} or H_2O_2 . (A) The DNA binding activity of *C. jejuni* Dps was analyzed by the capacity to retard the migration of supercoiled pGEM plasmid in 1% agarose gel. The DNA on the gel was stained with ethidium bromide. Binding reactions were performed in 50 mM Tris-HCl (pH 6.8), containing 50 μM FeCl_2 or 10 mM H_2O_2 as indicated. Lanes: 1, DNA only; 2, Dps only; 3, Dps plus DNA; 4, BSA plus DNA. Two different forms of the Dps-DNA complexes are indicated by arrows I and II. (B) Fifteen micrograms of purified Dps was incubated under the same conditions and run in the same agarose gel as in panel A, and the protein was stained with Coomassie blue. Lane 1, buffer only; lane 2, 50 μM FeCl_2 ; lane 3, 10 mM H_2O_2 .

binding was dose dependent. Concentrations of H_2O_2 as low as 10 μM were able to promote DNA binding (see Fig S3 in the supplemental material), and hence, it is likely that H_2O_2 affects Dps activity under relevant physiological conditions. Increasing concentrations of iron favored Dps-DNA interaction; a saturation point was observed when an iron/Dps dodecamer ratio of approximately 25:1 was achieved (see Fig. S4, lane 5, in the supplemental material). Interestingly, this saturation point is just above the 12-iron/Dps ratio, which is the expected ratio required to occupy all the 12 Fe^{2+} binding sites present within the Dps dodecamer. Other divalent metals tested, including Mn^{2+} , Mg^{2+} , and Ca^{2+} , were not able to induce Dps DNA binding (data not shown).

Effects of pH, salts, and Mg^{2+} on the *C. jejuni* Dps-DNA interaction. The DNA binding activity of Dps was further tested in a range of different buffer conditions. Higher pH decreased the Dps-DNA interaction for both Fe^{2+} and H_2O_2 treatments until no DNA binding was observed at pH 8.8 (Fig. 3, compare lanes 3 and 6 to 5 and 8). The H_2O_2 -induced DNA binding activity of Dps seems to be more sensitive to higher pH than that induced by Fe^{2+} : at pH 8, there was some DNA binding to Dps treated with Fe^{2+} , while no DNA binding was detected for Dps treated with H_2O_2 (Fig. 3, lanes 4 and 7).

The presence of NaCl or MgCl_2 decreased the ability of Dps to interact with DNA. In the presence of 0.1 M NaCl or 5 mM MgCl_2 , the Fe^{2+} -induced Dps-DNA complex formation decreased slightly (Fig. 3, lanes 9 and 15) but was significantly affected in the presence of H_2O_2 (Fig. 3, lanes 12 and 17). Throughout, Dps-DNA interactions were still observed for both Fe^{2+} and H_2O_2 treatments at NaCl and MgCl_2 concentrations up to 1 M and 25 mM, respectively (Fig. 3).

Effects of iodoacetamide, DTT, and the presence of cysteine residues on the Dps-DNA interaction. The DNA binding activity in the presence of H_2O_2 has never been reported for Dps proteins, making *C. jejuni* Dps unique. H_2O_2 is too simple structurally to be specifically recognized by a protein; however, this molecule can

FIG 3 DNA binding activity of *C. jejuni* Dps under different buffer conditions. The DNA binding activity of *C. jejuni* Dps was analyzed by the capacity to retard the migration of a supercoiled pGEM plasmid in 1% agarose gel. The DNA on the gel was stained with ethidium bromide. Binding reactions were performed under different conditions, as indicated in the table. Lane 1 is a control without the addition of Dps.

catalyze oxidation of a variety of protein groups (39, 40). Oxidation of cysteine residues and formation of disulfide bonds have been implicated in H₂O₂ sensing in both prokaryotes and eukaryotes (40–42).

Sequence alignments between *C. jejuni*, *E. coli*, and *H. pylori* Dps revealed that the *C. jejuni* Dps has 3 unique cysteine residues (see Fig. S2C in the supplemental material). Two of these (C68 and C85) are surface exposed and located in flexible loops at dimer interface in the protein’s 2-fold symmetry axis (see Fig. S2B). Furthermore, the presence of nearby lysine residues could decrease the pK_a of thiol, rendering C68 and C85 very sensitive to H₂O₂ oxidation in a similar fashion to that described for the archetypical *E. coli* OxyR H₂O₂ sensor transcriptional factor (43).

