The *dps* Gene of Symbiotic "Candidatus Legionella jeonii" in *Amoeba proteus* Responds to Hydrogen Peroxide and Phagocytosis†

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To survive in host cells, intracellular pathogens or symbiotic bacteria require protective mechanisms to overcome the oxidative stress generated by phagocytic activities of the host. By genomic library tagging, we cloned a *dps* (stands for DNA-binding protein from starved cells) gene of the symbiotic "Candidatus Legionella jeonii" organism (called the X bacterium) (*dps*<sub>X</sub>) that grows in *Amoeba proteus*. The gene encodes a 17-kDa protein (pI 5.19) with 91% homology to Dps and DNA-binding ferritin-like proteins of other organisms. The cloned gene complemented the *dps* mutant of Escherichia coli and conferred resistance to hydrogen peroxide. Dps<sub>X</sub> proteins purified from *E. coli* transformed with the *dps*<sub>X</sub> gene were in oligomeric form, formed a complex with pBluescript SKII DNA, and protected the DNA from DNase I digestion and H<sub>2</sub>O<sub>2</sub>-mediated damage. The expression of the *dps*<sub>X</sub> gene in "Candidatus Legionella jeonii" was enhanced when the host amoeba was treated with 2 mM H<sub>2</sub>O<sub>2</sub> and by phagocytic activities of the host cell. These results suggested that the Dps protein has a function protective of the bacterial DNA and that its gene expression responds to oxidative stress generated by phagocytic activities of the host cell. With regard to the fact that invasion of *Legionella* sp. into respiratory phagocytic cells causes pneumonia in mammals, further characterization of *dps*<sub>X</sub> expression in the *Legionella* sp. that multiplies in a protozoan host in the natural environment may provide valuable information toward understanding the protective mechanisms of intracellular pathogens.

The generation of various reactive oxygen species (ROS) by respiratory bursts during phagocytosis is the first line of defense against invading pathogens for neutrophils and macrophages (5, 12). When phagocytosis takes place, the oxygen uptake increases due to the activation of NADPH oxidase associated with the plasma membrane (5). This enzyme oxidizes NADPH and O<sub>2</sub> to form a superoxide anion (O<sub>2</sub><sup>-</sup>) and pours O<sub>2</sub><sup>-</sup> into the phagocytic vacuole (4). Subsequently, superoxide dismutase catalyzes the conversion of O<sub>2</sub><sup>-</sup> to H<sub>2</sub>O<sub>2</sub>. At the expense of H<sub>2</sub>O<sub>2</sub>, myeloperoxidase oxidizes halide ions to hypohalous acids (14). The additional production of oxidants, especially hydroxyl radical and singlet oxygen, is established by nonenzymatic reactions. Most phagocytes use these oxidants to kill invading microorganisms. ROS are potent cellular oxidizing agents that damage proteins and membrane lipids. They also attack bases or deoxyriboses of DNA to produce damaged bases or strand breaks (12, 23). Among these ROS, H<sub>2</sub>O<sub>2</sub> can penetrate cell membranes rapidly and react with Fe(II) ions to form various free radicals (37), causing various forms of oxidative damage to phagocytic cells. For their protection against oxidative damage during phagocytosis, phagocytic cells have two antioxidant enzymes, e.g., catalases and peroxidases. One of the typical antioxidants in neutrophils is glutathione peroxidase. In organisms lacking glutathione peroxidase, thioredoxin-dependent peroxidase (TPx) replaces glutathione peroxidase to reduce H<sub>2</sub>O<sub>2</sub> (22). TPx was first isolated from *Saccharomyces cerevisiae* (8, 17) and was later found to be widely distributed in prokaryotic and eukaryotic cells. In addition to removing H<sub>2</sub>O<sub>2</sub>, TPx has also been suggested to play a role as a direct free radical scavenger by reducing sulfur-containing radicals (8). Due to this activity, TPx can prevent oxidative damage induced by an oxidation system capable of generating ROS in the presence of a thiol-reducing equivalent (21).

