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$_2$O$_2$ 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$_2$O$_2$ 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$_2$O$_2$ 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$_2$O$_2$ in vivo. The concerted activation of Dps-DNA binding in response to low pH, H$_2$O$_2$, 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 (H$_2$O$_2$ + Fe$^{2+}$ $\rightarrow$ OH$^-$ + OH$^+$ + 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 an iron-oxide form Fe$_2$O$_3$, trapped in the protein cage (6).

One particular subfamily of ferritins is Dps (DNA binding protein from starved cells), first characterized in Escherichia coli (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 condensate the DNA is related to its capacity to self-aggregate under certain conditions (10). Dps-DNA binding activity correlates 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$_2$O$_2$ 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$_2$O$_2$ 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$_2$O$_2$ in vivo. The concerted activation of Dps-DNA binding in response to low pH, H$_2$O$_2$, and Fe$^{2+}$ may protect C. jejuni DNA during host colonization.

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$_2$O$_2$ 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
TABLE 1  C. jejuni strains, plasmids, and primers used in this study

<table>
<thead>
<tr>
<th>Strain, plasmid, or primer</th>
<th>Genotype, phenotype, or sequencea</th>
<th>Source or reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>11168-O</td>
<td>Wild type (human isolated strain), originally strain 5636/77</td>
<td>S1</td>
</tr>
<tr>
<td>LFH2</td>
<td>gj1534c::cat Cm’</td>
<td>This work</td>
</tr>
</tbody>
</table>

Plasmids

- pCR-BluntII-TOPO
- pLHPCRcj1534c
- pET19b
- pLHPCETcj1534c
- pLHPCETcj1534c-C-S
- pC6
- pGEM
- pLHPCRCj1534cCAT

C. jejuni plasmids, and oligonucleotides used in PCRs are listed in Table 1.

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 QIAprep Midi Kit (Qiagen). Enzymes were obtained from New England BioLabs (NEB) and used according to the manufacturer’s 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 Ndel 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 Ndel-BamHI sites, producing the plasmid pLHPCETcj1534c, which expressed C. jejuni Dps with an N-terminal His tag.

E. coli BL21 (ADE3) carrying pLHPCETcj1534c 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 4 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 gene carrying the C54S, C68S, and C85S mutations and flanked by the Ndel and BamHI restriction sites were synthesized and cloned into pDTSmarKm vector by Integrated DNA Technologies (IDT). The mutant genes were subcloned into the expression vector pET19b using the Ndel-BamHI sites, producing the plasmid pLHPCETcj1534c-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 plLHPCRcj1534c plasmid was amplified by inverted PCR using the Expand long template PCR system (Roche) and the Cj1534c InvF and 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 plLHPCRcj1534cCAT. 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 Ndel, 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 μM (final dodecamer concentration) in 2 mM pGEM (Promega). For the experiments in the presence of Fe^{3+}, preweighed FeCl_3 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 μM (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, di-thiothreitol (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 μl for 10 min at 25°C, the reaction was mixed (at 10:2 ratio) with 50% glycerol solution. Iodoacetamide and DTT were removed using a desalting column (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_3 was added to final concentration of 50 μM 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 bipyrildil to 5 mM. The integrity of the DNA was analyzed by 1% agarose gel electrophoresis.

(ii) Nuclease cleavage. Purified C. jejuni Dps (0.15 μM 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-μl reaction mixtures. When indicated, FeCl_3 or H_2O_2 was present at final concentrations of 50 μM 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 (pLHPETGC1534c) 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 μM or 0.3 μM (final dodecamer concentration) in 400 μl 50 mM Tris-HCl (pH 6.8). Freshly prepared FeCl_3 was added to a final concentration of 100 μM, 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 μl/min. Purified His-Dps was diluted to 0.15 μM (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 μM FeCl_3 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 ferric oxidase 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 ferric oxidase 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^{3+} under atmospheric oxygen conditions. Control experiments showed negligible changes in absorbance of an FeCl_3 solution in the absence of Dps. However, when Dps was incubated with FeCl_3, 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 ferric oxidase 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
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 Fe²⁺ or H₂O₂ 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 of Dps self-aggregates is well documented in C. jejuni 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 Fe²⁺ or H₂O₂ could be caused by the formation of disulfide bonds between different Dps dodecamers. However, the SDS-PAGE profiles of Dps treated with Fe²⁺ or H₂O₂ 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 Fe²⁺ and H₂O₂ to activate Dps-DNA binding was dose-dependent. Concentrations of H₂O₂ as low as 10 μM were able to promote DNA binding (see Fig S3 in the supplemental material), and hence, it is likely that H₂O₂ 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 Fe²⁺ binding sites present within the Dps dodecamer. Other divalent metals tested, including Mn²⁺, Mg²⁺, and Ca²⁺, were not able to induce Dps DNA binding (data not shown).

