



Review

The multifaceted capacity of Dps proteins to combat bacterial stress conditions: Detoxification of iron and hydrogen peroxide and DNA binding

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ABSTRACT

Background: The widely expressed Dps proteins, so named after the DNA-binding properties of the first characterized member of the family in *Escherichia coli*, are considered major players in the bacterial response to stress.

Scope of review: The review describes the distinctive features of the "ferritin-like" ferroxidation reaction, which uses hydrogen peroxide as physiological iron oxidant and therefore permits the concomitant removal of the two reactants that give rise to hydroxyl radicals via Fenton chemistry. It also illustrates the structural elements identified to date that render the interaction of some Dps proteins with DNA possible and outlines briefly the significance of Dps–DNA complex formation and of the Dps interaction with other DNA-binding proteins in relation to the organization of the nucleoid and microbial survival.

General significance: Understanding in molecular terms the distinctive role of Dps proteins in bacterial resistance to general and specific stress conditions.

Major conclusions: The state of the art is that the response to oxidative and peroxide-mediated stress is mediated directly by Dps proteins via their ferritin-like activity. In contrast, the response to other stress conditions derives from the concerted interplay of diverse interactions that Dps proteins may establish with DNA and with other DNA-binding proteins.

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1. Introduction

The first member of the Dps proteins family was isolated in 1992 by Roberto Kolter et al. from 3-day old cells of *Escherichia coli*. This model system reflects the prevailing state of bacteria during the majority of their lives in their natural environment where microorganisms face an intense competition for nutrients and are under starvation conditions. Kolter et al. observed that *in vitro* the protein they had isolated binds DNA without apparent sequence specificity and therefore named it Dps for DNA-binding protein from starved cells [1]. They observed also that Dps–DNA complexes are very stable so as to render DNA resistant to DNase and to oxidative damage attendant treatment with H₂O₂, and that mutant cells lacking Dps show a dramatically altered pattern of protein synthesis during starvation and do not develop starvation-induced resistance to H₂O₂ [1,2]. In turn, the strong induction of Dps expression during the stationary phase of *E. coli* growth renders Dps the most abundant protein in the bacterial cytoplasm where Dps–DNA crystals are formed. DNA is entrapped in these crystals and shielded physically by

the protein. On this basis DNA protection by *E. coli* Dps was attributed to its interaction with DNA [3,4]. However, the physical sequestration of DNA in Dps–DNA complexes did not explain the seminal observation that Dps expression is associated to starvation-induced resistance to H₂O₂ and increases during the log phase of growth under conditions of oxidative stress [5].

Understanding the molecular basis of the linkage between Dps expression and bacterial resistance toward H₂O₂-mediated and oxidative stress has been the focus of most subsequent studies on the Dps protein family. The fact that this linkage is provided by the highly conserved ferroxidase center [6], that confers a "ferritin-like" activity to all Dps proteins, was decisive for their assignment to the ferritin superfamily proposed initially by Evans et al. [7].

To date Dps proteins, after the characterization of over 20 family members, are considered major players in the bacterial response to oxidative stress. The molecular foundation of their protective action will be reviewed and discussed in terms of both the "ferritin-like" activity and the DNA binding capacity. The former nullifies the toxic combination of Fe(II) and H₂O₂ and allows protection not only of DNA, but also of other biological macromolecules under conditions of oxidative damage. In contrast, DNA binding provides a physical means of protecting specifically this macromolecule. Therefore, special emphasis will be given to the structural determinants on the Dps molecule that allow interaction with DNA.

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2. Structural features of Dps proteins

Dps proteins are distributed widely in the bacterial kingdom. Typically bacterial genomes encode for one Dps protein; some genomes encode for two (e.g. *Mycobacterium smegmatis*, *Deinococcus radiodurans*, *Thermosynechococcus elongatus*, and *Bacillus anthracis*) while two actinobacteria, *Streptomyces coelicolor* and *Streptomyces ghanaensis*, encode three of these proteins. Thus, a search in the PROSITE database using the *E. coli* protein as a probe identified over 300 Dps sequences. These are characterized by a central core and by N- and C-terminal regions of variable length and with a different number of positive charges (Fig. 1). The central core contains several conserved amino acids that are located at or near the ferroxidase center (respectively H51, H63, D78, E82, and W52, *E. coli* numbering) or are involved in structurally important interactions (e.g. W163, R83, D143—*E. coli* numbering— which participate in stabilization of the quaternary assembly).