To minimize damage caused by ROS, invading microorganisms have developed complex strategies to avoid contact with oxidants and to defend themselves from injury. These strategies include highly effective enzymatic systems to repair damaged DNA and rapid detoxification of the reactants that produce radicals (38). Participants in the protection system include ROS-scavenging enzymes, such as superoxide dismutases, catalases, peroxidases, oxidative-damage repair enzymes, and Dps (DNA-binding protein from starved cells), a nonspecific DNA-binding and -protecting protein (12).

The ability to escape oxidative damage from host-generated ROS has been extensively documented for parasitic infections (25, 35) and pathogenic infections (5, 12). The limiting step in the parasite’s detoxification process appears to be at the level of H<sub>2</sub>O<sub>2</sub> neutralization by TPx in *Entamoeba* spp. (30), *Onchocerca volvulus* (22), trypanosomatidae (25), and *Plasmodium falciparum* (35). In nature, there are many microorganisms that proliferate in phagocytic protozoans as intracellular pathogens or as symbionts. Since endosymbionts in phagocytic...
Plasmids

dps

Consistent threats from oxidative damage is provided. In this study, like amoeba, is possible only when a measure to escape con
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tive damage caused by the host cell during phagocytosis.

stress. Our findings explain how
cell. The free-living
overcome these adverse conditions to colonize within a host

Species, including the symbiotic bacterium "Ca.

worms. The symbiotic "Can
didatus Legionella jeonii" (referred to as the X bacterium)
enters an amoeba through phagocytosis and multiplies
within symbiotic vesicles separated from phagolysosomes
(16, 28). Symbiotic bacteria overproduce a stress protein,
GroEL, using strong promoters and unique transcription
terminators in the groE gene (the GenBank accession num-
ber for the groE gene of "Ca. Legionella jeonii" is M86549)
(1, 19). The presence of large amounts of GroEL in symbi-
otic "Ca. Legionella jeonii" suggests that even established
intraacellular symbionts are stressed, although the exact
ature of the stress is not known (16).

Previously, we cloned the peroxiredoxin (Prx II, a type of
TPx) gene from A. proteus and confirmed that the protein
coded by this gene provides protection against phagocytosis-
mediated damage (27). The accumulation of TPx in the
amoeba cytoplasm may allow the host cell to generate H₂O₂
to kill bacteria during phagocytosis. On the other hand, no
defense mechanism against H₂O₂ or ROS has been reported for
Legionella species, including the symbiotic bacterium "Ca.
Legionella jeonii." Yet survival within professional phagocytes,
like amoeba, is possible only when a measure to escape con-
sistent threats from oxidative damage is provided. In this study,
we report the cloning of a dps gene of "Ca. Legionella jeonii"
(dpsEc). We describe the functional properties of DpsEc protein
and demonstrate its involvement in the defense against H₂O₂
stress. Our findings explain how Legionella counteracts oxida-
tive damage caused by the host cell during phagocytosis.

MATERIALS AND METHODS

Amoeba culture. The D and XD strains of A. proteus were cultured in Chalkley’s solution in Pyrex baking dishes (35 by 22 by 4 cm) at 24°C (27). They were fed daily with axenically cultured Tetrahymena pyriformis as a food organism.

Bacterial strains and plasmids. The bacterial strains and plasmids used in this study are listed in Table 1. The XL1-Blue strain of Escherichia coli was used for cloning purposes, and the BL21(DE3) strain was used for protein purification. E. coli strain ZK126 (W3110 ts-2 ΔlacU169) and its dps mutant ZK1058 (ZK126 Δdps::kan) were kindly provided by R. Kolter at Harvard Medical School (2). For plasmid purification, a colony of E. coli on a solid agar LB plate was inoculated into 5 ml of LB medium and incubated at 37°C in rotary shakers for 16 h.

