Role of Dps (DNA-binding proteins from starved cells) aggregation on DNA

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1. ABSTRACT

The review outlines the experimental studies that have led to the current understanding at a molecular level of the protective role exerted by Dps proteins under stress conditions. After a brief description of the structural signatures and of the ferroxidase activity, which confers to all Dps proteins the capacity to decrease the hydroxyl radical induced DNA damage, the interaction of some family members with DNA is analysed. Special emphasis is given to the Dps structural elements that render the interaction with DNA possible and to the consequences that complex formation has on nucleoid organization and microbial survival.

2. INTRODUCTION

In prokaryotes genomic DNA is localized in the nucleoid that is analogous in function to the nucleus of a eukaryotic cell, but is not enclosed by a membrane. In each case, DNA is organized in higher order structures due to the interaction with distinct DNA-binding proteins, histones in eukaryotic cells and histone-like proteins in bacteria. The latter comprise proteins that bind DNA without sequence specificity and have a uniform localization within the nucleoid, such as H-NS (histone-like nucleoid structuring protein), HU (heat-unstable nucleoid protein), IHF (integration host factor), and Dps (DNA binding protein from starved cells), while others, like Fis
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(factor for inversion stimulation), bind DNA in a sequence-
specific manner and are confined to specific loci (1, 2). The
nucleoid proteins are distributed differently in the various
species and may be expressed at largely different
concentrations during the cell cycle. Thus, Fis occurs only in
gamma-Proteobacteria and represents the most abundant
protein during the log phase (~ 60,000 molecules per cell in
Escherichia coli), but is almost undetectable during the
stationary phase (3), whereas the widely distributed Dps
protein becomes the dominant nucleoid component precisely
during the latter phase of growth (~ 180,000-200,000
molecules per cell in E. coli; ref.1). Further, expression of the
same nucleoid protein may be under the control of different
promoters in different bacterial species and respond to different
environmental stimuli. In E. coli, for example, expression of the
dps gene is up-regulated by sigma s towards stationary
phase and by OxyR and sigma 70 in response to hydrogen
peroxide stress during the exponential phase of growth (4). In
contrast, in Staphylococcus aureus and other Gram-positive
bacteria, expression of the Dps homolog MrgA (Metallo
regulated gene A) is rather sustained throughout growth and is
up-regulated in response to oxidative stress under the control
of PerR (5).

Importantly, the formation and maintenance of
well organized higher order DNA structures is of biological
relevance per se as it affords protection against
environmental stresses by decreasing the accessibility of
genomic DNA to damaging agents (6, 7). It is therefore not
surprising that Dps proteins are part of the sophisticated
machinery whose role is to protect specifically bacterial
DNA not only during starvation and oxidative stress, but also from UV-light damage, nuclease cleavage, iron and
copper toxicity, thermal stress, and acid and base shock (8).

The seminal studies by Roberto Kolter and
collaborators on the family prototype, E. coli Dps, pointed also
to a global regulatory role of the protein as indicated by the
highly pleiotropic phenotype of mutant cells lacking Dps (4, 9,
10). Nevertheless, no experiments addressed the underlying
molecular mechanism(s) which must involve other DNA-
binding proteins that either interact directly with Dps or
compete with Dps for binding DNA. The subsequent
recognition that not all Dps proteins bind DNA, but are all
endowed with ferroxidase activity directed the interest of
several research groups towards understanding the relevance of
this activity in the oxidative stress response (11-15). These
studies evidenced the structural features of Dps proteins that
confers them iron detoxification-sequestration properties
resembling those of ferritins and led to the assignment of Dps
proteins to the ferritin superfamily. Only in recent years the
biological relevance of the Dps-DNA interaction is receiving
growing attention. The relevant data will be reviewed in the
present paper after examination of the structural motifs
identified to date that are used by Dps proteins in the
interaction with DNA.

