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*) 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. DpsX proteins purified from *E. coli* transformed with the *dps* gene were in oligomeric form, formed a complex with pBluescript SKII DNA, and protected the DNA from DNase I digestion and *H*₂*O*₂-mediated damage. The expression of the *dps* gene in *Candidatus Legionella jeonii* was enhanced when the host amoeba was treated with 2 mM *H*₂*O*₂ 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* 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*₂ to form a superoxide anion (*O*₂⁻) and pours *O*₂⁻ into the phagocytic vacuole (4). Subsequently, superoxide dismutase catalyzes the conversion of *O*₂⁻ into *H*₂*O*₂. At the expense of *H*₂*O*₂, 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 deoxyribose of DNA to produce damaged bases or strand breaks (12, 23). Among these ROS, *H*₂*O*₂ 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 antioxidant enzymes in neutrophils is glutathione peroxidase. In organisms lacking glutathione peroxidase, thioredoxin-dependent peroxidase (TPx) replaces glutathione peroxidase to reduce *H*₂*O*₂ (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*₂*O*₂, 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*₂*O*₂ 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
cells are under conditions of prolonged stress, they are required to develop efficient mechanisms to adapt and to survive in a hostile environment. Also, they must have fitness traits to overcome these adverse conditions to colonize within a host cell. The free-living Amoeba proteus is a professional phagocyte that feeds on ciliates and microorganisms. The symbiotic “Ca. 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 number for the groE gene of “Ca. Legionella jeonii” is M86549) (1, 19). The presence of large amounts of GroEL in symbiotic “Ca. Legionella jeonii” suggests that even established intracellular symbionts are stressed, although the exact nature 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 encoded 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 coincidence of defense against H₂O₂ stress is developed (27).

Results

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 mut-2 ΔlacU169) and its dps mutant ZK1058 (ZK126 dps–kan) were kindly provided by Dr. C.-S. Min at the College of Medicine, Seoul National University. 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-indolyl β-D-thiogalactopyranoside) 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. dpsX was amplified from the cloned plasmid using the primer pair DX-1 (5'-AGGAACTCATGAGTGAAGTATAC-3') and DX-2 (5'-TAAGCTTGGGATTCTATTTC-3') and ligated into the pGEM-T Easy vector (Promega, WI). Amplified products were digested with BamHI and HindIII, and ligated into the pGem-T Easy vector. The resulting plasmids were transformed into E. coli XL1-Blue cells for protein expression. The expression level of the C-terminal His-tag fusion protein was determined using Coomassie blue staining and Western blotting (Promega, WI).

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-dpsXc 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). Bacterial 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
column was washed with 2 ml binding buffer and 2 ml washing buffer (50 mM NaH₂PO₄, 300 mM NaCl, 20 mM imidazole). The protein was then eluted with 0.5 ml elution buffer (50 mM NaH₂PO₄, 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 purified DpsX proteins as antigens. Proteins (50 µg in 100 µl) were mixed in a 200-µl reaction mixture and incubation for 5 min at room temperature. DNA was allowed to interact with Dps proteins, as was done in the gel retardation assay. DNA damage was induced by the addition of 1 unit of DNase I (1 unit/ml volume. After dilution with 50 mN H₂O₂ for 30 min at room temperature. The exponentially growing cells were diluted 1:500 into 5 ml of fresh medium and incubated at 37°C, and left at 4°C overnight to allow the clot to contract. Serum was collected by centrifugation at 10,000 × g for 10 min at 20°C in aliquots of 100 µl.

Gel electrophoresis and Western blotting. An SDS-polyacrylamide gel was prepared using a discontinuous buffer system (18). A molecular mass standard was purchased from Sigma Chemical Co. After electrophoresis, gels were stained with 0.25% Coomassie brilliant blue (BCB) or processed for immunoblotting, and proteins that had been separated by gel electrophoresis were transferred to a nitrocellulose membrane (Schleicher & Schuell, Germany) using a semidry transfer apparatus and immunostained with anti-DpsX serum as the primary antibody and horseradish peroxidase-conjugated goat anti-mouse immunoglobulin G (diluted 1:5,000 in phosphate-buffered saline; Jackson ImmunoResearch Laboratories, PA) as the secondary antibody. Immunoblots were visualized using the ECL Western blotting detection system (Amersham Bioscience, United Kingdom). For nondenaturing PAGE, a 6% polyacrylamide gel was prepared according to the method of Laemmli (18) except that SDS and 2-mercaptoethanol were excluded. Samples (100 µg) were loaded with a dye (10% glycerol, 0.01% bromophenol blue, 62.5 mM Tris-HCl, pH 6.8), and the electrophoresis was carried out at a constant current of 12 mA. Gels were stained with BCB. Bovine serum albumin (BSA) and horse spleen ferritin were used as size markers.