2.1. The Dps fold and quaternary structure

The tertiary and quaternary structures of Dps proteins are highly conserved: the polypeptide chain folds into a four-helix bundle (A–D helices) formed by the central core. An additional small helix, the BC helix, is located in the loop that links the B and C helices. The stable molecule is a quasi-spherical, shell-like dodecamer assembled with 23 symmetry (external diameter ~9.0 nm) that defines an internal cavity ~4.5 nm in diameter (Fig. 2). The Dps fold and assembly were described first for *E. coli* Dps by Grant et al. [8] who evidenced the close resemblance of both structural features with those typical of ferritins, although the latter proteins are assembled from 24 identical or highly similar subunits related by 432 symmetry. At the junction of the 3-fold symmetry axes the Dps subunits, just like the ferritin ones, form small pores that connect the internal cavity with solvent and can be used for passage of ions and small molecules. Since the 23

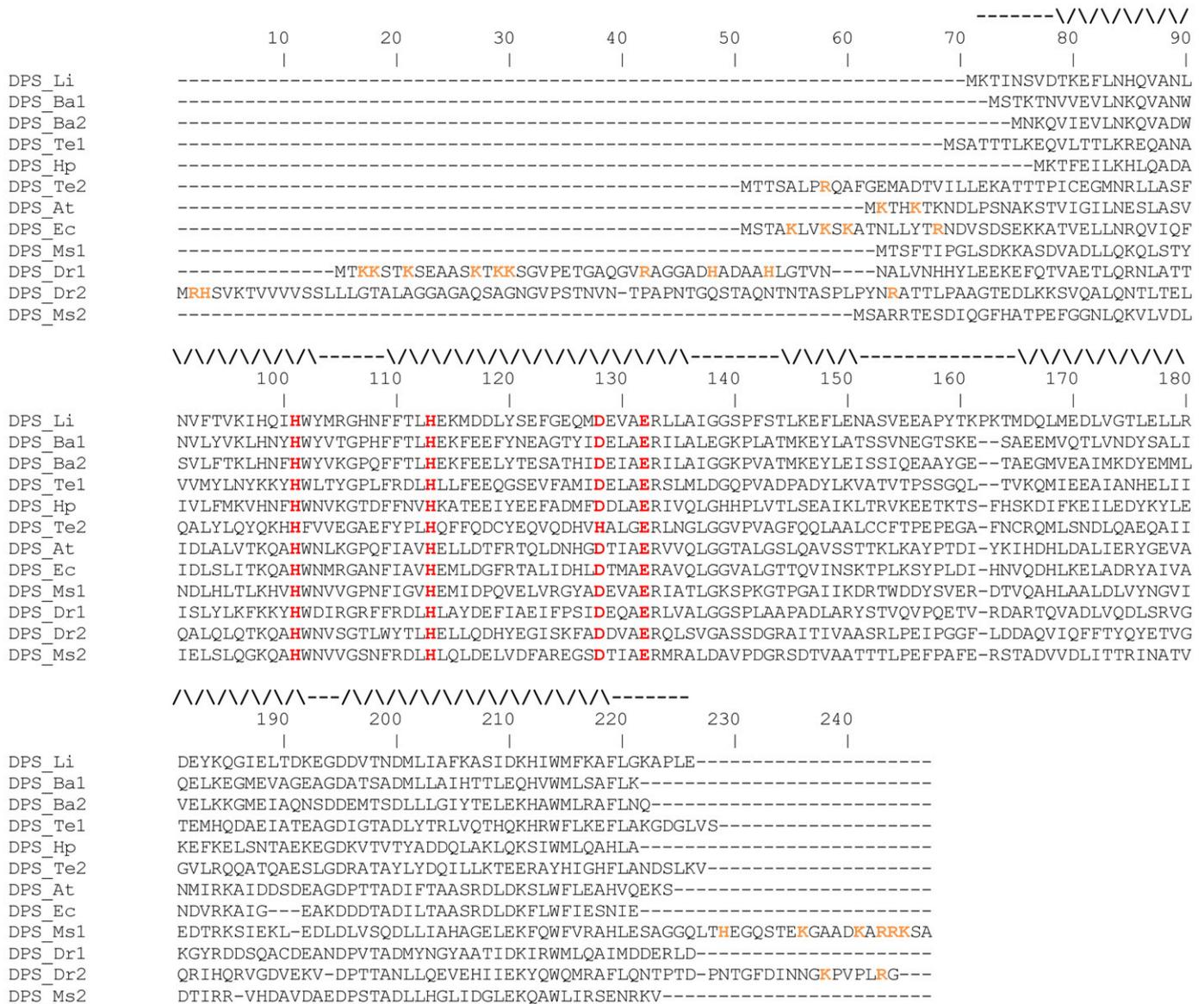


Fig. 1. Alignment of representative sequences of Dps proteins. The software Multalin (www.expasy.org) was used to align Dps sequences. The sequences were selected among proteins of known crystal structure: *L. innocua* (DPS_Li; PDB 1QGH), *B. anthracis* (DPS_Ba1 and DPS_Ba2; PDB 1J15 and 1J1G), *H. pylori* (DPS_Hp; PDB 1J14), *A. tumefaciens* (DPS_At; PDB 1O9R), *E. coli* (DPS_Ec; PDB 1DPS), *M. smegmatis* (DPS_Ms1 and DPS_Ms2; PDB 1UVH and 2Z90), *T. elongatus* (DPS_Te1 and DPS_Te2; PDB 2C41 and 2VXX) and *D. radiodurans* (DPS_Dr1 and DPS_Dr2; PDB 2C2U and 2C2J). Secondary structure elements: random coil (---), α -helix (|/|/|). The positively charged residues of N-terminal and C-terminal regions are in orange, whereas the ferroxidase center residues are in red.

