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Dps Proteins, an Efficient Detoxification and DNA Protection Machinery in the Bacterial Response to Oxidative Stress

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Abstract The proteins belonging to the Dps (DNA-binding proteins from starved cells) family play an important role within the bacterial defence system against oxidative stress. They act on Fe(II) and hydrogen peroxide that are potentially toxic in the presence of air. Fe(II) forms spontaneously insoluble Fe(III) and reacts with molecular oxygen or its reduced forms to yield the highly damaging hydroxyl radicals. All Dps proteins have the distinctive capacity to annul the toxic combination of iron and hydrogen peroxide as they use the latter compound to oxidise Fe(II). In addition to this intrinsic DNA protection capacity, several members of the family, including the archetypical *Escherichia coli* Dps, protect DNA physically by shielding it in large Dps-DNA complexes. The structural and functional characteristics that endow Dps proteins with the chemical and physical protection mechanism are presented and discussed also in the framework of the varied situations that may be encountered in different bacterial species.

Keywords Dps proteins, DNA-binding, proteins from starred cells, DNA protection

Subject codes L14005, L23004

The presence of oxygen on earth is beneficial, but poses two major problems to nearly all forms of life, namely the exposure to oxidative stress and the drastic decrease in the availability of iron, an essential element. These two problems are tightly correlated. Iron availability is decreased by the spontaneous oxida-

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tion of Fe(II) in the presence of air since Fe(III) is highly insoluble at neutral pH values. Moreover, the non-enzymatic production of reactive oxygen species is potentiated by the reaction of free intracellular Fe(II) with molecular oxygen and its reduced forms. In particular, Fe(II) and H_2O_2 generate the most toxic hydroxyl radicals, $^{\bullet}OH$, that can damage many biological macromolecules including DNA via the so-called Fenton reaction: Fe(II) + $H_2O_2 \rightarrow$ Fe(III) + $^{\bullet}OH + OH^-$. To counteract these problems, all organisms living in the presence of oxygen have developed an extensive array of specialized defence systems that detoxify the reactive oxygen species or remove iron from the cytoplasm and store it in a non-toxic, soluble form.

Dps proteins (DNA-binding proteins from starved cells) are increasingly recognized as important players in the complex antioxidant response system of bacterial cells. They belong to the ferritin superfamily and were discovered in relatively recent years despite the very wide distribution. The first Dps protein was identified in 1992 in *Escherichia coli* where it represents the most abundant chromatin component in stationary phase cells. *E. coli* Dps was found to protect DNA from oxidative damage both *in vitro* and *in vivo*, an action attributed to the binding of DNA without apparent sequence specificity (1–3). In turn, the interaction between the two macromolecules was shown to manifest itself in the formation of large Dps-DNA complexes that appear as co-crystals in late stationary phase cells (4, 5). A few years later, seminal studies on *Listeria innocua* Dps revealed that DNA protection from oxidative damage occurs even in the absence of binding and brought to light the ferritin-like activity of Dps proteins which endows them with the rather unique capacity to detoxify iron and H_2O_2 concurrently.

A brief account of the structural and functional properties that characterize authentic ferritins is in order with the aim of outlining similarities and differences with respect to Dps proteins. Ferritins are ubiquitous proteins whose structure and function are highly conserved throughout evolution (6). The molecule is tailored to prevent iron toxicity by means of a multi step process that involves binding and oxidation of Fe(II) and solubilization of Fe(III) within the protein cavity. To this end, all ferritins are spherical shells assembled from 24 identical or highly similar subunits, folded as a four-helix bundle and related by 432 symmetry (Fig. 1, top). Fe(II) is bound at the ferroxidase center which is formed by a set of conserved residues within the four-helix bundle of single subunits; it is oxidized pairwise by molecular oxygen with the production of hydrogen peroxide (7, 8). Assembly of the subunits along the three-fold symmetry axes forms negatively charged pores that allow passage of iron and small molecules (8, 9) and give rise to an electrostatic gradient which guides Fe(II) towards the ferroxidase center (10). After oxidation, iron moves to the internal protein cavity where it is deposited and solubilized as ferric hydroxide micelles. One ferritin molecule can harbour up to 4500 iron