To verify if cysteine oxidation could be involved in H₂O₂ sensing, DNA binding experiments were performed using Dps that was pretreated with iodoacetamide or DTT. In the presence of iodoacetamide or DTT, the H₂O₂ effect on the DNA binding activity of Dps was abolished (Fig. 4A); however, the iodoacetamide treatment did not affect the ability of Dps to bind DNA in the presence of Fe²⁺ (Fig. 4A, lane 7), suggesting that Fe²⁺ and H₂O₂ induce DNA binding through different mechanisms. The presence of DTT abolished the Dps-DNA interaction in the presence of Fe²⁺ (Fig. 4A, lane 8). However, the migration of the control DNA was also affected (Fig. 4A, lane 4), probably as result of DNA nicking due to radicals that are known to form when Fe²⁺, DTT, and oxygen are combined (44).

The fact that the H₂O₂-induced DNA binding activity of *C. jejuni* Dps was abolished by iodoacetamide or DTT suggests that cysteine oxidation could be involved in H₂O₂ sensing. To verify this hypothesis, a Dps variant in which all three cysteines were converted to serines (C54S C68S C85S) was prepared. This variant kept 75% of the iron oxidation activity (see Fig. S5 in the supplemental material). The DNA binding activity of this variant (induced by Fe²⁺ or H₂O₂) was comparable to that of the wild-type (Fig. 4B). As expected, iodoacetamide, which is not reactive toward the serine, did not affect DNA binding for the C54S C68S C85S Dps variant (Fig. 4B).

These observations exclude a role of cysteine oxidation in H₂O₂ sensing by *C. jejuni* Dps. However, the fact that iodoacetamide blocked DNA binding induced by H₂O₂ suggests that cys-

FIG 4 Effects of iodoacetamide, DTT, and cysteines on Dps-DNA interaction. The DNA binding activity of *C. jejuni* Dps was analyzed by the capacity to retard the migration of supercoiled pGEM plasmid in 1% agarose gel. The DNA on the gel was stained with ethidium bromide. (A) Reaction mixtures contained H₂O₂ or FeCl₂ as indicated (except lanes 1 and 5, which are controls); BSA was used as a negative control. Proteins in lanes 3 and 7 were pretreated with 10 mM iodoacetamide for 30 min. Proteins in lanes 4 and 8 were pretreated with 2 mM DTT for 30 min. (B) Reaction mixtures contained 10 mM H₂O₂. Lane 1, control DNA only; lane 2, Dps C54S C68S C85S mutant; lane 3, C54S C68S C85S mutant pretreated with iodoacetamide.

teine alkylation may sterically affect the interaction between the wild-type Dps and DNA. Hence, the Dps 2-fold symmetry (see Fig. S2B in the supplemental material) is likely to be the site for DNA binding, at least for the H₂O₂ response.

Analysis of the Dps-DNA interaction using surface plasmon resonance. We further analyzed the DNA binding activity of *C. jejuni* Dps using surface plasmon resonance, which is more sensitive than the DNA shift assays. Purified Dps protein samples were either left untreated or were pretreated with H₂O₂ or Fe²⁺ and then mobilized in three different flow cells of a Ni²⁺-NTA Biacore sensor chip; a fourth flow cell without any protein loaded was kept as a reference. Different concentrations of supercoiled plasmid DNA were used as the ligand.

When the experiments were performed at pH 8.8, no Dps-DNA interactions were detected, confirming the results of the electrophoresis mobility shift assays (data not shown). However, when buffer at pH 6.8 was used, Dps was able to interact with plasmid DNA (Fig. 5). Pretreatment of Dps with H₂O₂ or Fe²⁺ increased the affinity of binding to DNA by 8 to 9 orders of magnitude (Fig. 5). The Dps-DNA binding interaction was virtually irreversible in the presence of H₂O₂ and Fe²⁺, such that no dissociation was observed after the removal of the ligand DNA (Fig. 5B and C). However, in the absence of H₂O₂ and Fe²⁺, partial dissociation of the Dps-DNA complex was observed within 30 s of the removal of the DNA (Fig. 5A).

