Role of Dps (<u>D</u>NA-binding <u>p</u>roteins from <u>s</u>tarved 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 (<u>h</u>istone-like <u>n</u>ucleoid <u>s</u>tructuring protein), HU (<u>h</u>eat-<u>u</u>nstable nucleoid protein), IHF (<u>i</u>ntegration <u>h</u>ost <u>f</u>actor), and Dps (<u>D</u>NA binding <u>p</u>rotein from <u>s</u>tarved cells), while others, like Fis

(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 genes 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 confer 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, ferritin use O_2 as iron oxidant with the production of hydrogen peroxide, bacterioferritins can use both O_2 and H_2O_2, while Dps proteins prefer by far H_2O_2 which is in general about a 100-fold more efficient than O_2 in carrying out iron oxidation (19). This property accounts for the unique capacity of Dps

```
                1                                                 50
DPS_Li     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . MKTI NSVDTKEFLNH
DPS_Sa     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . MSNQQDVVKELNQ
DPS_Ba1    . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . MSTKTNVVEVLNK
DPS_Ba2    . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . MNKQVI EVLNK
DPS_Hp     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . MKTFEI LKH
DPS_Cj     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . MSVTKQLLQ
DPS_At     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . MKTHKTKNDLPS. NAKSTVI GI LNE
DPS_Ec     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . MSTAKLVKSKATNLLYTRNDVSD. SEKKATVELLNR
DPS_Ms1    . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . MTSFTI PGLSD. KKASDVADLLQK
DPS_Ms2    . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . MSARRTESDI QGFHATPEFGGNLQK
DPS_Dr1    . . . . . . . . . . . . MTKKKSTKSEAASKTKKSGVPETGAQGVRAGG ADHADAAHLGTVN. . . . NALVNHHYLEEKEFQTVAETLQR
DPS_Dr2    MRHSV KTVV VVSSLLLGTALAGGAGAQSAGNGVPSTNVN. TPAPN TGGSTAQNTNTASPLPYNRATTLPAAGTEDLKKSVQALQN
DPS_Te     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . MSNGTTATPVQAFGQI ADNPI GLEKSVTEPVCEGMNV
DPSA_Ll    . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . MI ELSI DEKYEAEVKKSEI DHHKPTAGAMLSH
DPSB_Ll    . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . MI TKLMI DEKYAKELDKAEI DHHKPTAGAMLGH

                100                                               150
DPS_Li     QVANL NVFT VKI HQI HWYMRGHNFFTL HEKMDDLYSEFGEQMDEV AERLLAI GGSPFSTLKEFLENAS. VEEAPYTKPKTMDDQLM
DPS_Sa     QVANWTVAY TKLHNF HWYVKGPNFFSL HVKFEELYNEASQYVDEL AERI LAVGGNPVGTLTECLEQSI . VKEA. . AKGYSAEQMV
DPS_Ba1    QVANWNVLY VKLHNYHWYVTQPHFFTL HEKFEEFYNEAGTYI DEL AERI LALEGKPLATMKEYLATSS. VNEG. . TSKESAEEMV