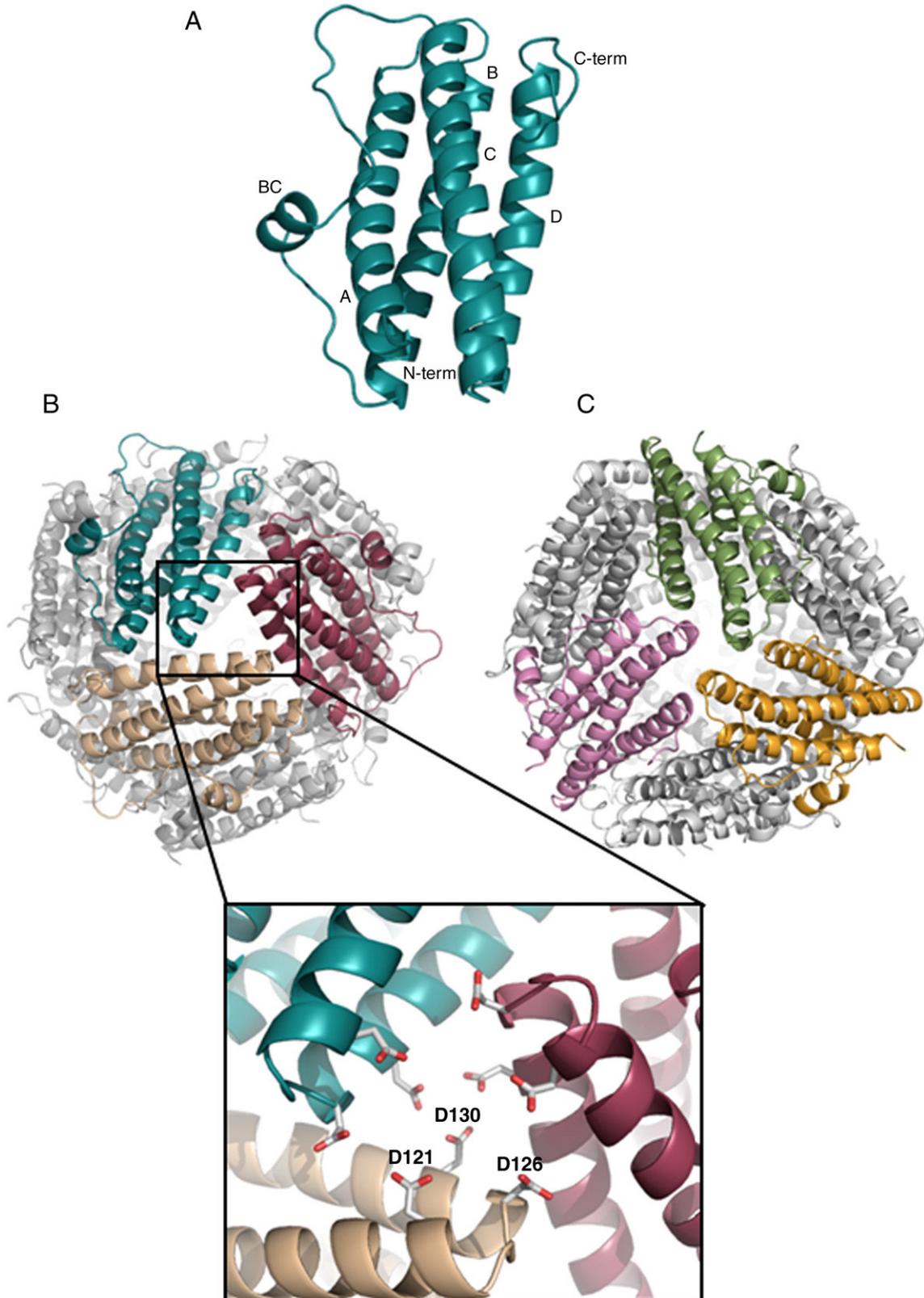


Fig. 2. Structural organization of Dps proteins. Dps fold (A), 12-mer assembly: view along the ferritin-like (B) and Dps-type (C) pores formed respectively by the N- and C-terminal regions of the subunits. In (B) the blow-up shows the aspartate residues lining of the ferritin-like pore in *L. innocua* Dps [10].

symmetry defines two kinds of environments at the 3-fold symmetry axes, two different types of pore can be distinguished. One is created by the N-terminal ends of the subunits and was named “ferritin-like” since it is lined by negatively charged residues similar to the pores formed at the 3-fold symmetry axes in ferritins. The structural

similarity carries over to function. Thus, in both protein families iron exploits the electrostatic potential created by the negatively charged residues lining the pores to enter and exit the protein cavity as shown by the presence of one metal ion in iron-soaked crystals of *D. radiodurans* Dps [9] and by recent experiments on *Listeria innocua* Dps

[10]. The other type of pore is formed by the C-terminal ends of the subunits, is unique to Dps proteins and has been called “Dps-type”. The function of these pores is still unknown. One of the reasons lies possibly in the variability of their dimensions and of the residues that line them.