Genomic DNA library tagging and gene cloning. Positive colonies of E. coli XL1-Blue containing a fragment of the genomic DNA of "Ca. Legionella jeonii" in the pBluescript SK (Stratagene, CA) vector were randomly selected from an IPTG (isopropyl-β-D-thiogalactopyranoside)-X-Gal (5-bromo-4-chloro-3-indolylβ-
D-galactopyranoside) plate (1). DNA inserted into each plasmid clone was tagged after both ends were sequenced using T3 and T7 primers. To define the full-length open reading frame (ORF) within genes of interest, the inserted DNA was subcloned in the pBluescript SKII (Stratagene, CA) vector and sequenced. Plasmid DNA was prepared using a plasmid purification kit (NucleoGen, Korea) by following the manufacturer’s instructions.

Cloning dps genes. dpsEc was amplified from the cloned plasmid using the primer pair DX-1 (5′-CTGCAGGCGTATACATCTCAG-3′) and DX-2 (5′-GAATTCGCATTCTCTCCCGC-3′) using the manufacturer’s instructions (Novagen). All steps were performed at 4°C. The PCR conditions for both dps genes included an initial denaturation at 95°C for 5 min; 30 cycles of denaturation for 30 s at 95°C, annealing for 1 min at 49°C, and extension for 1 min at 72°C; and a final extension for 5 min at 72°C in a model 2400 DNA thermal cycler (Perkin-Elmer, CT).

Purification of Dps proteins. C-terminal His-tagged Dps recombinant proteins were purified as follows. E. coli BL21(DE3) cells transformed with pET-24a-dpsEc or pET-24a-dpsX were grown at 37°C in LB medium to an A₆₀₀ of 0.5 and then induced with 1 mM IPTG, followed by incubation for 3 h to overproduce the recombinant Dps proteins (34). Bacillary cells were harvested by centrifugation, washed in 20 mM Tris-HCl buffer (pH 8.0), and resuspended in binding buffer (50 mM NaH₂PO₄, 300 mM NaCl, pH 7.8). Cells were broken by sonication and centrifuged at 14,000 × g for 20 min. Dps proteins were purified from the supernatant using Novagen Ni-nitrilotriacetic acid affinity matrix according to the manufacturer’s instructions (Novagen). All steps were performed at 4°C. The supernatant was added to Ni-nitrilotriacetic acid resin (0.12 the volume of the supernatant) and mixed gently by shaking for 1 h. The mixture was then loaded into a column with the bottom outlet capped with autoclaved glass wool. The

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TABLE 1. E. coli strains and plasmids used in this study

<table>
<thead>
<tr>
<th>Strain or plasmid</th>
<th>Relevant characteristic(s)</th>
<th>Source or reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>XL1-Blue</td>
<td>recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lacF' proAB lacB</td>
<td>Stratagene</td>
</tr>
<tr>
<td>BL21(DE3)</td>
<td>ompT hsdSBI with a λ prophage carrying the T7 RNA polymerase gene</td>
<td>Novagen</td>
</tr>
<tr>
<td>ZK126</td>
<td>Wild-type E. coli strain (W3110 ts-2 ΔlacU169)</td>
<td>23</td>
</tr>
<tr>
<td>ZK1058</td>
<td>ZK126 dps::kan mutant</td>
<td>23</td>
</tr>
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</table>