The addition of 1 mM DTT resulted in partial dissociation of the Dps-DNA complex that was induced by Fe²⁺ or H₂O₂, as analyzed by SPR (data not shown); this confirms the results obtained in the gel shift assays (Fig. 4A, lane 8). Hence, the Dps-DNA interaction is abolished in the presence of DTT; however, the molecular mechanism underlying such a response remains unknown.

The SPR data essentially confirmed the results obtained using the gel shift. Furthermore, the higher sensitivity of the SPR allowed the detection of low-affinity Dps-DNA interactions that

FIG 5 Interaction between *C. jejuni* Dps and plasmid DNA at pH 6.8 observed by SPR. The response difference between the treatment and the reference flow cell is shown (Δ RU). The arrows indicate the addition (time zero) and removal (time 60 s) of the ligand DNA. (A) Interaction between DNA and Dps without any pretreatment of the protein. (B) Interaction between DNA and the Dps pretreated with Fe^{2+} . (C) Interactions between DNA and Dps pretreated with H_2O_2 . The concentrations of DNA tested were 6.25, 12.5 (in duplicate), 25, and 50 ng/ml. The K_d s were determined using the Biacore Evaluation software.

could not be observed using gel shifts: the interaction between untreated Dps and DNA under pH 6.8 (Fig. 5A) but not at pH 8.8 (data not shown). The Dps-DNA interaction was also detected by SPR when a linear relaxed PCR product DNA was used as ligand at pH 6.8. Hence, the Dps-DNA complex formation occurs independently of the DNA's overall topological structure.

Dps protects DNA from DNase I and binds DNA under oxidative stress conditions. The ability of Dps to interact with DNA suggested that it could protect DNA from enzymatic and/or oxidative damage when the Fe^{2+} and H_2O_2 levels are high. Hence, we evaluated the capacity of Dps to protect plasmid DNA from both DNase I and from hydroxyl radicals. Treatment of purified pGEM (Promega) plasmid DNA with DNase I resulted in nearly complete DNA degradation (Fig. 6A). However, when Dps and Fe^{2+} or H_2O_2 were present, the DNA signal was still observed in the well of the gel after agarose gel electrophoresis (Fig. 6A), supporting the hypothesis that Dps can physically protect DNA.

The combination of Fe^{2+} and H_2O_2 leads to the formation of hydroxyl radicals, which in turn cause double-stranded DNA breaks that can be accessed by the conversion of supercoiled circular plasmid DNA to linear DNA by electrophoresis (12). Indeed, when pGEM plasmid DNA was treated with Fe^{2+} and H_2O_2 , it was converted to a slower-migrating band that corresponds to the mo-

FIG 6 The *C. jejuni* Dps protein protects DNA from DNase I and hydroxyl radicals. (A) Reactions were performed in 50 mM Tris-HCl (pH 6.8) containing 0.25 mM MgCl_2 and 0.01 mM CaCl_2 at 25°C. The purified Dps protein was pretreated with FeCl_2 or H_2O_2 for 10 min followed by addition of 0.01 U DNase I. After 15 min, reactions were quenched with 100 mM EDTA and analyzed on an agarose gel stained with ethidium bromide. (B) Supercoiled pGEM plasmid was incubated with BSA (lanes 1 and 3) or with Dps (lanes 2 and 4) in 50 mM Tris-HCl (pH 6.8) for 30 min at 25°C. Hydroxyl radical generation through the Fenton reaction was achieved by adding 100 μM FeCl_2 and 10 mM H_2O_2 (lanes 3 and 4). Reactions were quenched with 5 mM the iron chelator bipyridyl and analyzed on an agarose gel stained with ethidium bromide.

lecular mass of linear plasmid DNA, suggesting double-stranded DNA breaks (Fig. 6B). However, when Dps was present, all DNA signal was retained in the well of the gel (Fig. 6B), suggesting the formation of a protein-DNA complex under oxidative stress conditions that could potentially protect the DNA from degradation. Unfortunately, we could not access the integrity of the DNA in isolation after Dps treatment because all attempts to dissociate the Dps-DNA complex using organic solvent extraction, DTT, hot SDS, or DNA cleanup kits were unsuccessful.