3. PROPERTIES OF DPS PROTEINS

3.1. Structure and ferroxidase activity

Over 300 Dps sequences have been identified by
a search in the PROSITE database using the E. coli protein
as probe. They are characterized by a common central core
and N-terminal and C-terminal regions of variable length
(Figure 1). The sequences contain highly conserved
residues that belong to the ferroxidase center (H51, H63,
D78, E82, E. coli numbering) or are involved in
interactions that stabilize the quaternary assembly (e.g.
W163, R83, D143, E. coli numbering).

The central core of the polypeptide chain folds into a four-helix bundle (A-D helices with an additional
small helix, the BC helix, located in the middle of the loop
that connects the B and C helices), resembling the typical
ferritin one (16). In all Dps proteins the polypeptide chains
assemble into an almost spherical, shell-like dodecameric
structure (external diameter, 9 nm) which is endowed with
23 symmetry and defines a central cavity of 4.5 nm in
diameter (Figure 2). The external surface of the Dps
molecule is rich in negatively charged residues (typical pI
values are 4.8-5.1) and is interrupted by pores that are
formed at the junction of the 3-fold symmetry related
subunits and allow passage of ions and small molecules in
and out of the protein shell. Two different types of pores
can be distinguished since the symmetry of the molecule
creates two kinds of environments at the 3-fold symmetry
axes. One type is formed by the N-terminal ends of the
subunits. It has been named “ferritin-like” since it is rich in
negatively charged residues like the pores formed at the 3-
fold symmetry axes in ferritins and likewise provides the
route for iron to enter and exit the protein cavity (17). The
other type of pore is formed by C-terminal ends; it is
typical of Dps-proteins and can thus be called “Dps-type”.
Its precise function has not been elucidated as yet.

The signature of the Dps family however is
represented by the ferroxidase center which has a most
unusual location since it is positioned at the interface of 2-
fold symmetry related subunits rather than within the four-
helix bundle of a single subunit as in all known proteins
with ferroxidase activity (18). The ferroxidase center of
Dps-proteins contains two metal binding sites with different
affinity for iron, like all such centers. The amino acid
residues that provide the iron ligands are highly conserved
and are furnished by both symmetry related subunits, H51
and H63 by one subunit, D78 and E82 by the other (E. coli
numbering). Despite the high conservation of the iron
ligands, the occupancy of the two metal binding sites with
iron in the known crystal structures varies significantly (12,
16, 18). In L. innocua Dps, the ferroxidase center contains
one iron and one water molecule, while in E. coli Dps two
water molecules take the place of the metal. This variability
in turn indicates that the nature of the amino acids forming
the second metal coordination shell influences the affinity
of the ferroxidase center for iron.

The mechanism of iron oxidation at the
ferroxidase centre distinguishes Dps proteins from the other
members of the ferritin superfamily. Thus, ferritins use O2
as iron oxidant with the production of hydrogen peroxide,
bacterioferritins can use both O2 and H2O2, while Dps
proteins prefer by far H2O2 which is in general about a 100-
fold more efficient than O2 in carrying out iron oxidation
(19). This property accounts for the unique capacity of Dps
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Figure 1. Alignment of representative sequences of Dps proteins. The software Multalin was used to align the proteins from: *L. innocua* (DPS_Li), *S. aureus* (DPS_Sa), *B. anthracis* (DPS_Ba 1 and DPS_Ba 2), *H. pylori* (DPS_Hp), *C. jejuni* (DPS_Cj), *A. tumefaciens* (DPS_At), *E. coli* (DPS_Ec), *M. smegmatis* (DPS_Ms 1 and DPS_Ms 2), *D. radiodurans* (DPS_Dr 1 and DPS_Dr 2), *T. erythraeum* (DPS_Te), *L. lactis* (DPSA_Ll and DPSB_Ll). The positive residues of N-terminal and C-terminal regions are in blue, whereas the ferroxidase center residues are in red. Note that the *L. lactis* Dps sequences lack the canonical ferroxidase center residues and that the corresponding catalytic activity has not been assayed to date (30).

proteins to detoxify concurrently Fe(II) and H₂O₂ which are both removed from solution and hence can not give rise to the highly toxic hydroxyl radicals via the Fenton reaction: 

\[ \text{Fe(II)} + \text{H}_2\text{O}_2 \rightarrow \text{Fe(III)} + \text{OH}^- + \text{OH} \cdot \]

As in the case of ferritins and bacterioferritins, Fe(III) is directed towards the protein internal cavity where it is deposited as ferric hydroxide micelles and wherefrom it can be mobilized after reduction in order to meet metabolic needs.