Plasmids

pGEM-T Easy

Ap′, PCR TA cloning vector plasmid

pBluescript SKII

Ap′, Tc′, cloning vector plasmid

pET-24a

Ap′, gene expression vector by T7 RNA polymerase containing a C-terminal His tag sequence

pBluescript SKII-dpsX

Ap′, 3-kbp EcoRI-EcoRI fragment containing “Ca. Legionella jeonii” dps gene from the genomic library in pBluescript SKII

pBAD18-E. coli-Dps

Ap′, 500-bp PCR-amplified fragment of E. coli dps in pBAD18

pET-24a-dpsX

Ap′, 480-bp PCR-amplified fragment of “Ca. Legionella jeonii” dps in pET-24a

pET-24a-dpsEc

Ap′, 500-bp PCR-amplified fragment of E. coli dps in pET-24a

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* Ap′, ampicillin resistance; Tc′, tetracycline resistance.
column was washed with 2 ml binding buffer and 2 ml washing buffer (50 mM NaOHPO₄, 300 mM NaCl, 20 mM imidazole). The protein was then eluted with 0.5 ml elution buffer (50 mM NaOHPO₄, 300 mM NaCl, 500 mM imidazole). After the purity of the protein was checked by 10% sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE), protein concentration was determined by the method of Lowry et al. (21).

Production of antisera. Female BALB/c mice (4 weeks old) were immunized with purified DpsX proteins as antigens. Proteins (50 µg in 100 µl) emulsified with an equal volume of complete Freund’s adjuvant (GIBCO BRL Life Technology, NY) were injected intraperitoneally. For booster injections, an equal volume of complete Freund’s adjuvant (GIBCO BRL Life Technology, NY) were injected intraperitoneally. For nondenaturing PAGE, a 6% polyacrylamide gel was used.

**Results**

**Characteristics of dpsX**. Through massive random sequencing of the genomic DNA library, a 629-bp DNA fragment of “Ca. Legionella jeonii” (GenBank accession no. AY598720) was sequenced (7). In contrast to most Dps proteins, which are involved in DNA protection against DNA damage caused by DNase I or by oxidation, pBluescript SKII DNA (1 µl) was used for the cloning of the genomic DNA sequence database under the accession number AY598720 for the dpsX gene and DO187950 for the dnaK gene.

**TABLE 2. RT-PCR primer sets**

<table>
<thead>
<tr>
<th>Primer</th>
<th>n-mer</th>
<th>Sequence</th>
<th>PCR fragment size (bp)</th>
<th>Source</th>
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<tr>
<td>dpsX-forward</td>
<td>22</td>
<td>5′-AGG AGT TCA CAA TGA GTG AAG T-3′</td>
<td>501</td>
<td>This study</td>
</tr>
<tr>
<td>dpsX-reverse</td>
<td>19</td>
<td>5′-GCG GCT TCA ACT GCT TT-3′</td>
<td>501</td>
<td>This study</td>
</tr>
<tr>
<td>16SX-forward</td>
<td>20</td>
<td>5′-CTG GGG ACC TTA TGG CCT GG-3′</td>
<td>500</td>
<td>1</td>
</tr>
<tr>
<td>16SX-reverse</td>
<td>20</td>
<td>5′-CAT TTC ACC GCT ACA CCG GA-3′</td>
<td>502</td>
<td>“Ca. Legionella jeonii”</td>
</tr>
<tr>
<td>groELX-forward</td>
<td>20</td>
<td>5′-CTA AGC GGC AAG TGA TTT CT-3′</td>
<td>502</td>
<td>genomic library</td>
</tr>
<tr>
<td>groELX-reverse</td>
<td>20</td>
<td>5′-TGA TCC GTA GCC AGC ATT AG-3′</td>
<td>502</td>
<td></td>
</tr>
<tr>
<td>dnaKX-forward</td>
<td>22</td>
<td>5′-CAT GTC GTG TTG ATT TAG C-3′</td>
<td>502</td>
<td></td>
</tr>
<tr>
<td>dnaKX-reverse</td>
<td>17</td>
<td>5′-CCT CGC CGC CTG TTT CT-3′</td>
<td>502</td>
<td></td>
</tr>
</tbody>
</table>