Dps enhances resistance to H_2O_2 in vivo. In order to analyze if Dps could protect *C. jejuni* against H_2O_2 in vivo, a *C. jejuni* dps knockout strain was constructed and its resistance to H_2O_2 was compared to that of the parental 11168-O strain. The parental strain viable cell count was reduced by 2 logs after challenge with 5 mM H_2O_2 , while the dps mutant strain was much less resistant to H_2O_2 as a 6-log reduction in cell count was observed (Table 2), confirming previous observations (17).

E. coli cells lacking Dps showed a significant change in protein expression profile, as indicated by two-dimensional gel electrophoresis (7). Hence, the reduced resistance of the *C. jejuni* dps mutant to H_2O_2 could be a pleiotropic effect of the dps mutation. However, we noted that expression of the *C. jejuni* Dps protein in host *E. coli* cells was able to increase the resistance of *E. coli* to H_2O_2 stress by 1 log (Table 2), suggesting that, at least in part, the H_2O_2 Dps protective effect is direct.

TABLE 2 The *C. jejuni* Dps protein confers resistance to H₂O₂

Strain	No. of viable cells ^a	
	Control	+5 mM H ₂ O ₂
<i>C. jejuni</i>		
11168-O	4.2×10^8	4.6×10^6
LFH2 <i>dps</i> mutant	4.9×10^8	1.9×10^2
<i>E. coli</i>		
BL21(pET19b)	1.6×10^7	3.5×10^4
BL21(pLHPETcj1534c)	1.2×10^7	5.0×10^5

^a The *C. jejuni* and *E. coli* strains were diluted in LB to OD₆₀₀s of 0.25 and 0.35, respectively. Cells were incubated at 37°C in the absence (control) or presence of H₂O₂ (5 mM). After 30 min, viable cells were determined by serial dilution CFU counting.

DISCUSSION

In this work, we used gel shift, DNase I protection, and SPR to show that *C. jejuni* Dps has the unique ability to sense the Fe²⁺ and H₂O₂ levels and bind to DNA in response to increasing concentrations of Fe²⁺ or H₂O₂. Furthermore, the Dps-DNA interaction was maximized at pHs below 7. When low pH and Fe²⁺ or H₂O₂ were combined, the Dps-DNA interaction was very tight, with *K_d*s in the picomolar range (Fig. 5), the Dps-DNA interaction was virtually irreversible, such that no dissociation was observed after the removal of the ligand DNA in SPR (Fig. 5B and C), and all attempts to dissociate the Dps-DNA complex formed *in vitro* were unsuccessful.

It is well established that Dps proteins confer resistance to reactive oxygen species. This is achieved by removal of Fe²⁺ from the cytoplasm and storing this metal in the oxidized form inside the protein cage, thereby reducing Fenton chemistry (4). Here we show that the *C. jejuni* Dps is able to oxidize iron (Fig. 1B); however, unlike typical Dps proteins that preferentially use H₂O₂, the *C. jejuni* Dps can efficiently use O₂ as an oxidant. Notably, ferroxidase activity was also detected in aerated buffers without added H₂O₂, and furthermore, addition of H₂O₂ did not alter this activity (Fig. 1B).

Dps proteins from several organisms can physically interact with DNA (7, 22, 25, 28, 45). In this study, we demonstrated that *C. jejuni* Dps can bind DNA in the presence of Fe²⁺ or H₂O₂ using electrophoretic mobility shift and DNase I protection assays (Fig. 2A and 5A). A previous report failed to detect *C. jejuni* Dps-DNA interaction, probably because no Fe²⁺ or H₂O₂ was used (17). We further analyzed the *C. jejuni* Dps-DNA interaction by SPR, and we were able to conclusively demonstrate that Dps protein was able to interact with DNA in the absence of Fe²⁺ or H₂O₂; however, much stronger binding was observed when Dps was pre-treated with Fe²⁺ or H₂O₂ (Fig. 5), further validating the results obtained by electrophoretic mobility shift assays.