E. coli dpsX mutant complementation assay. Overnight LB cultures of the desired strains were diluted 1:500 into 5 ml of fresh medium and incubated at 37°C with shaking for 3 h. The exponentially growing E. coli cells were exposed to various concentrations of H₂O₂ for 30 min at room temperature. The treatment was stopped by the addition of 1,595 units of catalase (specific activity, 31,900 units/mg protein). Cultures were then diluted 100-fold in LB medium and plated on LB plates to determine the number of CFU and calculate the number of survivors (24). Sensitivity to oxidant chemicals was also tested using colony patch assays. After overnight growth, dilutions were made, and several concentrations of E. coli cells of each stained culture (10⁶ to 10⁷ cells in a volume of 4 µl) were spotted onto LB agar plates containing H₂O₂ and were incubated at 37°C for 2 days.

Gel retardation assay. Supercoiled plasmid DNA (1 µg) was mixed with DpsX or DpsY proteins at a DNA-to-protein molar ratio of 1:200 or 1:1,000 in 50 mM Tris-HCl (pH 7.9) containing 50 mM NaCl and incubated for 30 min at 30°C. Aliquots of the reaction mixture were loaded on a 1% agarose gel in Tris-acetate-EDTA buffer, run at a constant voltage of 50 V, and visualized by ethidium bromide staining (7, 11).

RESULTS

Characteristics of DpsX. Through massive random sequencing of the genomic DNA library, a 629-bp DNA fragment of Ca. Legionella jeonii DNA described here were deposited in the GenBank nucleotide sequence database under the accession number AJ598720 for the dpsX gene and DQ187950 for the dnak gene.

Characteristics of dpsX. Through massive random sequencing of the genomic DNA library, a 629-bp DNA fragment of Ca. Legionella jeonii DNA described here were deposited in the GenBank accession no. AJ598720. The gene encoded a putative polypeptide of 147 amino acids with a molecular mass of 17 kDa (pI 5.19), as predicted by the ProtParam program. In a BLAST search, DpsX was found to be a homologue of E. coli Dps (18.56 kDa, pI 5.78) (7) and other proteins in the Dps family. It showed 68% identity to Dps protein of Legionella pneumophila (GenBank accession no. AUA26778). The degree of homology between these proteins is higher in conserved domains, showing 91.0% similarity for DNA-binding proteins and 80.6% similarity for ferritin-binding proteins (3). DpsX also contained the so-called DNA-binding signature that is highly conserved among various Dps proteins (Fig. 1) (7). In contrast to most Dps proteins, which display an N-terminal extension containing various lengths of positively charged lysine residues, DpsX has two lysine residues in the corresponding region. A phylogenetic analysis of the basis for the deduced amino acid sequences indicated that “Ca. Legionella jeonii” DpsX forms a clade with L. pneumophila.
Dps. 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**<sub>X</sub> gene complements the *E. coli* **dps**<sub>::kan</sub> null mutant.

To investigate the role of the **dps**<sub>X</sub> gene in vivo, we introduced the pBluescript SKII plasmid containing the cloned **dps**<sub>X</sub> gene into the **dps**<sub>::kan</sub> 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 H<sub>2</sub>O<sub>2</sub> (Fig. 3A). At 10 mM H<sub>2</sub>O<sub>2</sub>, the survival rate of the **dps**<sub>::kan</sub> 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**<sub>X</sub>. The presence of the pBluescript SKII vector alone had no effect on the survival of...
the *dps::kan* mutant strain. A significant recovery from its sensitivity to oxidant chemicals was also observed in patch assays of the *dps::kan* mutant strain transformed with the *dps*<sub>X</sub> gene (Fig. 3B). Together, these results imply that the *dps*<sub>X</sub> gene is a functional homologue of the *dps* gene of *E. coli*.

**Dps<sub>X</sub> is an oligomeric protein.** The recombinant proteins of Dps<sub>X</sub> and *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<sub>X</sub> and *E. coli* Dps were resolved as 21 kDa and 23 kDa, respectively. In Western blotting, the antiserum raised against Dps<sub>X</sub> showed specificity to Dps<sub>X</sub> proteins but not to *E. coli* Dps proteins. In nondenaturing gel electrophoresis, both recombinant proteins were resolved as individual bands by BCB staining (Fig. 5). Dps<sub>X</sub> 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<sub>X</sub> 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 *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 *E. coli* Dps, the majority of Dps<sub>X</sub> proteins were in a high-number oligomeric form. It appeared that most of the Dps<sub>X</sub> 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.