DPS_Ba2    QVADWSVLF TKLHNF HWYVKGPQFFTL HEKFEELYTESATHI DEI AERI LAI GGKPVATMKEYLEI SS. I QEA. . AYGETAEGMV
DPS_Hp     LQADA I VLF MKVHNF HWNVKGTDFFNVHKATEEI YEEFADMFDDL AERI VQLGHHPLVTLSEAI KLTR. VKEETKTSFHSKD. I F
DPS_Cj     MQADA HHLWVKFHNYHWNVKGLQFFSI HEYTEKAYEEMAELFDSC AERVLQLGEKAI TCQKVLMENAK. SPKVAKDCFTPLE. VI
DPS_At     SLASV I DLA LVTKQAHWNLKGPQFI AVHELLDTFRTQLDNHGDTI AERVVQLGGTALGSLQAVSSTTK. LKAYPTDI YKI HD. HL
DPS_Ec     QVI QFI DLS LI TKQAHWNMRGANFI AVHEMLDGFRTALI DHLDTMAERAVQLGGVALGTTQVI NSKTP. LKSYPLDI HNVQD. HL
DPS_Ms1    QLSTY NDLHLTLKHVHWNVVGPNFI GVHEMI DPQVELVRGYADEV AERI ATLGKSPKGTPGAI I KDRT. WDDYSVERDTVQA. HL
DPS_Ms2    VLVDL I ELS LQGKQAHWNVVGSNFRDL HLQLDELVDFAREGSDTI AERMRALDAVPDGRSDTVAATTT. LPEFPAFERSTAD. VV
DPS_Dr1    NLATT I SLY LKFKKYHWDI RGRFFRDL HLAYDEFI AEI FPSI DEQ AERLVALGGSPLAAPADLARYST. VQVPQETVRDART. QV
DPS_Dr2    TLTEL QALQLQTKQAHWNVSGTLWYTL HELLQDHYEGI SKFADDV AERQLSVGASSDGRAI TI VAASR. LPEI PGGFLDDAQ. VI
DPS_Te     ALASC QALY LQYEKHHF VVEGAEFNQL HEFFKESYEEVKEHVHEMAERLNGLGGVPVASFAKLAEMCC. FTPEEDGSFNARN. MI
DPSA_Ll    VLSNI FYEKI SLMQAGL YAKSANY. . . RI KFREI ALKEDEWFYLI SEQLLDENELVPTTLDEFVSNHKFI ENDPKAKYWTDEALI
DPSB_Ll    VLSNL FI ENI RLTQAGI YAKSPVK. . . CEYLREI AQREVEYFFKI SDLLLLDENEI VPSTTEEFLKYHKFI TEDPKAKYWTDEDLL

                200                                               250
DPS_Li     EDLVGTLEL LRDEYKQGI ELTDKEGDDVTNDMLI AFKASI DKHI WMFKAFLGKAPLE. . . . . . . . . . . . . . . . . . . . . . . . .
DPS_Sa     EELSQ DFTNI SKQLENAI EI AGNAGDDVSEDMFI GMQTSVDKHNWMFKSYLS. . . . . . . . . . . . . . . . . . . . . . . . . . . .
DPS_Ba1    QTLVN DYSA LI QELKEGMEVAGEAGDATSADMLLAI HTTLEQHVWMLSAFLK. . . . . . . . . . . . . . . . . . . . . . . . . . .
DPS_Ba2    EAI MK DYEMMLVELKKGMEI AQNSDDEMTSDLLLGI YTELEKHAWMLRAFLNQ. . . . . . . . . . . . . . . . . . . . . . . . . .
DPS_Hp     KEI LE DYKY LEKEFKEL SNTAEKEGDKVTVTYADDQLAKLQKSI WMLQAHLA. . . . . . . . . . . . . . . . . . . . . . . . . . .
DPS_Cj     ELI KQ DYEY LLAEFKKLNEAAEKESDTTTAAFAQENI AKYEKSLWMI GATLQGACKM. . . . . . . . . . . . . . . . . . . . . . .
DPS_At     DALI E RYGE VANMI RKAI DDSDEAGDPTTADI FTAASRDLDKSLWFLEAHVQEKS. . . . . . . . . . . . . . . . . . . . . . . .
DPS_Ec     KELAD RYAI VANDVRKAI G. . . EAKDDDTADI LTAASRDLDKFLWFI ESNI E. . . . . . . . . . . . . . . . . . . . . . . . . .
DPS_Ms1    AALDL VYNGVI EDTRKSI EKL. EDLDLVSQDLLI AHAGELEKFQWFVRAHLESAGGGLTHEGQSTEKGAADKARRKSA. . . . . . .
DPS_Ms2    DLI TT RI NA TVDTI RR. VHDAVDAEDPSTADLLHGLI DGLEKQAWLI RSENRKV. . . . . . . . . . . . . . . . . . . . . . . . .
DPS_Dr1    ADLVQ DLSR VGKGYRDDSQACDEANDPVTADMYNGYAATI DKI RWMLQAI MDDERLD. . . . . . . . . . . . . . . . . . . . . . .
DPS_Dr2    QFFTY QYET VGQRI HQRVGDVEKV. DPTTANLLQEVEHI I EKYQWQMRAFLQNTPTDPNT. GFDI NNGKPVPLRG. . . . . . . . . .