**RESULTS**

**Characteristics of dpsX**. Through massive random sequencing of the genomic DNA library, a 629-bp DNA fragment of “Ca. Legionella jeonii” containing a putative dpsX gene was cloned (GenBank accession no. AY598720). The gene encoded a putative polypeptide of 147 amino acids with a molecular mass of 17 kDa (pI 5.19), as predicted by the GenBank database (1). The expression of the dpsX gene upon exposure to H₂O₂ or during phagocytosis was monitored by reverse transcription (RT)-PCR. Oligonucleotide primers (Table 2) were designed by using the PRIMERESELECT program (DNASTAR, Inc., WI). Total RNA was extracted directly from the 1D strain of A. proteus using RNAwiz (Ambion, TX) according to the manufacturer’s protocol. To remove contaminating DNA, 20 µl total RNA was incubated at 37°C for 30 min with 30 units of RNase-free DNase (Promega) and 1 unit of RNasin-RNAse inhibitor (Promega) in a 30-µl volume. After residual DNase was inactivated by heat at 80°C for 5 min, an aliquot (100 ng) of RNA was used for the RT-PCR, using Promega’s RT-PCR system. Conditions were as follows. Reverse transcription was performed at 50°C for 60 min, and denaturation was performed at 72°C for 10 min. PCR amplification of cDNA was performed using 3 µl of each reverse transcriptase reaction mixture, followed by 25 cycles of denaturation at 95°C, annealing at 54°C, and an extension for 45 s at 72°C, with a final extension at 72°C for 5 min. PCR products were analyzed by electrophoresis using 1% agarose gels, and the band intensity was measured using a Kodak film 44CF image station (Kodak Digital Science, NY).

**Nucleotide sequence accession numbers.** The sequences of the cloned “Ca. Legionella jeonii” DNA described here were deposited in the GenBank nucleotide sequence database under the accession number AY598720 for the dpsX gene and DO187950 for the dnaK gene.

**Downloaded from http://jb.asm.org/ on October 15, 2017 by guest**
This result indicates that the protein that we cloned belongs to the Dps family, rather than to the ferritin or bacterioferritin group (Fig. 2).

The **dps** gene complements the *E. coli* **dps**::**kan** null mutant. To investigate the role of the **dps** gene in vivo, we introduced the pBluescript SKII plasmid containing the cloned **dps** gene into the **dps**::**kan** mutant (ZK1058) of *E. coli*. The survival rate of the Dps-deficient mutant was distinguishable from that of the wild-type strain (ZK126) at 10 mM H₂O₂ (Fig. 3A). At 10 mM H₂O₂, the survival rate of the **dps**::**kan** mutant strain was significantly lower than that of the wild type. However, the phenotype of hydrogen peroxide sensitivity was restored to near wild-type levels by introducing **dps**. The presence of the pBluescript SKII vector alone had no effect on the survival of the Dps-deficient mutant was distinguishable from that of the wild-type strain (ZK126) at 10 mM H₂O₂ (Fig. 3A). At 10 mM H₂O₂, the survival rate of the **dps**::**kan** mutant strain was significantly lower than that of the wild type. However, the phenotype of hydrogen peroxide sensitivity was restored to near wild-type levels by introducing **dps**. The presence of the pBluescript SKII vector alone had no effect on the survival of...
the \textit{dps:kan} mutant strain. A significant recovery from its sensitivity to oxidant chemicals was also observed in patch assays of the \textit{dps:kan} mutant strain transformed with the \textit{dps\textsubscript{X}} gene (Fig. 3B). Together, these results imply that the \textit{dps\textsubscript{X}} gene is a functional homologue of the \textit{dps} gene of \textit{E. coli}.