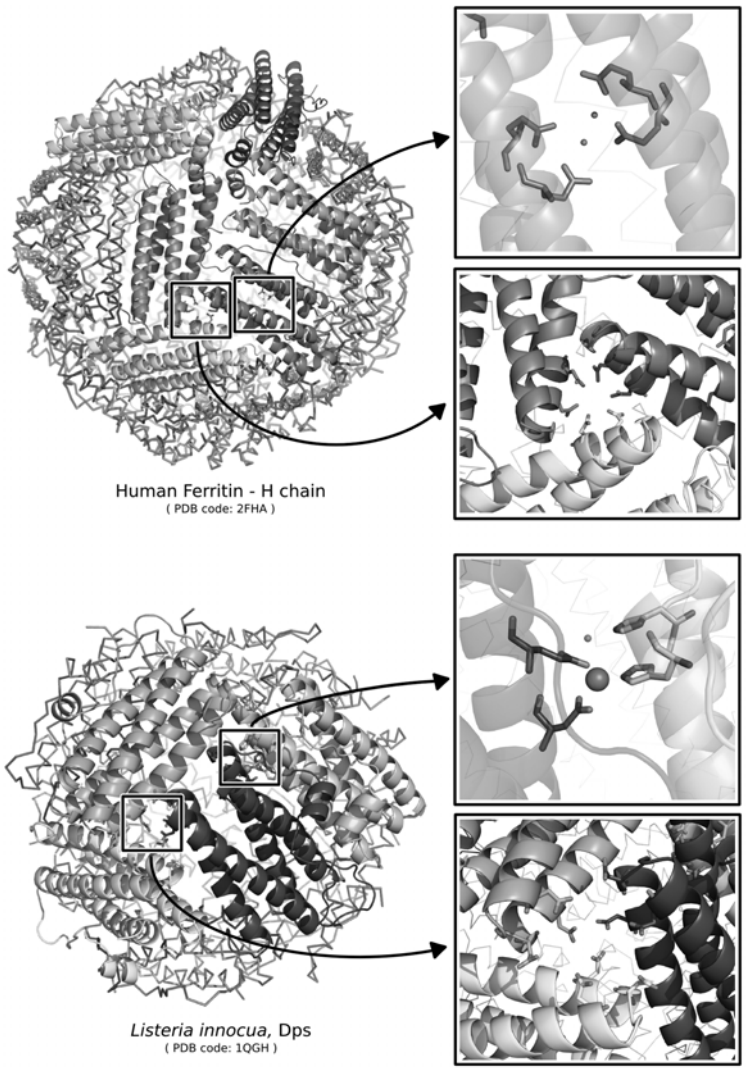

Fig. 1 X-ray crystal structure of the human ferritin H-chain 24-mer (top) and of the *Listeria innocua* Dps 12-mer (bottom). In both structures the subunits related by symmetry elements (4-fold, 3-fold and 2-fold axes) are indicated with different colors. The insets on the right are enlargements of (from top to bottom): the ferritin ferroxidase center embedded in a single subunit with the carboxylate groups that ligate iron and two water molecules (small red spheres) in the position occupied by the metal; the channels along the ferritin 3-fold symmetry axes surrounded by negatively charged residues; the Dps ferroxidase center shared by two symmetry-related subunits with the histidine and carboxylate groups that ligate iron (large red sphere) and one water molecule (small red sphere) in the position occupied by the second metal atom; the channels along the Dps 3-fold symmetry axes surrounded by negatively charged residues and resembling closely those of ferritin.

atoms that can be released after reduction to Fe(II) when the metal is required to meet the metabolic needs of the organism (6).