The presence of Fe²⁺ or H₂O₂ could induce the Dps-DNA interaction by affecting the DNA, Dps, or both. When DNA was preincubated with Fe²⁺ or H₂O₂ and then purified, no interaction with Dps was detected by electrophoretic mobility shift assay in the absence of Fe²⁺ or H₂O₂ (data not shown). During the SPR analysis, Dps was preincubated with Fe²⁺ or H₂O₂ and these molecules were washed away before the addition of the DNA. The increase in binding affinity for the Fe²⁺- and H₂O₂-treated Dps samples (Fig. 5) suggests that both Fe²⁺ and H₂O₂ have an effect on the Dps structure. This is corroborated by the fact that Dps self-aggregated when treated with Fe²⁺ or H₂O₂ (Fig. 2B). Indeed,

the ability of the *H. pylori* and *E. coli* Dps proteins to bind DNA closely correlated with the capacity of Dps to form self-aggregates *in vitro* (22, 24).

High NaCl concentrations reduced the Dps-DNA interactions (Fig. 3), suggesting that ionic interactions could be involved in Dps-DNA binding. The predicted surface of the *C. jejuni* Dps resembles the *H. pylori* orthologue (i.e., it is highly positively charged due to the presence of lysine side chains) (33); furthermore, both *C. jejuni* Dps and *H. pylori* Dps do not carry the N-terminal extension present in the *E. coli* Dps that has been implicated in DNA binding (see Fig. S2C in the supplemental material). The positively charged *C. jejuni* and *H. pylori* Dps surfaces are likely to be responsible for the interaction with the negatively charged DNA (22). This model could explain why Mg²⁺ negatively affected the Dps-DNA interaction (Fig. 3) as Mg²⁺ can bind to the DNA phosphates counterbalancing its intrinsic negative charge. The Dps-DNA binding is strongly influenced by the pH, such that DNA interaction was strong in pH 6.8 and not detected at pH 8.8 (Fig. 3). Similar results have been reported for *H. pylori* and *E. coli* Dps (20, 22). We speculate that the rise in pH from 6.8 to 8.8 could result in deprotonation of the lysine side chains on the *C. jejuni* Dps surface reducing its overall positive charge, thereby reducing its ability to interact with DNA. Even though the ε-amino group of lysine has typical p*K_a* in the range of 10, the presence of nearby lysine residues could decrease the p*K_a*, resulting in lysine deprotonation at pHs around 8.

The activation of Dps-DNA binding by Fe²⁺ observed here has also been reported in *H. pylori* (16). Fe²⁺ titration experiments suggest that the Dps-DNA interaction is maximized when all of the 12 Fe²⁺ binding sites of Dps are occupied (see Fig. S4 in the supplemental material). The binding of Fe²⁺ could increase the overall positive charge of Dps protein, favoring interaction with the negatively charged DNA. Alternatively, Fe²⁺ binding could affect the overall Dps structure, enhancing the affinity for DNA.

The identification of H₂O₂-induced DNA binding of *C. jejuni* Dps is novel, and it remains to be determined if this is a unique feature of the *C. jejuni* Dps. In *E. coli*, Dps is induced in the presence of H₂O₂ by the transcriptional factor OxyR (46); once accumulated, Dps has default DNA binding activity (7). In contrast, *C. jejuni* Dps expression does not respond to H₂O₂ (17), but Dps DNA binding activity does (Fig. 2A). The final output is the same in both species: Dps will bind DNA whenever H₂O₂ accumulates. The fact that *C. jejuni* lacks an OxyR orthologue might explain why *C. jejuni* Dps has evolved to directly sense H₂O₂.

Even though we could not determine how H₂O₂ activates the *C. jejuni* Dps-DNA binding at the molecular level, our results excluded the participation of cysteine oxidation in this process (Fig. 4B). The fact that iodoacetamide blocked the H₂O₂-induced (but not the Fe²⁺-induced) Dps-DNA binding (Fig. 4A) indicates that these molecules may activate DNA binding through different mechanisms. Furthermore, it suggests the 2-fold-symmetry surface of Dps (see Fig. S2B in the supplemental material) as the site for DNA interaction in the presence of H₂O₂. Similarly, this Dps surface has been considered to be the DNA binding site for *Deinococcus radiodurans* Dps-1 (32, 47).

The sum of the ferroxidase and DNA binding activities of *C. jejuni* Dps is likely to confer the DNA protection against hydroxyl radicals observed *in vitro* (Fig. 5B) and to be responsible for the reduced survival of *C. jejuni* *dps* mutants to H₂O₂ *in vivo* (Table 2) and inside macrophages (34). An H₂O₂ burst is the primary host

response against pathogens. The *C. jejuni* Dps binds DNA in response to H_2O_2 concentrations as low as $10\ \mu\text{M}$ (see Fig. S3 in the supplemental material), which is far below the amount generated inside macrophages.