DPS_Te     EHDLK AEQDVI KLLRRLAAQAESLGDRATRYLYEKI LLETEERAY HLDHFLAPDTLVVLN. . . . . . . . . . . . . . . . . . . . .
DPSA_Ll    ENFI N DFQNQNLFI GRAI KLAQKEEKFSLELAI RKLYGYNLSI I P YFAGELGKTI GEFI EEEDED. . . . . . . . . . . . . . . . . .
DPSB_Ll    ESFI V DFQA QNMFI TRAI KLANKEEKFALAAGVVELYGYNLQVI R NLAGDLGKSVADFHDEDEDNDN. . . . . . . . . . . . . . . . . .
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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 H_2O_2 which are both removed from solution and hence can not give rise to the highly toxic hydroxyl radicals via the Fenton reaction: $Fe(II) + H_2O_2 \rightarrow Fe(III) + OH^- + OH^-$. 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 H_2O_2 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 the name to 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
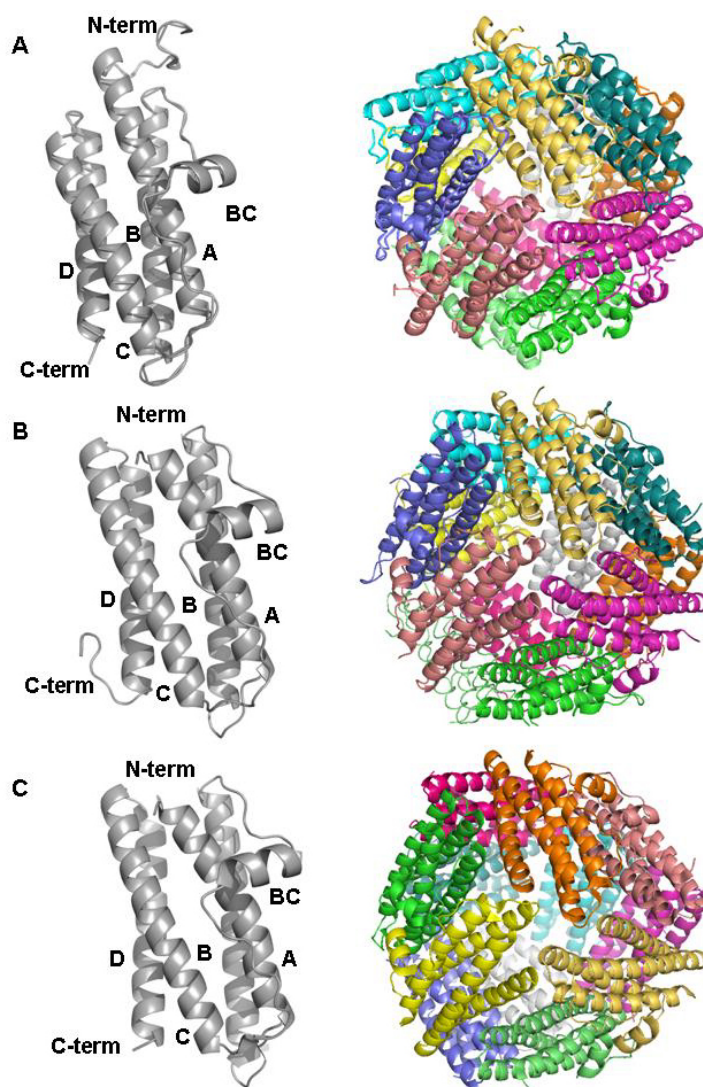
Figure 2. Structure of the *E. coli* (A), *L. innocua* (B) and *H. pylori* (C) Dps monomers and of the assembled 12-mers. Characteristic 4-helix bundle monomer structure (left) and view of the 12-mer (right) which evidences the 3-fold symmetry at the ferritin-like pores. Iron enters through these pores 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
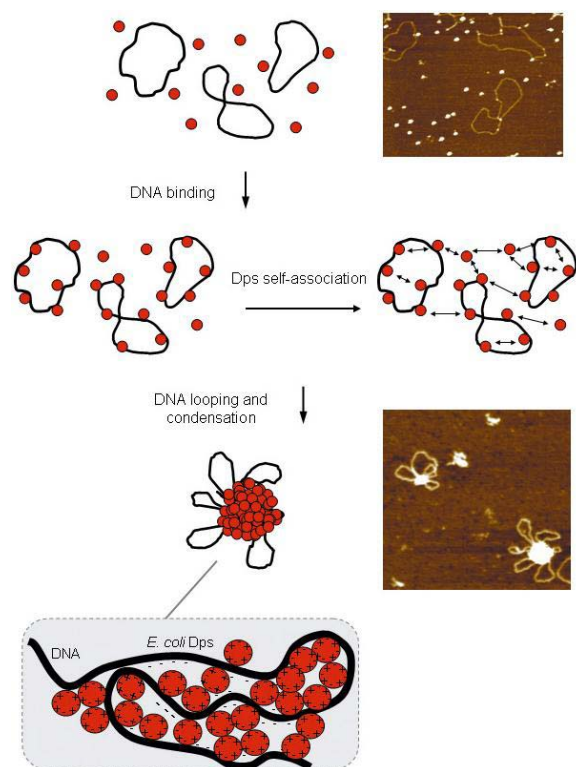
Figure 3. Model and demonstrative AFM images of the interaction between *E. coli* Dps with DNA at pH 6.5. The positively charged lysine residues at the Dps N-terminus drive DNA condensation as they interact concomitantly with DNA and with negatively charged regions of adjacent Dps molecules. For further details see text.