\textbf{Dps\textsubscript{X} is an oligomeric protein.} The recombinant proteins of Dps\textsubscript{X} and \textit{E. coli} Dps were purified under native conditions using His trap resins and analyzed by SDS gel electrophoresis (Fig. 4). In the gel, the molecular masses of Dps\textsubscript{X} and \textit{E. coli} Dps were resolved as 21 kDa and 23 kDa, respectively. In Western blotting, the antiserum raised against Dps\textsubscript{X} showed specificity to Dps\textsubscript{X} proteins but not to \textit{E. coli} Dps proteins. In nondenaturing gel electrophoresis, both recombinant proteins were resolved as individual bands by BCB staining (Fig. 5). Dps\textsubscript{X} proteins appeared as one major band representing a high-number oligomer and two minor bands representing lower-number oligomers. In this gel electrophoresis, we used BSA (pI 4.8) and ferritin (pI 4.5) as markers (11). The high-number oligomeric form of Dps\textsubscript{X} migrated near the ferritin monomer (450 kDa), while two low-number oligomeric forms were resolved between the BSA monomer (66 kDa) and dimer (132 kDa). In the meantime, the one high-number oligomeric form of \textit{E. coli} Dps proteins was resolved near the BSA trimer (198 kDa), and the low-number oligomeric form was resolved near the BSA monomer (66 kDa). In contrast to \textit{E. coli} Dps, the majority of Dps\textsubscript{X} proteins were in a high-number oligomeric form. It appeared that most of the Dps\textsubscript{X} proteins were in a 12-meric complex, since the monomeric form that resolved by SDS-PAGE had a molecular mass of 21 kDa (Fig. 4). Some of the proteins may be in 3-meric or 6-meric form, as we noticed two minor bands between monomeric (66-kDa) and dimeric (132-kDa) BSA.

\textbf{Dps\textsubscript{X} binds and protects DNA in vitro.} The DNA binding capacity of Dps\textsubscript{X} was analyzed by gel retardation assay using

![FIG. 3. The \textit{dps\textsubscript{X}} gene complements the \textit{dps:kan} mutant of \textit{E. coli} in the assay of numbers of CFU (A) and colony patches (B). The presence of the \textit{dps\textsubscript{X}} gene increases the survival of the \textit{dps:kan} mutant strain, but the pBluescript SKII vector alone has no effect on survival. The wild-type strain was ZK126 (\textit{E. coli} K-12); the \textit{dps:kan} mutant was ZK1058 (\textit{E. coli} K-12). Error bars represent the standard deviations based on three experiments.]

![FIG. 4. Purification of His-tagged Dps\textsubscript{X} and \textit{E. coli} Dps proteins. An SDS-polyacrylamide gel (10\%) was stained with BCB, and Western blotting (WB) with antiserum against His-tagged Dps\textsubscript{X} protein was performed. Lanes: M, molecular mass markers (masses in kilodaltons appear at the left); 1, crude extract for Dps\textsubscript{X}; 2, purified Dps\textsubscript{X}; 3, crude extract for \textit{E. coli} Dps; 4, purified \textit{E. coli} Dps. The arrow and the arrowhead indicate the positions of His-tagged \textit{E. coli} Dps (23 kDa) and Dps\textsubscript{X} (21 kDa), respectively.]
BSA as a negative control (Fig. 6A). Stable DpsX-DNA complexes were detected when purified DpsX proteins were added to supercoiled pBluescript SKII plasmid DNA; at a DNA-to-protein molar ratio of 1:1,000, a complex was formed that did not enter the agarose gel. Similar DNA-binding properties have been observed for the Dps proteins from *E. coli* (2, 24), *Agrobacterium tumefaciens* (7), and *Mycobacterium smegmatis* (11). When the molar ratio between DNA and protein was reduced to 1:200, neither DpsX nor *E. coli* Dps formed complexes with DNA whose mobility was not affected in the 1% agarose gel (Fig. 6A).