**Effects of pH, salts, and Mg²⁺ 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 Fe²⁺ and H₂O₂ treatments until no DNA binding was observed at pH 8.8 (Fig. 3, compare lanes 3 and 6 to 5 and 8). The H₂O₂-induced DNA binding activity of Dps seems to be more sensitive to higher pH than that induced by Fe²⁺: at pH 8, there was some DNA binding to Dps treated with Fe²⁺, while no DNA binding was detected for Dps treated with H₂O₂ (Fig. 3, lanes 4 and 7).

The presence of NaCl or MgCl₂ decreased the ability of Dps to interact with DNA. In the presence of 0.1 M NaCl or 5 mM MgCl₂, the Fe²⁺-induced Dps-DNA complex formation decreased slightly (Fig. 3, lanes 9 and 15) but was significantly affected in the presence of H₂O₂ (Fig. 3, lanes 12 and 17). Throughout, Dps-DNA interactions were still observed for both Fe²⁺ and H₂O₂ treatments at NaCl and MgCl₂ 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 of Dps in the presence of H₂O₂ has never been reported for Dps proteins, making C. jejuni Dps unique. H₂O₂ is too simple structurally to be specifically recognized by a protein; however, this molecule can
catalyze oxidation of a variety of protein groups (39, 40). Oxida-
tion of cysteine residues and formation of disulfide bonds have
been implicated in H$_2$O$_2$ sensing in both prokaryotes and eu-
karyotes (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).

FIG 3  DNA binding activity of C. jejuni Dps under different buffer condi-
tions. 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.

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 H$_2$O$_2$ or FeCl$_3$ as indicated (except lanes 1 and 5, which are con-
trols); 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 H$_2$O$_2$. Lane 1, control DNA only; lane 2, Dps C54S/C68S/C85S mutant;
lane 3, C54S/C68S/C85S mutant pretreated with iodoacetamide.

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 sensi-
tive than the DNA shift assays. Purified Dps protein samples were
either left untreated or were pretreated with H$_2$O$_2$ or Fe$^{2+}$
and then mobilized in three different flow cells of a Ni$^{2+}$-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 H$_2$O$_2$ or Fe$^{2+}$
increased the affinity of binding to DNA by 8 to 9 orders of mag-
nitude (Fig. 5). The Dps-DNA binding interaction was virtually
irreversible in the presence of H$_2$O$_2$ and Fe$^{2+}$, such that no disso-
ciation was observed after the removal of the ligand DNA (Fig. 5B
and C). However, in the absence of H$_2$O$_2$ and Fe$^{2+}$, 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 Fe$^{2+}$ or H$_2$O$_2$, as
analyzed by SPR (data not shown); this confirms the results ob-
tained in the gel shift assays (Fig. 4A, lane 8). Hence, the Dps-DNA
interaction is abolished in the presence of DTT; however, the mol-
ecular 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 al-
lowed the detection of low-affinity Dps-DNA interactions that
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\(^{2+}/H_2O_2\) 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 molecular 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 protected 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
<table>
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<tbody>
<tr>
<td>C. jejuni</td>
<td>Control +5 mM H2O2</td>
</tr>
<tr>
<td>11168-O</td>
<td>4.2 × 10⁶</td>
</tr>
<tr>
<td>LHF2-dps mutant</td>
<td>4.9 × 10⁶</td>
</tr>
<tr>
<td>E. coli</td>
<td></td>
</tr>
<tr>
<td>BL21(pET19b)</td>
<td>1.6 × 10⁷</td>
</tr>
<tr>
<td>BL21(pLHPETcj1534c)</td>
<td>1.2 × 10⁷</td>
</tr>
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</table>

aThe C. jejuni and E. coli strains were diluted in LB to OD₆₀₀ 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 Kₛ 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, ferrooxidase 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 way 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 pKₐ in the range of 10, the presence of nearby lysine residues could decrease the pKₐ, 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 ferrooxidase 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$_2$O$_2$ concentrations as low as 10 μM (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$_2$O$_2$, 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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