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. 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 requirement for long DNA fragments may possibly be imposed by the decreased flexibility of the N-terminus inherent in the helical conformation.

The studies on *M. smegmatis* Dps1 (MsDps1) revealed that DNA binding can be mediated also by a second structural motif, namely a freely mobile and positively charged C-terminus (31-33). In fact, MsDps1 is characterized by a truncated, uncharged N-terminus and by a 26 amino acids long C-terminal region that contains both positively and negatively charged amino acid side chains (3 lysine and 2 arginine residues plus 1 aspartic and 3 glutamic acid residues). The observation that removal of the last 16 C-terminal residues results in loss of the DNA binding activity without affecting folding or the ferroxidase activity proves the involvement of the C-terminal region in the interaction with DNA (33). It is of interest that *M. smegmatis* Dps1 binds DNA but is unable to promote its condensation and does not self-aggregate in its absence, a behaviour attributed to the four negatively charged residues of the C-terminus that partly compensate the positive charges carried by the lysine and arginine residues (34). It is not known whether *M. smegmatis* Dps1 is able to condense DNA *in vivo* also because the bacterial genome contains a second Dps protein, MsDps2, identified in very recent studies (27). At variance with MsDps1, MsDps2 does not possess a long C-terminal region but is characterized by an extended N-terminus that appears to be involved not only in the interaction with DNA but also in stabilization of the dodecameric assembly.