As mentioned above, when first characterized, *L. innocua* Dps was shown to oxidize iron, even though the kinetics of oxidation by molecular oxygen was rather slow, and to store Fe(III) in the protein shell. Actually, due to this dual function, *Listeria* Dps was considered to be a ferritin, though unusual since the conserved iron ligands at the ferroxidase center were lacking and the molecular mass of the oligomer was significantly smaller than expected and pointed to an assembly of only 12 subunits (11). The dodecameric construction was confirmed by the resolution of the X-ray crystal structure which established that the four-helix bundle subunits form a spherical shell with 23 symmetry and give rise to negatively charged pores along the three-fold symmetry axes as in ferritins (12). However, the most important feature of the structure is the unusual nature of the ferroxidase center that is shared by two-fold symmetry related subunits rather than being embedded in single subunits as all canonical ones (Fig. 1, bottom). Significantly, the iron ligands are conserved in all Dps proteins, e.g. those from *Bacillus anthracis* (13), *Halobacterium salinarum* (14), *Deinococcus radiodurans* (15–17), *Streptococcus suis* (18) and *Bacillus brevis* (19), such that the inter-subunit ferroxidase center can be taken as a family signature.

Similarly to ferritins, iron has access to the ferroxidase center and the internal cavity through the negatively charged pores at the three-fold axes as indicated by the presence of one metal ion in iron-soaked crystals of *D. radiodurans* Dps (16). The iron oxidation mechanism at the structurally atypical inter-subunit ferroxidase center is likewise distinctive. The Dps ferroxidase center, unlike the ferritin one, does not use O_2 as the physiological iron oxidant, but H_2O_2 which is about 1000-fold more efficient than O_2 (20, 21). The only exceptions to the striking preference for H_2O_2 as an iron oxidant known to date are the two Dps proteins from *B. anthracis*, Ba Dps1 and Ba Dps2. Ba Dps2 can use both O_2 and H_2O_2 as oxidants since the relevant rates differ only 3-fold, whereas Ba Dps1 has no detectable ferroxidase activity with H_2O_2 either aerobically or anaerobically, but resembles Ba Dps1 in its activity with O_2. The structural basis for this behaviour has not yet been elucidated, but is likely to reside in differences in the second coordination shell given the conservation of the ferroxidase center iron ligands (22).

The later steps in the iron detoxification process carried out by Dps proteins, namely the storage/solubilization of Fe(III) as ferric hydroxide crystalline cores in the protein cavity (23), resemble those carried out by ferritins, although the storage capacity corresponds to about 500 Fe(III)/dodecamer (11). Likely sites of mineralization are apparent in the crystal structure of iron-loaded *D. radiodurans* Dps2 (17). Just as in ferritins, iron reduction renders the stored metal available to the microorganism (21).