A further structural similarity of the Dps and ferritin molecule concerns the internal cavity which has a predominantly negative charge in both assemblages and is used in an analogous manner to furnish iron core nucleation sites.

2.2. The ferroxidase center

The ferroxidase center represents the most distinctive structural signature of the Dps family. It is highly conserved and has a most unusual location at the interface of 2-fold symmetry related subunits rather than within the four-helix bundle of a single subunit as in all known ferroxidases ([6], Fig. 3). This signature is not present in the Dps-like dodecameric proteins from two Archaea, *Pyrococcus furiosus* [11] and *Sulfolobus solfataricus* [12], which possess an intrasubunit ferroxidase center resembling that of bacterioferritins and apparently belong to a different branch of the ferritin superfamily.

Typical ferroxidase centers are bimetallic. In ferritins, the two iron atoms are at a distance of about 3 Å and are connected by an oxo-bridge. The so-called A site uses a histidine and carboxylates as iron coordinating ligands and binds iron with higher affinity than the so-called B site where the metal is coordinated by carboxylates only [13]. In Dps proteins, both symmetry related subunits furnish the iron ligands (Fig. 3). In particular, one subunit provides two histidine residues (H51 and H63, *E. coli* numbering) and the other subunit two carboxylate ligands (D78 and E82, *E. coli* numbering). The only known exception is *T. elongatus* DpsA where the ferroxidase center comprises three histidine residues since the canonical aspartate (Asp78, *E. coli* numbering) is substituted by a histidine, His78 (Fig. 1).

Despite the high conservation of the iron coordinating residues, the occupancy of the two metal binding sites with iron varies significantly in the known crystal structures. In *L. innocua* Dps, where the ferroxidase center was identified, it contains one iron and one water molecule, which lies at about 3 Å from the iron and could be replaced by the second iron atom to give rise to a canonical bimetallic ferroxidase center [6]. The ferroxidase center of *Helicobacter pylori* Dps [14], *D. radiodurans* Dps1 [9,15], and *Agrobacterium tumefaciens* Dps [16] likewise contains one bound iron; in *D. radiodurans* Dps2 [17] and *Bacillus brevis* Dps [18] it contains two iron atoms, while in *E. coli* Dps [8] and *T. elongatus* Dps [19] it is occupied by two water

molecules. This variability in iron occupancy in turn indicates that the amino acids forming the second metal coordination shell influence the affinity of the ferroxidase center for iron. In *E. coli* Dps the decreased affinity for iron has been attributed to the presence of a lysine residue which forms a salt bridge with the iron coordinating Asp residue [20]. Notably, anaerobic titration of *E. coli* Dps with Fe(II) leads to full saturation of both the A and the B sites with the metal [21].

2.3. DNA binding signatures

The striking DNA binding ability displayed by the prototypic *E. coli* Dps [1,3,4] is not shared by all members of the family since the establishment of effective electrostatic interactions with the negatively charged DNA backbone depends on the presence of specific structural elements on the Dps molecule.

The N-terminal region was recognized as a DNA-interacting element in *E. coli* Dps based on the X-ray crystal structure which displays highly mobile N-terminal regions that reach out from the surface of the molecule into solvent such that the three lysine residues they contain can interact with the phosphate groups on DNA [8]. The role played by the multiplicity of positively charged residues and by the N-terminus mobility in determining the strength and pH dependence of the interaction was evaluated in later studies that employed also other techniques, namely electrophoretic mobility shift assays and atomic force microscopy, to assess Dps–DNA complex formation. The DNA binding behaviour of wild type *E. coli* Dps and its N-terminus deletion mutants pointed out the role of lysine multiplicity on the DNA binding ability at various pH values [22]. Thus, the wild type protein carries three positively charged lysines at the intact N-terminus at physiological pH values and forms large complexes that enclose many Dps molecules and one or more DNA plasmids, the so-called DNA condensates. When pH increases and the lysine residues become deprotonated, large complexes are no longer formed and simple DNA binding takes place. On the other hand, the mutant lacking 2 out of 3 N-terminal lysine residues binds DNA, but is unable to condense it, while the mutant lacking all 3 lysine residues has no DNA binding or condensation capacity. Mobility of the N-terminus is essential for an effective Dps–DNA interaction just as the presence of positive charges. Thus, *A. tumefaciens* Dps, which carries at the N-terminus three lysine residues like *E. coli* Dps, is unable to interact with DNA since the N-terminal regions are immobilized on the dodecamer surface by means of hydrogen bonding and salt bridge interactions established with the 3-fold symmetry related subunits