**Dps<sub>X</sub> binds and protects DNA in vitro.** The DNA binding capacity of Dps<sub>X</sub> was analyzed by gel retardation assay using

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

**FIG. 4.** Purification of His-tagged Dps<sub>X</sub> and *E. coli* Dps proteins. An SDS-polyacrylamide gel (10%) was stained with BCB, and Western blotting (WB) with antiserum against His-tagged Dps<sub>X</sub> protein was performed. Lanes: M, molecular mass markers (masses in kilodaltons appear at the left); 1, crude extract for Dps<sub>X</sub>; 2, purified Dps<sub>X</sub>; 3, crude extract for *E. coli* Dps; 4, purified *E. coli* Dps. The arrow and the arrowhead indicate the positions of His-tagged *E. coli* Dps (23 kDa) and Dps<sub>X</sub> (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 dpsX gene responds to H₂O₂. To analyze the expression of the dpsX 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 Dps X 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, and gene expression was analyzed by RT-PCR using specific primers for the dpsX, groELX, dnaKX, and 16S rRNAX genes. The PCR products were analyzed by electrophoresis in a 1% agarose gel, and the relative levels of gene expression were the same as those shown in Fig. 7.

FIG. 7. Effects of 2 mM H₂O₂ on the expression of the dps, groEL, and dnaK genes from “Ca. Legionella jeonii” of xD amoebae. Total RNA was extracted from xD amoebae treated with 2 mM H₂O₂ and analyzed by RT-PCR using primers specific to the dpsX, groELX, dnaKX, and 16S rRNAX genes of the symbiotic “Ca. Legionella jeonii” organism. The PCR products were analyzed by electrophoresis on a 1% agarose gel, and the band density was read by a Kodak model 440CF image station (Kodak Digital Science, NY) for comparisons of the levels of gene expression to that of 16S rRNA. The bars represent the means ± standard deviations from three experiments.

FIG. 8. Effects of the phagocytosis of the host on the expression of the dps, groEL, and dnaK genes from “Ca. Legionella jeonii” of xD amoebae. Total RNA was extracted from xD amoebae at time intervals after feeding with Tetrahymena organisms, and gene expression was analyzed by RT-PCR using specific primers for the dpsX, groELX, dnaKX, and 16S rRNAX genes. The PCR products were analyzed by electrophoresis in a 1% agarose gel, and the relative levels of gene expression were the same as those shown in Fig. 7.

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...
protecting DNA from oxidative damage both in vitro and in vivo by directly binding to DNA (10, 11, 24, 31, 36, 38).

The amino acid sequences of some Dps proteins display N-terminal extensions of various lengths that contain two or three positively charged lysine residues (7). In E. coli, the self-aggregation of Dps and Dps-driven DNA condensation are parallel phenomena that are associated with this lysine-rich and highly mobile N terminus (6). DNA protection against the toxic action of Fe(II) and H₂O₂ is not affected by N-terminal deletions, either in vitro or in vivo (6). In our experiments, we also observed that the substitution of N-terminal or C-terminal Lys or Arg residues of DpsX did not affect its ability to bind DNA and to protect DNA against the Fenton reaction (data not shown).

All Dps proteins are known to form multimers and different oligomeric forms, which may have different DNA-binding abilities (2, 11). For example, in Mycobacterium smegmatis, the dodecameric form is capable of binding DNA and forming large crystalline arrays with DNA, whereas the trimeric form has the capacity to protect DNA against Fenton reaction-mediated damage (11). We found that the majority of DpsX proteins exist as a 12-mer complex, while some are in a 3-meric or 6-meric form (Fig. 5). We presume that these isoforms may partake in different venues in the protection of symbiotic bacteria during phagocytosis.

The expression patterns of dps and dnaK genes upon H₂O₂ treatment were similar to that induced by phagocytosis (Fig. 7 and 8). Interestingly, the expression of the groEL gene was not affected by H₂O₂ and was activated at 2 h after the onset of phagocytosis. It is possible that the phagocytic activities of host amoebae may generate other stimulants in addition to H₂O₂. Alternatively, the delayed expression of dps genes is regulated by the stationary-phase sigma factor RpoS (σ^70), OxyR, and integration host factor (13). In Legionella spp., the hierarchical organization of the dps, dnaK, and groE genes is not known. Our data show a much higher level of induction for dps than for groEL and dnaK by both H₂O₂ and phagocytic activities. Such differences in gene expression may indicate that Dps plays a pivotal role in the protection of DNA from oxidative damage.

In a study of gene expression profiling by microarray analysis, a whole set of 919 in vivo-regulated genes belonging to Salmonella enterica serovar Typhimurium (9). In this experiment, dps was one of the early genes to be induced following Salmonella ingestion by macrophages. It has been shown that the dps gene plays an important role in the ability of Salmonella enterica serovar Typhimurium to resist killing by host phagocytes and to cause a productive infection (13). Also, a Dps-like protein conferring resistance to hydrogen peroxide is required for the proliferation of Listeria monocytogenes in specific host cells, or compartments (26). These data suggest that Dps proteins are essential for pathogenic bacteria during infection and proliferation.

From a previous study, it has been speculated that amoebae having PtoX II may generate hydrogen peroxide as a bacterialic 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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REFERENCES