The activities of Dps proteins in the protection of DNA from DNase I digestion or damage mediated by hydrogen peroxide were examined in vitro using pBluescript SKII plasmid DNA. In the absence of DpsX or the *E. coli* Dps protein, pBluescript SKII DNA was easily digested by DNase I (Fig. 6B). At a DNA-to-protein molar ratio of 1:1,000, DNA was protected from DNase I digestion and also showed retardation in gel mobility. At a 1:200 molar ratio, although DNA mobility was not retarded, DNA was protected from DNase I digestion. These observations imply that DpsX protein can protect DNA from DNase I digestion. One important function of Dps in vivo is to protect the DNA from oxidative radicals. Hydrogen peroxide in the presence of ferrous ions generates hydroxyl radicals through the Fenton reaction. As a result, these radicals cause DNA strand breaks by oxidizing the sugar and base moieties. As seen in Fig. 6C, in the presence of 50 μM FeCl₂ and 5 mM H₂O₂, supercoiled pBluescript SKII DNAs were mostly degraded, whereas DNAs remained intact in the presence of DpsX or *E. coli* Dps, even at a 1:200 molar ratio. These results demonstrate that Dps has the ability to protect DNA against Fenton reaction-mediated hydroxyl radical damage. Taken together, our results confirm that DpsX protein is a functional homologue of *E. coli* Dps in the protection of DNA from DNase I digestion and damage caused by hydroxyl radicals.

The *dps* gene responds to H₂O₂. To analyze the expression of the *dps* gene from “Ca. Legionella jeonii” in symbiosis with *A. proteus*, we performed RT-PCR analysis using *groEL*, *dnaK*,

FIG. 5. Comparisons of oligomeric properties of His-tagged DpsX and *E. coli* Dps in vitro. Purified His-tagged DpsX and *E. coli* Dps proteins were analyzed by nondenaturing gel (6%) electrophoresis and stained with BCB and polyclonal antiserum against DpsX protein (Western blotting [WB]). Lanes: 1, BSA (66 kDa, pI 4.8); 2, horse spleen ferritin (450 kDa, pI 4.5); 3, His-tagged DpsX; 4, His-tagged *E. coli* Dps. Masses in kilodaltons appear at the left. Bands I, II, and III represent two putative lower-number oligomers (I and II) and one higher-number oligomer (III) of DpsX. The arrow and arrowhead indicate the putative low- and high-number oligomers of *E. coli* Dps, respectively.

FIG. 6. Comparisons of His-tagged DpsX and *E. coli* Dps in DNA binding and protection of DNA. (A) Formation of protein-DNA complexes. The pBluescript SKII DNA incubated with proteins at 30°C for 30 min was analyzed for gel retardation in 1% agarose gel and stained with ethidium bromide. Lanes: 1, pBluescript SKII DNA alone; 2 to 6, pBluescript SKII DNA incubated with BSA, DpsX, and *E. coli* Dps, respectively. (B) Protection of DNA from DNase I digestion. The pBluescript SKII DNA (1 µg) was incubated with proteins at 30°C for 30 min and then treated with 1 U DNase I for 5 min. (C) Protection of DNA from H₂O₂-mediated damage. The pBluescript SKII DNA (1 µg) was incubated with proteins at 30°C for 30 min and then processed for the Fenton reaction. The DNA-to-protein molar ratios were 1:1,000 for additions marked “a” and 1:200 for additions marked “b.”
and 16S rRNA genes of “Ca. Legionella jeonii” as references (Fig. 7 and 8). The groEL and dnaK genes were chosen since they are associated with a heat shock response regulon (20, 32, 33). The 16S rRNA gene was chosen as a representative of abundant cellular housekeeping genes. The levels of mRNAs of these genes were compared by RT-PCR using total RNA prepared at time intervals from the xD strain of A. proteus treated with 2 mM H₂O₂ (Fig. 7) (28). The level of dpsX transcription was highest (4.5-fold higher than that of the untreated control) after the 0.5-h treatment and gradually decreased to a 3.5-fold level at 1.5 h. The level of dnaK expression was increased to a maximum of 2.5-fold at 1 h of treatment. However, the effect of H₂O₂ on the expression of the groEL gene was not significantly different from that of untreated control cells until the 0.5-h incubation period (Fig. 7).