The distinctive iron oxidation/uptake mechanism of Dps proteins, by preventing the Fe(II) and H₂O₂ mediated oxidative damage, furnishes a generalized protection to DNA and the other biological macromolecules and provides the molecular explanation for the increased synthesis of Dps proteins in response to hydrogen peroxide stress. This contention is demonstrated by a large number of investigations on the different sensitivity to peroxide stress of several bacterial species and their dps deletion mutants (10, 20, 21). In this connection the studies on *Streptococcus mutans* and *Porphyromonas gingivalis* are of special interest as these species lack catalase but display resistance to peroxide stress, a property which is reduced drastically upon deletion of the *dps* gene (13, 22).

3.2. DNA-binding mechanisms

The capacity to bind DNA has given to Dps proteins the family, but is not shared by all Dps proteins. The interaction takes place without sequence specificity (9) in accordance with the uniform distribution of Dps in the nucleoid (23). It leads to formation of large Dps-DNA complexes that shield DNA physically. Dps-DNA complex formation therefore protects DNA from a variety of toxic agents, but does not protect proteins and lipids at variance with the chemical protection mechanism just illustrated.

The capacity to bind DNA is correlated to the presence of structural elements that permit the unique strategy of *Streptococcus mutans* and *Porphyromonas gingivalis* to ensure survival in the midst of toxic agents, but does not protect proteins and lipids at variance with the chemical protection mechanism just illustrated.

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Figure 2. Structure of the *E. coli* (A), *L. innocua* (B) and *H. pylori* (C) Dps monomers and of the assembled 12-mers. Characteristic 4-helix bundle monomer structure (left) and view of the 12-mer (right) which evidences the 3-fold symmetry at the ferritin-like pores. Iron enters through these pores and reaches the bimetallic ferroxidase center, which is occupied by one iron and one water molecule in the X-ray structure of *L. innocua* (18) and *H. pylori* (45) Dps and by two water molecules in the *E. coli* Dps structure (16).

establishment of electrostatic interactions between positively charged amino acid residues on the Dps surface and the negatively charged DNA backbone. Different structural motifs have been identified to date by means of *in vitro* experiments on the purified proteins and plasmid DNA. These involve simple gel shift assays, where the interaction manifests itself in the retardation of the band(s) pertaining to plasmid DNA and therefore can be used to determine the affinity between the two macromolecules, and atomic force microscopy (AFM) experiments which visualize the Dps-DNA complexes and provide insight into the molecular details of the interaction between the two macromolecules.

DNA binding was reported first in *E. coli* Dps where it involves the flexible, lysine-rich N-terminal regions which depart from the four-helix bundle core of each subunit and face solvent in the assembled molecule. Their regular disposition in space, that is dictated by the 23 symmetry of the dodecamer, allows formation of ordered Dps-DNA complexes resembling those observed in stationary phase *E. coli* cells that over-produce Dps to levels that are fourfold higher than those accumulated in starved wild-type bacteria (24). In the latter bacteria such crystalline structures are detected only rarely.

A recent work on *E. coli* Dps N-terminus deletion mutants, that monitored Dps-DNA complex formation by means of agarose gel electrophoresis and AFM, unveiled that the capacity to form large Dps-DNA complexes is linked to the presence of multiple lysine residues in the N-terminus and to their state of protonation (25). At
The situation depicted in Figure 3 resembles that occurring in starved wild-type bacteria (24) where the cellular Dps concentration does not allow formation of crystals embedding DNA.