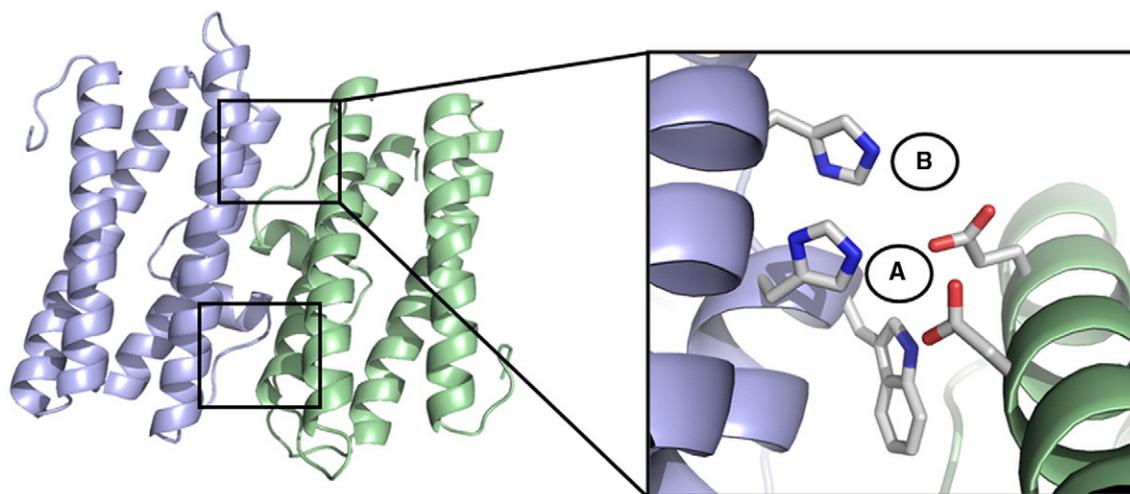


Fig. 3. Dimeric interface and ferroxidase center of Dps proteins. Two-fold symmetry interface (left) and blow-up of the intersubunit ferroxidase center located at the interface (right). The positions of the high affinity A and low affinity B iron binding site are indicated along with those of the conserved metal ligands, namely two histidine residues, one aspartic acid and one glutamic acid.

[16]. A decreased flexibility of the N-terminus may account also for the observation that the DNA binding/condensation capacity of *Lactococcus lactis* DpsA and DpsB is limited to long stretches (>4000 bp) of linearized DNA. In both lactococcal Dps proteins, the N-terminal sequences do face solvent, but are folded into an α helix which is intrinsically less mobile than an extended conformation [23,24].

The N-terminus is likely to mediate the interaction with DNA in several Dps proteins since some 20 family members have N-terminal sequences resembling that of *E. coli* Dps. The studies on *M. smegmatis* Dps2 (MsDps2), and on *D. radiodurans* Dps1 have shown this contention to be true [25–27].

Two other DNA binding signatures have been identified to date. The C-terminal region, provided it has the appropriate charge and mobility characteristics, can be used to advantage to bind DNA. The only known example is furnished by *M. smegmatis* Dps1 which has a truncated N-terminus and a freely mobile, 26 amino acid long C-terminus containing five positively and four negatively charged amino acids. Removal of the last 16 C-terminal residues abolishes the DNA binding activity and proves the use of this region for the interaction between the two macromolecules [28,29]. *M. smegmatis* Dps1 is of interest also because it provides insight into the fine interplay between electrostatic charges that regulate the Dps–DNA interaction. Thus, *M. smegmatis* Dps1 is unable to promote DNA condensation despite the presence of multiple positive charges on the C-terminus, likely due to a compensatory effect of the negatively charged residues on the very same region [30].

The third DNA binding signature is operative in *H. pylori* Dps, also named HPNAP because of its neutrophil-activating properties. At pH values around neutrality, HPNAP unlike the other Dps proteins is characterized by a positively charged protein surface that is used to bind DNA. The HPNAP–plasmid DNA complexes appear as “beads-on-a-string” at pH 8.0 in atomic force microscopy images and as large condensates at pH values around 7.0. This pH-dependent, fully reversible change in DNA binding mode is believed to represent a physiologically relevant mechanism that can be used by the bacterium to protect DNA during infection in the human stomach, where the significant decrease in cytoplasmic pH puts this macromolecule in danger [31]. A similar mechanism may be operative in the Dps protein from *Trichodesmium erythraeum*, a marine N_2 -fixing cyanobacterium. This Dps protein has a truncated C-terminus, no positively charged residues on the N-terminus, but binds DNA at pH 8.0 without condensing it into large Dps–DNA complexes [32].

This scrutiny of the various structural elements used by Dps molecules to bind DNA is believed to provide an exhaustive picture of the modes of interaction between the two macromolecules. Thus, the presence or absence of these signatures in the sequence of a given Dps protein can be used to predict its DNA binding ability with reasonable confidence. Indeed, Dps proteins characterized by an N-terminus of reduced length, like *L. innocua* Dps [33], *B. anthracis* Dlp-1 and Dlp-2, [34] and *Campylobacter jejuni* [35] do not interact with DNA, according to predictions. However, when inspection of the sequence evidences extended, positively charged N- or C-terminal regions, in view of their possible immobilization on the protein surface as in *A. tumefaciens* Dps [16], the predicted DNA binding activity should be confirmed experimentally, for example by means of electrophoretic mobility shift assays.