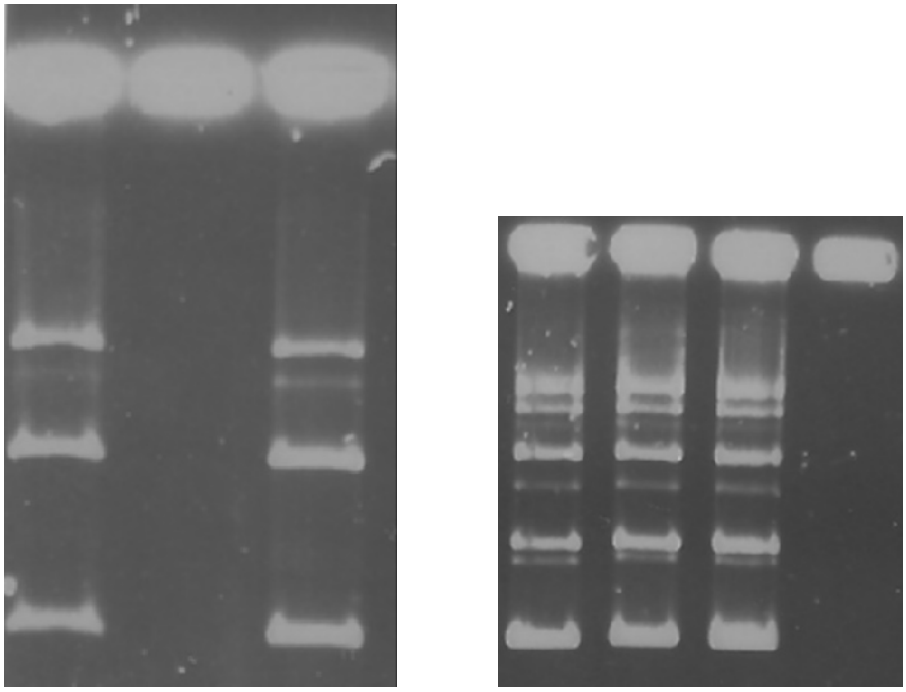

Fig. 2 DNA binding (left panel) and protection (right panel) properties of *Escherichia coli* and *Listeria innocua* Dps. Left panel (from the left): lane 1, plasmid DNA – the bands correspond to different degrees of supercoiling. In the presence of *E. coli* Dps (lane 2), large Dps-DNA complexes are formed that do not leave the loading well on the top of the gel. In contrast, in the presence of *L. innocua* Dps (lane 3), which does not interact with DNA, the electrophoretic pattern is unaltered. Right panel (from the left): lane 1: plasmid DNA – the bands correspond to different degrees of supercoiling. Exposure of DNA to Fe(II) and H_2O_2 fully degrades it such that no bands are apparent (lane 4). The presence of *E. coli* Dps (lane 2) or *L. innocua* Dps (lane 3) prevents DNA degradation as indicated by the electrophoretic pattern which is unaltered relative to lane 1. Taken together with the data in the left panel, this behaviour demonstrates that DNA protection takes place irrespective of the occurrence of Dps-DNA interaction. Conditions: 20 nM plasmid DNA, 3 μM Dps, 50 mM Tris-HCl buffer at pH 7.3, addition where indicated of 100 μM $FeSO_4$ and 10 mM H_2O_2.

The distinctive capacity of Dps proteins to utilize simultaneously Fe(II) and H_2O_2 reduces the toxicity of these two compounds drastically since they can no longer give rise to hydroxyl radicals (20, 21, 24, 25). Bacterial DNA, given the absence of nucleosomes, is particularly exposed to the damaging action of these radicals which induce breaks in one or both DNA strands. *In vitro*, Dps proteins fully protect DNA from this Fe(II) and H_2O_2 mediated DNA degradation as can be assessed easily by means of DNA damage assays (20). Importantly, those Dps proteins that do not bind DNA, like *L. innocua* (Fig. 2 and ref. 25) are able to impair DNA degradation just as efficiently as those which form Dps-DNA complexes like *E. coli* Dps (Fig. 2).

Given that Dps proteins protect DNA from oxidative damage even in the absence of interaction between the two macromolecules, one may ask what the full biological significance of Dps-DNA complex formation is. The two protection mechanisms differ in terms of distribution and structural basis: "chemical" protection is conferred by all Dps proteins since it is due to the highly conserved ferroxidase center, whereas "physical" DNA protection is imparted only by some Dps proteins as it is related to the presence of specific structural elements. To date, several such elements have been identified by means of *in vitro* studies.

The archetypical *E. coli* Dps, as hypothesized upon resolution of the X-ray crystal structure (26) and demonstrated later (27), binds DNA via the highly flexible and positively charged N-terminal region which departs from the four-helix bundle core of each subunit. In the dodecamer, the N-terminal regions face the solvent and therefore are able to interact with the negatively charged DNA backbone. Their regular disposition in space, which is dictated by the 23 symmetry of the dodecamer, allows formation of ordered Dps-DNA complexes such as those observed in stationary phase cells (4, 5). In these complexes, *E. coli* Dps forms a physical shield that segregates DNA and protects it from the action of damaging agents. However, many Dps proteins do not bind DNA because the N-terminal region is either very short, as in *L. innocua* Dps (12), or lacks flexibility due to interactions with amino acid residues on the protein surface as in *Agrobacterium tumefaciens* Dps (28), or lacks positively charged amino acids. Other Dps proteins adopt different binding strategies: *Mycobacterium smegmatis* Dps employs the C-terminal region, which is particularly long and rich in positively charged amino acids (29, 30), while *Helicobacter pylori* Dps uses the whole molecular surface which carries an overall positive charge at the slightly acid pH values reached by the bacterial cytoplasm during host infection (31). These limited examples show that the electrostatic interaction between Dps proteins and DNA is realized by means of an unexpected variety of molecular mechanisms which operate precisely under the stress conditions specific to the bacterium, as in *H. pylori*. Complex formation therefore does contribute to DNA protection, but it is not known why this additional factor is not required by some bacteria.