The dpsX gene responds to phagocytosis. To characterize the mode of dpsX expression by symbiotic “Ca. Legionella jeonii” in response to the phagocytic activities of the host amoebae, we performed RT-PCR analyses using total RNA extracted from xD amoebae at time intervals after the feeding of Tetrahymena organisms (Fig. 8). In xD amoebae fed with Tetrahymena organisms for 0.5 h, dpsX gene expression rapidly increased to 4-fold that in unfed amoebae, and the level was maintained until 1 h. The level of dnaK expression increased to 2- or 5-fold that in unfed amoebae, and the level persisted until 2 h. In contrast, the level of groEL expression did not change until 1 h and then increased to 2.5-fold that in unfed amoebae at 2 h. The effect of phagocytosis on the expression of dpsX and dnaK was similar to that of 2 mM H₂O₂ treatment, while a different response was induced in the expression of groEL.

Together, these observations imply that amoebae may generate hydrogen peroxide within phagosomes as well as other unidentified signaling molecules during phagocytosis. The “Ca. Legionella jeonii” in symbiotic vacuoles may respond to these signals in this period by expressing the dpsX, dnaK, and groEL genes.

DISCUSSION

The mechanism underlying the protection of endosymbiotic microorganisms against biochemical attacks generated by a host is intriguing in that it may shed insight into how an association between endocellular organisms and the host evolved. Elucidating the biochemical basis of protection may also provide a means to the effective eradication of endocellular pathogens such as Mycobacteria and Legionella. Yet, little is known about the mode of protection exerted by the symbionts to counterbalance the oxidative stress generated in the process of phagocytosis. In this study, we demonstrated that the DpsX protein of symbiotic “Ca. Legionella jeonii” is a functional homologue of Dps of E. coli. We found that the expression of the dpsX gene by symbiotic bacteria is activated in response to H₂O₂ and confers protection against DNA damage.

Dps proteins are members of a superfamily that includes ferritins and bacterioferritins, which are known to have evolved as divergent homologues from a common ancestor (29). Close homologues of Dps have been identified in distantly related bacteria (7, 11, 15, 24, 36, 38), implying that this protein maintains a general and crucial function. Dps proteins found in many eubacterial and archaeabacterial species appear to protect cells from oxidative stress by sequestering iron and limiting Fenton reaction-catalyzed oxyradical formation (13). Although being DNA-binding proteins, Dps proteins lack any of the known DNA-binding motifs (7, 11). They play a key role in...
From a previous study, it has been speculated that amoebae having Prx II may generate hydrogen peroxide as a bactericidal mechanism (27). Thus, “Ca. Legionella jeonii” in endosymbiosis may be in a state of prevailing stress and may require a protective mechanism for survival in a professional phagocytic host. Since “Ca. Legionella jeonii” accumulates large amounts of GroEL proteins during symbiosis, the protein itself has been considered a molecular component relevant to the survival of “Ca. Legionella jeonii” in symbiosis. However, little is known about the molecular target of GroEL or the mechanism by which GroEL affects the process of phagocytosis. The DpsX protein that we characterized in this study marks the first protein shown to respond to the hydrogen peroxide and phagocytic activities of the host. In most cases, ROS toxicity is exerted via direct damaging effects on DNA. A number of preventive measures evolved to counteract such ROS-mediated toxicity (11, 13). One important question that has been frequently addressed is how DNA is protected from ROS in a bacterial cell under stationary-phase conditions or under some kind of nutritional stress. This question attains further importance in Legionella-like pathogens multiplying in protozoans or obligate endosymbionts, such as “Ca. Legionella jeonii,” which invade through the phagocytic pathway and reside in the host cytoplasm. Our data suggest that the expression of DpsX enables the symbiotic bacterium to efficiently adapt to protect itself and its genetic material under such stressful conditions.

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