During its life cycle, *C. jejuni* has to cope with the acidic environment found in its host's digestive tract and inside phagolysosomes. Our data suggest that Dps could act to protect DNA under low pH; this hypothesis is supported by the fact that *C. jejuni* Dps expression is induced under acid stress (48) and during host colonization (35). Furthermore, it was shown that *E. coli* Dps contributes to acid tolerance by protecting DNA under low pH (20), and a *dps* gene isolated from a metagenome library conferred acid resistance to *E. coli* (49).

The concerted activation of *C. jejuni* Dps DNA binding in response to low pH, H_2O_2 , and Fe^{2+} would effectively protect DNA during host colonization and explain, at least in part, the described inability of *C. jejuni* *dps* mutants to colonize poultry and piglets (34, 35). Other animal pathogens, such as *Haemophilus influenzae* and *Salmonella enterica*, also rely on Dps for bacterial resistance during host colonization (21, 50). The critical protective role played by bacterial Dps during host colonization indicates that Dps could be an interesting target for the development of vaccines and/or antimicrobial drugs.

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REFERENCES

- Blaser MJ. 1997. Epidemiologic and clinical features of *Campylobacter jejuni* infections. *J. Infect. Dis.* 176:S103–S105.
- Allos BM. 1997. Association between *Campylobacter infection* and Guillain-Barre syndrome. *J. Infect. Dis.* 176:S125–S128.
- Israeli E, Agmon-Levin N, Blank M, Chapman J, Shoenfeld Y. 2012. Guillain-Barre syndrome, a classical autoimmune disease triggered by infection or vaccination. *Clin. Rev. Allergy Immunol.* 42:121–130.
- Zeth K. 2012. Dps biomineralizing proteins: multifunctional architects of nature. *Biochem. J.* 445:297–311.
- Carrondo MA. 2003. Ferritins, iron uptake and storage from the bacterioferritin viewpoint. *EMBO J.* 22:1959–1968.
- Bou-Abdallah F. 2010. The iron redox and hydrolysis chemistry of the ferritins. *Biochim. Biophys. Acta* 1800:719–731.
- Almiron MA, Link J, Furlong D, Kolter R. 1992. A novel DNA-binding protein with regulatory and protective roles in starved *Escherichia coli*. *Genes Dev.* 6:2646–2654.
- Roy S, Saraswathi R, Chatterji D, Vijayan M. 2008. Structural studies on the second *Mycobacterium smegmatis* Dps: invariant and variable features of structure, assembly and function. *J. Mol. Biol.* 375:948–959.
- Chiancone E, Ceci P. 2010. The multifaceted capacity of Dps proteins to combat bacterial stress conditions: detoxification of iron and hydrogen peroxide and DNA binding. *Biochim. Biophys. Acta* 1800:798–805.
- Grant RA, Filman DJ, Finkel SE, Kolter R, Hogle JM. 1998. The crystal structure of Dps, a ferritin homolog that binds and protects DNA. *Nat. Struct. Biol.* 5:294–303.
- Ilari A, Stefanini S, Chiancone E, Tsernoglou D. 2000. The dodecameric ferritin from *Listeria innocua* contains a novel intersubunit iron-binding site. *Nat. Struct. Biol.* 7:38–43.
- Martinez A, Kolter R. 1997. Protection of DNA during oxidative stress by the nonspecific DNA-binding protein Dps. *J. Bacteriol.* 179:5188–5194.
- Ceci P, Ilari A, Falvo E, Chiancone E. 2003. The Dps protein of *Agrobacterium tumefaciens* does not bind to DNA but protects it toward oxidative cleavage: X-ray crystal structure, iron binding, and hydroxyl-radical scavenging properties. *J. Biol. Chem.* 278:20319–20326.
- Tu WY, Pohl S, Gizynski K, Harwood CR. 2012. The iron-binding protein Dps2 confers peroxide stress resistance on *Bacillus anthracis*. *J. Bacteriol.* 194:925–931.
- Nair S, Finkel SE. 2004. Dps protects cells against multiple stresses during stationary phase. *J. Bacteriol.* 186:4192–4198.
- Wang G, Hong Y, Olczak A, Maier SE, Maier RJ. 2006. Dual roles of *Helicobacter pylori* NapA in inducing and combating oxidative stress. *Infect. Immun.* 74:6839–6846.
- Ishikawa T, Mizunoe Y, Kawabata S, Takade A, Harada M, Wai SN, Yoshida S. 2003. The iron-binding protein Dps confers hydrogen peroxide stress resistance to *Campylobacter jejuni*. *J. Bacteriol.* 185:1010–1017.