3. Protection mechanisms operative in Dps proteins

3.1. Chemical mode of protection: ferroxidase activity

The ferroxidase activity of Dps proteins has distinctive features with respect to the other members of the ferritin superfamily. Ferritins in general use O_2 as iron oxidant with the production of hydrogen peroxide [36], bacterioferritins can employ both O_2 and

H_2O_2 [37], whereas Dps proteins typically prefer H_2O_2 which is usually about 100-fold more efficient than O_2 in carrying out iron oxidation [19,21,38].

The first indications in this respect were provided by the protein isolated from *L. innocua*, which at the time was believed to be a novel, unusual ferritin due to its dodecameric assembly and the inefficiency of O_2 as iron oxidant [33]. However, the decisive observation that H_2O_2 is by far better than O_2 in carrying out Fe(II) oxidation was made on *E. coli* Dps [21] and was thereafter shown to be valid for practically all Dps proteins assayed. Thus, in the presence of H_2O_2 the ferroxidation rate is between 100- and 1000-fold faster with respect to O_2 [19,21,39]. The only reported exception refers to Dps2 (also named Dlp2) from *B. anthracis* which reacts with Fe(II) and H_2O_2 three-fold faster than with O_2 . The structural basis for this unusual behaviour remains unexplained.

The concomitant removal of Fe(II) and H_2O_2 from solution results in a strong inhibition of the Fenton reaction: $Fe(II) + H_2O_2 \rightarrow Fe(III) + OH^- + OH^\cdot$. As a result, formation of the highly toxic hydroxyl radicals is attenuated significantly as confirmed by spin trapping experiments using EMPO (5-ethoxycarbonyl-5-methyl-1-pyrroline-*N*-oxide) as a probe. These experiments not only proved that the release of OH^\cdot into solution decreases significantly in the presence of *E. coli* and *L. innocua* Dps, but showed that it is practically nil if Fe(II) is added to the protein before hydrogen peroxide [21,39]. The inhibition of Fenton chemistry requires an intact ferroxidase center whereas oxidation of Fe(II) by molecular oxygen does not. Thus, in *Listeria* Dps variants that carry substitutions at the iron coordinating ligands, the rate of iron oxidation and the capacity to inhibit Fenton chemistry are decreased significantly in the presence of H_2O_2 but are affected only slightly under aerobic conditions [40].

After oxidation, iron moves to the internal Dps protein cavity and gives rise to a microcrystalline core that may contain up to 400–500 iron atoms [20]. At variance with the ferroxidation step, the iron uptake one has similar characteristics in both ferritins and Dps proteins. Thus, a slow nucleation step is followed by fast, cooperative growth of the crystal as manifest in the sigmoid kinetics of core formation. The nucleation step takes place at the internal cavity and is likely to involve specific residues as in ferritins. On the other hand, cooperativity in the crystal growth step is modulated by the strength of the electrostatic gradient at the pores and is linked to the size distribution of the iron mineral as shown recently by the behaviour of *L. innocua* Dps and site-specific variants of negatively charged pore residues ([10] and Fig. 2B, blow-up).

The decreased production of OH^\cdot has obvious consequences on the protection of DNA, RNA, proteins and lipids, the biological targets of these highly reactive oxygen species. The experimentalists have concentrated their attention on the protection of DNA which in bacteria can be attacked directly by damaging agents due to the absence of nucleosomes. DNA protection against Fe(II)- and H_2O_2 -mediated oxidative damage has been assessed by means of simple *in vitro* assays that utilize agarose gel electrophoresis and supercoiled or linearized DNA plasmids. The effect of *L. innocua* and *E. coli* Dps in tests entailing exposure of pet-11a DNA to 1 mM H_2O_2 and 24 Fe(II)/dodecamer at pH 7.0 is depicted in Fig. 4. In the absence of Dps, DNA is fully degraded. In contrast, Dps in sufficient amounts to saturate DNA yields essentially complete protection irrespective of the occurrence of DNA binding. The order of iron and H_2O_2 addition does not appear to have an influence in this experiment which therefore appears to have a lower sensitivity than that employing EMPO described in [21,39] and mentioned above.