Before reviewing data obtained on bacterial cells, the Dps-like proteins identified recently in Archaea (32–34) and in *Lactococcus lactis* MG1363 (35) should be mentioned. To date, the protein from the hyperthermophilic acidophile *Sulfolobus solfataricus* is the best characterized archeon one (32, 33). Intriguingly, it shares similarities with both Dps proteins and ferritins. The dodecameric cage-like assemblage of four-helix bundle subunits that utilizes H_2O_2 preferentially in the oxidation of Fe(II) is similar to authentic Dps proteins, whereas the ferroxidase center harboured within single subunits resembles the typical ferritin one. A unique signature is represented by a pair

of cysteine residues adjacent to the ferroxidase site, a motif that may play a structural role or a redox active one. Further biochemical investigations will elucidate how this distinct combination of structural features functions at a molecular level. The dodecameric protein from *L. lactis* binds DNA, but lacks a ferroxidase center, be it a conventional intra-subunit ferritin-like one or the inter-subunit one characteristic of Dps proteins; moreover its iron sequestration and H_2O_2 detoxification capacity has not yet been demonstrated (35).

In bacterial cells, the unique capacity of Dps proteins to combat Fe(II) and H_2O_2 dependent oxidative stress manifests itself clearly, even though different situations can be encountered due to the expression of different defence proteins in various species. Taking solely ferritins and Dps proteins into consideration, some bacteria (e.g. *E. coli*) possess both, others (e.g. *Listeria* spp.) contain only Dps proteins. Ferritins, when present, have an iron storage role, whereas Dps proteins serve to inhibit the production of reactive oxygen species via Fenton chemistry. The latter function is of special importance in pathogenic bacteria since production of H_2O_2 by macrophages and neutrophiles is one of the first defence mechanisms of the host. The diversity of situations and the distinctive role of Dps proteins are illustrated by the following examples.

The Gram-negative obligate anaerobe *Porphyromonas gingivalis*, which is associated with chronic periodontites, displays a high degree of aerotolerance even though by definition it can not grow in aerobic conditions. It lacks catalase and thus is unable to break down H_2O_2 into O_2 and water, but possesses a "canonical" ferritin and a Dps protein that is espressed under the control of the transcription factor OxyR, like other hydrogen peroxide inducible activities. The behaviour of the relevant gene-deletion mutants demonstrates that aerotolerance is conferred both by ferritin and the Dps protein, whereas resistance to peroxide stress depends solely on the presence of the latter protein (36, 37). In the closely related anaerobic opportunistic pathogen *Bacteroides fragilis*, which colonizes the lower intestinal tract, *dps* expression in ΔoxyR strains is induced approximately fourfold by O_2 exposure but not by H_2O_2, indicating that Dps expression is also under the control of an oxygen-dependent OxyR-independent mechanism (38). Given that both *P. gingivalis* and *B. fragilis* are well adapted to strictly anaerobic environments, one must question the role of such complex oxidative stress response systems. Perhaps they provide resistance to the oxidative burst of human phagocytes until appropriate anaerobic conditions are established at the site of infection (38).

The case of *Streptococcus mutans*, a Gram-positive, facultative anaerobe that is found commonly in the human oral cavity and contributes significantly to tooth decay, is likewise of interest. *S. mutans* can grow in the presence of oxygen due to the action of flavoenzymes, even though, like all lactic acid bacteria, it does not synthesize heme and therefore lacks catalase and heme-peroxidase that are required for oxygen tolerance. *S. mutans* expresses a Dps-like protein

named Dpr (Dps-like peroxide resistance) that protects the bacterium from peroxides, as the name indicates, and hence confers oxygen tolerance. Accordingly, *dpr* disruption mutants form about 10^4-fold less colonies with respect to wild type in the presence of air (39, 40).

The protection that the highly conserved ferritin-like activity of Dps proteins imparts against oxidative stress and the associated damage to DNA and other cellular components therefore can be evidenced unambiguously. The understanding of the metabolic conditions that promote formation of Dps-DNA complexes in some bacterial species, taken together with the elucidation of all the factors that affect expression of Dps proteins, will provide a complete picture of the significance of these fascinating proteins in the bacterial response to stress conditions.

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