- Cooksley C, Jenks PJ, Green A, Cockayne A, Logan RP, Hardie KR. 2003. NapA protects *Helicobacter pylori* from oxidative stress damage, and its production is influenced by the ferric uptake regulator. *J. Med. Microbiol.* 52:461–469.
- Olczak AA, Wang G, Maier RJ. 2005. Up-expression of NapA and other oxidative stress proteins is a compensatory response to loss of major *Helicobacter pylori* stress resistance factors. *Free Radic. Res.* 39:1173–1182.
- Jeong KC, Hung KF, Baumler DJ, Byrd JJ, Kaspar CW. 2008. Acid stress damage of DNA is prevented by Dps binding in *Escherichia coli* O157:H7. *BMC Microbiol.* 8:181. doi:10.1186/1471-2180-8-181.
- Pang B, Hong W, Kock ND, Swords WE. 2012. Dps promotes survival of nontypeable *Haemophilus influenzae* in biofilm communities in vitro and resistance to clearance *in vivo*. *Front. Cell Infect. Microbiol.* 2:58. doi:10.3389/fcimb.2012.00058.
- Ceci P, Mangiarotti L, Rivetti C, Chiancone E. 2007. The neutrophil-activating Dps protein of *Helicobacter pylori*, HP-NAP, adopts a mechanism different from *Escherichia coli* Dps to bind and condense DNA. *Nucleic Acids Res.* 35:2247–2256.
- Su M, Cavallo S, Stefanini S, Chiancone E, Chasteen ND. 2005. The so-called *Listeria innocua* ferritin is a Dps protein. Iron incorporation, detoxification, and DNA protection properties. *Biochemistry* 44:5572–5578.
- Ceci P, Cellai S, Falvo E, Rivetti C, Rossi GL, Chiancone E. 2004. DNA condensation and self-aggregation of *Escherichia coli* Dps are coupled phenomena related to the properties of the N-terminus. *Nucleic Acids Res.* 32:5935–5944.
- Timoteo CG, Guilherme M, Penas D, Folgosa F, Tavares P, Pereira AS. 2012. *Desulfovibrio vulgaris* bacterioferritin uses H_2O_2 as a co-substrate for iron oxidation and reveals DPS-like DNA protection and binding activities. *Biochem. J.* 446:125–133.
- Zhao G, Ceci P, Ilari A, Giangiacomo L, Laue TM, Chiancone E, Chasteen ND. 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.
- Wolf SG, Frenkiel D, Arad T, Finkel SE, Kolter R, Minsky A. 1999. DNA protection by stress-induced biocrystallization. *Nature* 400:83–85.
- Castruita M, Saito M, Schottel PC, Elmegreen LA, Myneni S, Stiefel EI, Morel FM. 2006. Overexpression and characterization of an iron storage and DNA-binding Dps protein from *Trichodesmium erythraeum*. *Appl. Environ. Microbiol.* 72:2918–2924.
- Stillman TJ, Upadhyay M, Norte VA, Sedelnikova SE, Carradus M, Tzokov S, Bullough PA, Shearman CA, Gasson MJ, Williams CH, Artymiuk PJ, Green J. 2005. The crystal structures of *Lactococcus lactis* MG1363 Dps proteins reveal the presence of an N-terminal helix that is required for DNA binding. *Mol. Microbiol.* 57:1101–1112.
- Ceci P, Ilari A, Falvo E, Giangiacomo L, Chiancone E. 2005. Reassessment of protein stability, DNA binding, and protection of *Mycobacterium smegmatis* Dps. *J. Biol. Chem.* 280:34776–34785.
- Bhattacharyya G, Grove A. 2007. The N-terminal extensions of *Deinococcus radiodurans* Dps-1 mediate DNA major groove interactions as well as assembly of the dodecamer. *J. Biol. Chem.* 282:11921–11930.
- Nguyen KH, Grove A. 2012. Metal binding at the *Deinococcus radiodurans* Dps-1 N-terminal metal site controls dodecameric assembly and DNA binding. *Biochemistry* 51:6679–6689.
- Zanotti G, Papinutto E, Dundon W, Battistutta R, Seveso M, Giudice G, Rappuoli R, Montecucco C. 2002. Structure of the neutrophil-activating protein from *Helicobacter pylori*. *J. Mol. Biol.* 323:125–130.
- Theoret JR, Cooper KK, Glock RD, Joens LA. 2011. A *Campylobacter jejuni* Dps homolog has a role in intracellular survival and in the development of campylobacteriosis in neonate piglets. *Foodborne Pathog. Dis.* 8:1263–1268.
- Theoret JR, Cooper KK, Zekarias B, Roland KL, Law BF, Curtiss R, Joens LA. 2012. The *Campylobacter jejuni* Dps homologue is important for *in vitro* biofilm formation and cecal colonization of poultry and may