These findings provide the molecular explanation for the initial observations by Kolter et al. that *E. coli* dps deletion mutants display an enhanced susceptibility to oxidative stress and that under these conditions Dps synthesis increases [2]. The data obtained thereafter on a number of bacterial species brought out clearly the unique capacity of Dps proteins to combat the Fe(II) and H_2O_2 dependent

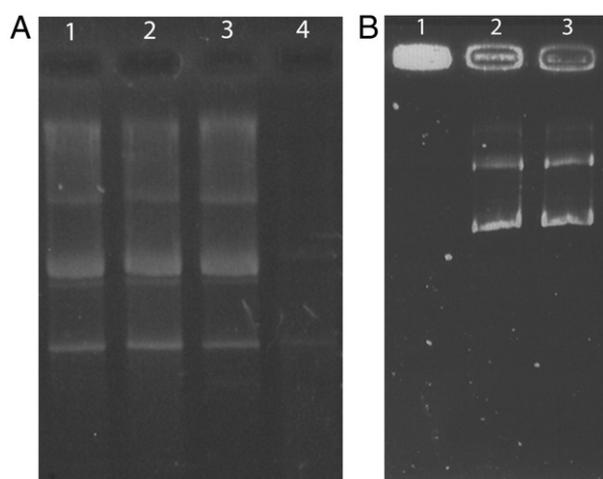


Fig. 4. DNA protection exerted by *L. innocua* and *E. coli* Dps as manifest in a DNA protection assay at pH 7.0. Panel A, lanes in the 1% agarose gel (from left to right): 1, native plasmid DNA (pet-11a, 5600 bp, 20 nM); 2, plasmid DNA with 3 μ M *E. coli* Dps exposed to 72 μ M FeSO_4 and subsequently to 1 mM H_2O_2 ; 3, plasmid DNA with 3 μ M *L. innocua* Dps exposed to 72 μ M FeSO_4 and subsequently to 1 mM H_2O_2 ; and 4, plasmid DNA exposed to 72 μ M FeSO_4 and subsequently to 1 mM H_2O_2 . In panel B, the 1% agarose gel shows that migration of plasmid DNA (lane 3) is not affected by the presence of *L. innocua* Dps (lane 2) but forms large complexes that do not enter the gel in the presence of *E. coli* Dps (lane 1).

oxidative stress even though different situations may be encountered in the various species due to the expression of different defence proteins. Thus, if one takes solely ferritins and Dps proteins into consideration for the sake of simplicity, some bacteria (e.g. *E. coli*, *H. pylori*, *T. longatus*, *B. anthracis*, and *A. tumefaciens*) possess both, while others (e.g. *Listeria* spp.) contain only Dps proteins. In the species which possess both proteins, ferritins have a prominent role in iron storage while Dps proteins provide resistance to peroxide stress as evidenced by the behaviour of the respective deletion mutants and by the nature of the transcription factors that control protein synthesis. It is worth stressing that the unique capacity of Dps proteins to inhibit the production of reactive oxygen species via Fenton chemistry is of special importance in pathogenic bacteria. Since production of H_2O_2 in macrophages and neutrophils represents one of the host first defence mechanisms, Dps proteins contribute to or are required for bacterial survival in macrophages and for virulence. In *Salmonella enterica* serovar Typhimurium for example, Dps protects the bacterium from iron-dependent killing by H_2O_2 , promotes its survival in macrophages and enhances its virulence in mice [41].

Numerous literature data bring out the different roles of ferritin and Dps proteins in bacteria. Only a few examples will be presented. *C. jejuni*, a microaerophilic Gram-negative bacterium that represents a major cause of bacterial diarrhea in undeveloped and developing countries, is likely to encounter growth-limiting or potentially lethal conditions such as iron limitation and oxidative stress in the natural habitat, be it the gastrointestinal tract of the host or the external environment during transmission. *C. jejuni* possesses a canonical 24-mer ferritin and a Dps protein that does not bind DNA and is expressed constitutively. As expected, ferritin plays an important role in iron storage under iron-restricted conditions while the Dps protein confers hydrogen peroxide stress resistance as demonstrated by the enhanced sensitivity to H_2O_2 of a *dps* mutant strain and by the re-establishment of peroxide resistance upon addition of the iron-chelator Desferal in the culture medium [35].

The relationships with other oxidative response systems are apparent in the following two examples. *Porphyromonas gingivalis* is a Gram-negative obligate anaerobe associated with chronic periodontitis that by definition cannot grow in aerobic conditions, but displays a high degree of aerotolerance. It has no catalase and therefore

is not able to break down H_2O_2 into O_2 and water, but contains a ferritin and a Dps protein. While aerotolerance is conferred both by ferritin and the Dps protein, resistance to peroxide stress depends solely on the presence of the latter protein that is expressed under the control of the transcription factor OxyR, like other hydrogen peroxide inducible activities [42,43]. Likewise of interest is *Streptococcus mutans*, a Gram-positive, facultative anaerobe that occurs frequently in the human oral cavity and is a major cause of tooth decay. *S. mutans* does not synthesize heme like all lactic acid bacteria and therefore lacks catalase and heme-peroxidase that are required for oxygen tolerance. However, *S. mutans* can grow in the presence of oxygen due to the action of flavoenzymes and of a Dps-like protein named Dpr (Dps-like peroxide resistance) that protects the bacterium from peroxides. The specific contribution of Dpr in conferring oxygen tolerance is proven by the $\sim 10^4$ -fold decreased formation of colonies by *dpr* disruption mutants with respect to wild type *S. mutans* in the presence of air [44,45].