Some 20 other Dps proteins have an N-terminal sequence resembling that of E. coli Dps in terms of flexibility, number and distribution of positively charge residues. Thus, most likely they have similar DNA binding/condensation capabilities as exemplified by Mycobacterium smegmatis Dps2 (MsDps2), and by Dps1 and Dps2 from Deinococcus radiodurans (27-29). A variation on the theme of the N-terminus mediated interaction with DNA is utilized by the two Dps proteins from Lactococcus lactis, named DpsA and DpsB. Interestingly, the N-terminal sequence of the lactococcal proteins forms an alpha helix that extends from the subunit core into solvent (30) and is able to bind/condense only long stretches of linearized DNA (> 4000 bp). The reasons for this size limitation are still unclear and call for additional work to be elucidated.

The physiological pH values, native E. coli Dps carries 3 protonated lysine residues at the intact N-terminus. It gives rise to large Dps-DNA aggregates that contain many Dps molecules and one or more DNA plasmids and do not enter the agarose gels. This situation is reminiscent of the rapid formation of Dps-DNA co-crystals in starved E. coli cells overexpressing Dps (24, 26). In contrast, the mutant lacking 2 out of 3 N-terminal lysine residues binds DNA but is unable to condense it into large aggregates, while the mutant lacking all 3 lysine residues has no DNA binding or condensation capacity. The study also revealed that, in the absence of DNA, E. coli Dps has a strong tendency to self-aggregate and precipitate out of solution under all experimental conditions leading to DNA condensation. This behaviour was attributed to the propensity of the positive charges on the N-terminus to interact with the negatively charged surface of adjacent Dps molecules. The tight linkage between protein self-aggregation and DNA condensation is presented schematically in Figure 3: the positively charged N-termini positioned regularly within the negative protein surface promote on the one hand E. coli Dps self-aggregation through interaction between adjacent dodecamers and on the other DNA binding. In turn, DNA-bound protein aggregates are able to interact with other protein molecules and/or with other DNA molecules and thereby give rise to large DNA condensates.

A third type of Dps-DNA interaction mechanism was identified in Helicobacter pylori Dps, also named HP-NAP due to its neutrophil-activating properties. Unlike the other members of the family, H. pylori Dps is characterized by a positively charged protein surface at pH values around neutrality. AFM experiments by Ceci et al. (35) disclosed that HP-NAP binds DNA in a “beads-on-a-string fashion” at pH 8.0 and condenses it into large aggregates at lower pH values. DNA condensation was attributed to protonation of amino acid residues on the protein surface, most likely
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Figure 4. Model and demonstrative AFM images of the interaction between *H. pylori* Dps with DNA and its pH-dependence. From top to bottom: the reduction in pH over the range 8.5-6.5 leads to a progressive increase of the overall positive on the protein surface. Consequently, *H. pylori* Dps becomes able to bind to DNA and condense it.

Histidines, since their typical pK value is near neutrality and the transition between the DNA condensation and binding modes occurs between pH 7.5 and 7.0. These observations, taken together with the fact that HP-NAP does not self-aggregate, point to a direct involvement of DNA in the condensation process. It may be envisaged that the negatively charged DNA filaments act as an adhesive between adjacent HP-NAP molecules when these are positively charged (Figure 4). In other words, it is the DNA polyanion itself which drives the condensation process by bridging a large number of positively charged HP-NAP molecules. This mechanism therefore differs markedly from that operative in the case of *E. coli* Dps where the driving force for Dps-DNA complex formation is provided by the protein. It is worth pointing out that the pH-dependent capacity of HP-NAP to either condense or simply bind DNA can be used to advantage by *H. pylori* to survive in the human stomach during infection when cytoplasmic pH decreases significantly (35). It follows that DNA binding-condensation may be envisaged as an important, fully reversible mechanism used by this organism to protect DNA from environmental attacks other than low pH, such as radicals and nucleases.

The recently described Dps from the marine N₂-fixing cyanobacterium *Trichodesmium erythraeum*, Dps<sub>tery</sub>, may use a mechanism resembling that operative in *H. pylori* Dps. Thus, although the N-terminal region does not carry positive charges and the C-terminal one is truncated, Dps<sub>tery</sub> binds DNA at 8.0, protects it from degradation by DNase, but is unable to condense it into large Dps-DNA complexes (36).