- serve as a protective antigen for vaccination. Clin. Vaccine Immunol. 19: 1426–1431.
36. Sambrook J, Fritsch EF, Maniatis T. 1989. Molecular cloning. a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
37. Laemmli UK. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680–685.
38. Bradford MM. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72:248–254.
39. Shacter E. 2000. Quantification and significance of protein oxidation in biological samples. Drug Metab. Rev. 32:307–326.
40. Rhee SG, Bae YS, Lee SR, Kwon J. 2000. Hydrogen peroxide: a key messenger that modulates protein phosphorylation through cysteine oxidation. Sci. STKE 2000:e1. doi:10.1126/stke.2000.53.pe1.
41. Toledano MB, Delaunay A, Monceau L, Tacnet F. 2004. Microbial H₂O₂ sensors as archetypical redox signaling modules. Trends Biochem. Sci. 29:351–357.
42. Zheng M, Aslund F, Storz G. 1998. Activation of the OxyR transcription factor by reversible disulfide bond formation. Science 279:1718–1721.
43. Choi H, Kim S, Mukhopadhyay P, Cho S, Woo J, Storz G, Ryu SE. 2001. Structural basis of the redox switch in the OxyR transcription factor. Cell 105:103–113.
44. Netto LE, Stadtman ER. 1996. The iron-catalyzed oxidation of dithiothreitol is a biphasic process: hydrogen peroxide is involved in the initiation of a free radical chain of reactions. Arch. Biochem. Biophys. 333:233–242.
45. Grove A, Wilkinson SP. 2005. Differential DNA binding and protection by dimeric and dodecameric forms of the ferritin homolog Dps from *Deinococcus radiodurans*. J. Mol. Biol. 347:495–508.
46. Altuvia S, Almiron M, Huisman G, Kolter R, Storz G. 1994. The dps promoter is activated by OxyR during growth and by IHF and sigma S in stationary phase. Mol. Microbiol. 13:265–272.
47. Nguyen KH, Smith LT, Xiao L, Bhattacharyya G, Grove A. 2012. On the stoichiometry of *Deinococcus radiodurans* Dps-1 binding to duplex DNA. Proteins 80:713–721.
48. Birk TT, Wik MT, Lametsch R, Knochel S. 2012. Acid stress response and protein induction in *Campylobacter jejuni* isolates with different acid tolerance. BMC Microbiol. 12:174. doi:10.1186/1471-2180-12-174.
49. Guazzaroni ME, Morgante V, Mirete S, González-Pastor JE. 15 October 2012. Novel acid resistance genes from the metagenome of the Tinto River, an extremely acidic environment. Environ. Microbiol. [Epub ahead of print.] doi:10.1111/1462-2920.12021.
50. Halsey TA, Vazquez-Torres A, Gravidahl DJ, Fang FC, Libby SJ. 2004. The ferritin-like Dps protein is required for *Salmonella enterica* serovar Typhimurium oxidative stress resistance and virulence. Infect. Immun. 72:1155–1158.
51. Skirrow MB. 1977. *Campylobacter* enteritis: a “new” disease. Br. Med. J. 2:9–11.