The data just presented demonstrate unambiguously that the ferroxidase activity of Dps proteins is essential in conferring bacterial protection against H_2O_2 -promoted damage. Having established this, the illustration of the most recent advancements in the understanding of the underlying reaction mechanism is in order. The overall scheme of the reaction of Fe(II) with H_2O_2 has been presented in several papers [21,39,40,46–49]. It leads to the production of water and entails the simultaneous transfer of two electrons to account for the very small release of hydroxyl radicals into solution [21,39]. However, intraprotein hydroxyl radicals can be formed upon transfer of single electrons as in other proteins [50,51]. The formation of hydroxyl radicals was indeed shown to occur in *E. coli* and *L. innocua* Dps after partial saturation of their ferroxidase centers with Fe(II) [52]. When such solutions are mixed rapidly with H_2O_2 in a stopped-flow apparatus, transient bands appear after 2.5 ms in both proteins and are no longer apparent after about 1 min. The transient *E. coli* Dps spectrum is characterized by a band centered at 512–536 nm attributable to a long-lived Trp radical, whereas the *L. innocua* Dps one contains additional bands at 410 nm, attributable to a Tyr radical, and at ~ 600 nm due to a Trp radical cation. With the aid of site-specific variants the bands have been assigned respectively to Trp52 and Trp32, which occupy the same position in the vicinity of the ferroxidase center in both *E. coli* and *L. innocua* Dps, and to Tyr50 which is present near this center only in the latter protein. Significantly, Trp52 (*E. coli* numbering) is conserved in over 90% of the known Dps sequences, while Tyr50 (*L. innocua* numbering) is present in about 50%. DNA protection assays carried out on the relevant site-specific mutants provided a reason for their conservation since they proved their role as a trap that limits release of hydroxyl radicals in solution and the consequent oxidative damage to DNA.

3.2. Physical mode of protection: DNA binding

At variance with ferroxidase activity, the DNA binding ability is not shared by all members of the Dps family as discussed under Section 2.3. Consequently, the protective function of Dps–DNA complexes is not common to all bacterial species. In those species where such complexes are formed, they play a key role under stress conditions occurring during starvation or vegetative growth, or induced by acid and base shock, iron and copper toxicity, high salt or radiation, but have no major relevance in resistance towards hydrogen peroxide [53]. Importantly, the binding of Dps to DNA without apparent sequence specificity described originally [1] represents only part of the picture since Dps proteins can bind specifically other nucleoid-associated proteins and DNA itself thus creating a complex, readily reversible interaction network with global regulatory functions.

In these types of studies *E. coli* served as a major model system. The observation of individual nucleoids by atomic force microscopy and

the use of *dps* deletion mutants have brought out the correlation between the cell cycle dependent changes in nucleoid compaction and the variations in the amount of Dps and other nucleoid-associated proteins, while the pleiotropic effects due to gene deletion have been accounted for in terms of direct effects on the transcription machinery [1]. In particular, the different effects of Dps expression on nucleoid compaction during the exponential and stationary phase of growth have been ascribed to the involvement of Fis and H-NS, two other nucleoid-associated proteins, that act by repressing Dps expression [54,55]. Fis binds at the *dps* promoter and prevents transcription initiation by σ^{70} containing RNA polymerase, while H-NS displaces this very same polymerase. Further, the abrupt decrease in Fis and H-NS concentration [55] when cells enter stationary phase explains the concomitant occurrence of Dps-dependent DNA super-compaction. The direct interaction between Dps and another DNA-binding protein was likewise reported to occur in *E. coli* [56]. The interaction involves DnaA, a replication initiation factor which promotes unwinding or denaturation of DNA at *oriC*. As a result, Dps prevent DnaA binding to DNA, hampers strand opening of the replication origin and blocks initiations partially. In turn, this block may provide a useful opportunity to repair damaged DNA under stress conditions and hence favour bacterial survival.

A most recent paper on *S. coelicolor* revealed a novel, unsuspected function of Dps proteins in bacterial cell division [57]. *S. coelicolor*, which has to adapt to various experimental stresses in situ, encodes three Dps proteins, DpsA, DpsB, and DpsC. Disruption of the respective genes results in a gene-specific, irregular condensation of spore nucleoids that is correlated with changes to the spacing between sporulation septa. Other stress conditions, notably heat and osmotic stress, induced expression of the various Dps proteins in a stimulus-dependent manner, suggesting stress-induced, protein-specific protective functions in addition to their role during reproductive cell division. Intriguingly, the *S. coelicolor* Dps proteins are not induced under oxidative stress suggesting that other enzymatic activities mediate the response to these conditions.

In conclusion, whereas the response to oxidative and peroxide-mediated stress finds its molecular basis in the ferritin-like activity of Dps proteins, the response to other stress conditions derives from the concerted interplay of diverse interactions that Dps proteins establish with DNA and with other DNA-binding proteins. Their study promises to disclose other roles of the exciting Dps family and may explain why DNA binding takes place only in some bacterial species.

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