The affinity for DNA that is associated to the Dps structural motifs just presented has been estimated on a few Dps proteins by means of gel shift assays and therefore refers only to the DNA binding mode. For this reason, the measurements were carried out under different conditions of pH and salt composition and cannot be compared easily. Despite this limitation, the data compiled in Table 1 indicate that the most effective mode of interaction is that established between *E. coli* Dps and DNA.
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Table 1. Affinity of Dps proteins for DNA as estimated by means of gel shift assays

<table>
<thead>
<tr>
<th>Protein</th>
<th>$K_D$ (µM)</th>
<th>Conditions</th>
<th>DNA</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli Dps</td>
<td>0.18</td>
<td>10 mM Tris-HCl, 1 mM EDTA, 100 mM NaCl, and 1 mM dithiothreitol, pH 7.5</td>
<td>linear duplex DNA (64 bp)</td>
<td>1</td>
</tr>
<tr>
<td>E. coli Dps</td>
<td>0.04</td>
<td>40 mM BisTris-acetate and 2 mM EDTA, pH 6.3</td>
<td>linear duplex DNA (150 bp)</td>
<td>25</td>
</tr>
<tr>
<td>L. lactis DpsA</td>
<td>0.3</td>
<td>10 mM Tris-HCl, 50 mM KCl and 1.5 mM MgCl$_2$, pH 8.3</td>
<td>linearized lactococcal shuttle vector pF2171 DNA (8000 bp)</td>
<td>30</td>
</tr>
<tr>
<td>L. lactis DpsB</td>
<td>4</td>
<td>10 mM Tris-HCl, 50 mM KCl and 1.5 mM MgCl$_2$, pH 8.3</td>
<td>linearized DNA lactococcal shuttle vector pF2171 (8000 bp)</td>
<td>30</td>
</tr>
<tr>
<td>T. erythraeum Dps</td>
<td>16</td>
<td>50 mM Tris-HCl, pH 7.9</td>
<td>pUC19 plasmid (2700 bp)</td>
<td>36</td>
</tr>
<tr>
<td>H. pylori Dps</td>
<td>1.1</td>
<td>50 mM Tris-HCl, ± 3 mM MgCl$_2$, pH 7.5</td>
<td>linearized pUC9-5S plasmid DNA (3100 bp)</td>
<td>35</td>
</tr>
</tbody>
</table>

Lastly, it is worth pointing out that the DNA-binding motifs identified to date most likely are at the basis of all possible modes of Dps-DNA interaction. In fact, Dps proteins devoid of such motifs are unable to interact with DNA as shown by the studies on *Listeria innocua* Dps (37), *Bacillus anthracis* Dlp-1 and Dlp-2 (38), *Campylobacter jejuni* (20) that are characterized by an N-terminus of reduced length, and *Agrobacterium tumefaciens* Dps, where the N-terminus is immobilized on the protein surface (12).

4. THE DPS-DNA INTERACTION AND THE ORGANIZATION OF THE BACTERIAL NUCLEOID

The importance of ordered Dps-DNA assemblies that are generated in response to stress conditions, such as those occurring during starvation, emerged soon after the discovery of Dps proteins (24, 26). However, only recent AFM studies that allowed observation of individual *E. coli* nucleoids during cell growth started revealing the precise role played by the Dps protein (2). The *E. coli* nucleoid, just as the eukaryotic chromosome, appears to be organized hierarchically in fundamental structural units that undergo dynamic changes during the cell cycle. Thus, the log phase nucleoid is characterized by loosely packed, thin 40 nm fibers folded into 80 nm fibers. Upon entry into stationary phase, the 80 nm fibers in turn fold in a superhelical manner into more compact globular structures, the “coral-reef structures”. In the late stationary phase, nucleoid packing becomes even tighter and resistant to lysis of the bacterial cell.

The cell cycle dependent changes in nucleoid compaction are correlated to variations in the amount of nucleoid-associated proteins, such as the Dps protein which becomes the most abundant nucleoid component in the late stationary phase. A comparison of the nucleoid organization in *wt E. coli* and in a *dps* deficient strain brought out that the *dps* deficient cells are unable to form “coral-reef” structures and compacted nucleoids during the early and late stationary phases of growth, respectively. Further, in the *dps* deficient strain overexpression of Dps induces nucleoid compaction during stationary phase but does not affect the organization of the nucleoid during log phase, suggesting that other proteins come into play. In principle these proteins can either bind to DNA or interact with Dps and thereby prevent its binding to DNA. Later studies on the *S. aureus* (39, 40) and *E. coli* nucleoid (41) were revealing in this respect and showed that both situations may occur.

In contrast to *E. coli*, where over-expression of Dps during log phase never induces nucleoid compaction, the induction of MrgA, the staphylococcal ortholog of Dps, leads to nucleoid compaction in both log and stationary phase. Oxidative stress-promoted MrgA expression likewise causes nucleoid condensation, again at variance with the behavior of *E. coli* (39). In the search for potential inhibitors of nucleoid compaction in *E. coli*, Ohniwa et al. (40) directed their attention towards Fis, a log phase nucleoid protein (3) which is present in *E. coli* (and in all gamma-Proteobacteria) but not in *S. aureus*. Fis, which binds preferably to its 15 bp consensus sequence, when in large amounts interacts with DNA in a sequence aspecific manner (41, 42). Therefore it may act as a physical barrier that interferes with the Dps-DNA interaction during log phase and, in addition, may affect DNA topology as it represses the expression of topoisomerase I and DNA gyrase (39). The organization of the nucleoid in *fis* deficient strains, where the *topA* and *gyrA/B* genes are both upregulated, indeed indicates that in *E. coli* the control of DNA topology, exerted by the interplay of Fis, Topo I and DNA gyrase (41-43), is critical for the Dps-induced nucleoid condensation.

The occurrence of direct interactions between Dps and other DNA-binding proteins was reported in a very recent paper by Chodavarapu et al. (44). These authors demonstrate that in *E. coli* Dps interacts with DnaA, a replication initiation factor which promotes unwinding or denaturation of DNA at oriC (around 240 bp) during DNA replication. As a result, Dps interferes with strand opening of the replication origin and causes less frequent initiations. The authors suggest that Dps may act as a checkpoint during oxidative stress to reduce new rounds of DNA replication, providing an opportunity to repair damaged DNA. Further, because Dps does not block initiations completely, the duplication of damaged DNA could be used to advantage to introduce mutations that increase the genetic variation of some bacterial populations and the survival of part of the bacterial cells.

5. THE DPS-DNA INTERACTION AND MICROBIAL SURVIVAL

The picture which emerges is that Dps proteins are important in sustaining microbial survival under a variety of conditions like vegetative growth, stationary phase, nutrient deprivation and oxidative stress. At a molecular level, the usefulness of Dps proteins can be traced back to two distinctive features of the protein family: i) the ferroxidase activity which endows all Dps proteins with the capacity to decrease the hydroxyl radical induced DNA damage, and ii) the capacity to interact with DNA and/or DNA binding proteins, which is limited to some
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members though it has given the name to the family. Most recently, the effects of Dps proteins on the organization of the nucleoid revealed that the Dps-DNA interaction can both modulate DNA structure and affect the transcription machinery, possibly recruiting or inhibiting transcription factors, and thereby play a role in regulating gene expression. These molecular mechanisms therefore underlie factors, and thereby play a role in regulating gene machinery, possibly recruiting or inhibiting transcription both modulate DNA structure and affect the transcription the nucleoid revealed that the Dps-DNA interaction can  

6. ACKNOWLEDGEMENTS

The authors thank Prof. Claudio Rivetti for the AFM images and local grants of the Ministero Universitá e Ricerca Scientifica (MIUR) to EC for support.

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**Key Words**: Dps (DNA-binding proteins from starved cells) proteins, Dps-DNA interaction, ferroxidase activity, nucleoid organization, microbial survival, Review

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