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
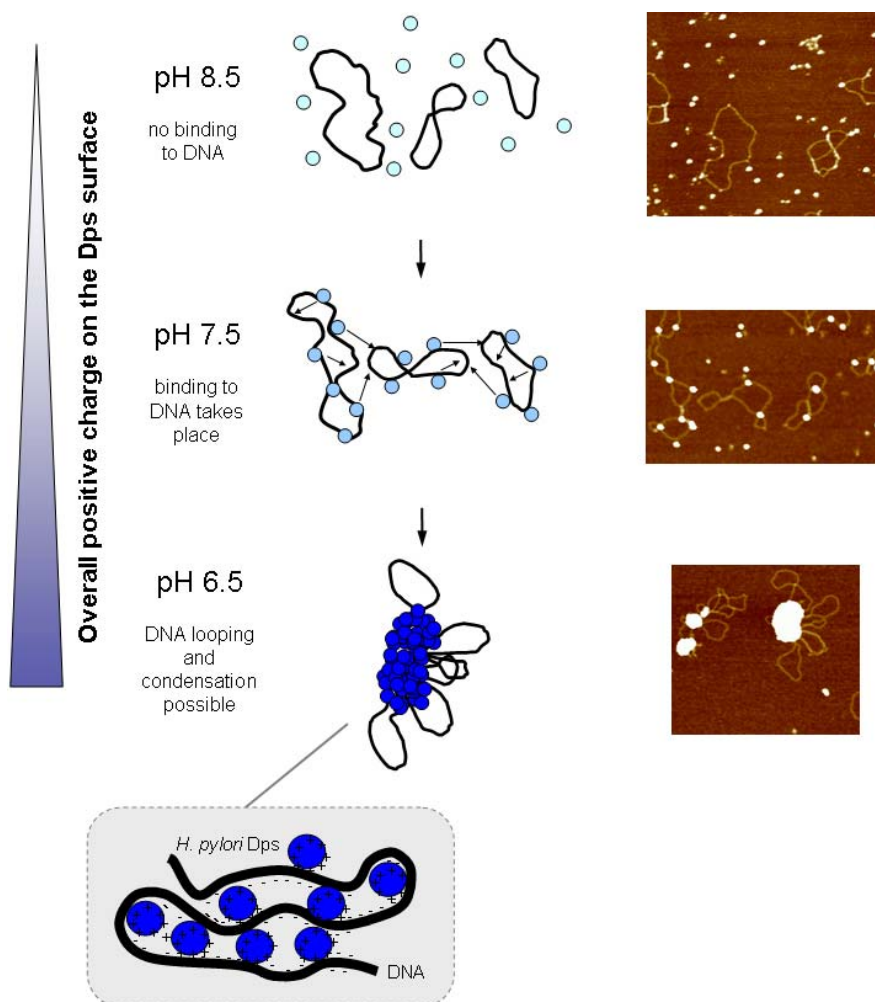
Figure 4. Model and demonstrative AFM images of the interaction between *H. pylori* Dps with DNA and its pH-dependence. From top to bottom: the reduction in pH over the range 8.5-6.5 leads to a progressive increase of the overall positive on the protein surface. Consequently, *H. pylori* Dps becomes 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 N_2-fixing cyanobacterium *Trichodesmium erythraeum,* Dps_{tery}, 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_{tery} 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.

Table 1. Affinity of Dps proteins for DNA as estimated by means of gel shift assays

Protein	K_D (μM)	Conditions	DNA	Ref
E. coli Dps	0.18	10 mM Tris-HCl, 1 mM EDTA, 100 mM NaCl, and 1 mM dithiothreitol, pH 7.5	linear duplex DNA (64 bp)	1
E. coli Dps	0.04	40 mM BisTris-acetate and 2 mM EDTA, pH 6.3	linear duplex DNA (500 bp)	25
L. lactis DpsA	0.3	10 mM Tris-HCl, 50 mM KCl and 1.5 mM MgCl₂, pH 8.3	linearized lactococcal shuttle vector pFI2171 DNA (8000 bp)	30
L. lactis DpsB	4	10 mM Tris-HCl, 50 mM KCl and 1.5 mM MgCl₂, pH 8.3	linearized DNA lactococcal shuttle vector pFI2171 (8000 bp)	30
T. erythraeum Dps	16	50 mM Tris-HCl, pH 7.9	pUC19 plasmid (2700 bp)	36
H. pylori Dps	1.1	50 mM Tris-HCl, ± 3 mM MgCl₂, pH 7.5	linearized pUC9-5S plasmid DNA (3100 bp)	35

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

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 the original observations of Kolter and collaborators on the global regulatory function of *E. coli* Dps (8, 9).

The interplay between DNA binding and the involvement of Dps proteins in response to oxidative stress requires a last comment because the widely distributed *dps* gene is generally induced under oxidative stress through different transcription factors, namely OxyR, which is present in alpha-, beta-, and gamma-Proteobacteria (including *E. coli*) and Actinobacteria, and PerR, which acts as a repressor of the *mrgA* gene and is found in Firmicutes (including *S. aureus*), Spirochete, alpha-Proteobacteria, Cyanobacteria, Chlamidia and hyperthermophilic bacteria. Ohniwa *et al.* (39) observed that the distribution of Fis is restricted to gamma-Proteobacteria and suggested that for this reason oxidative stress can not induce nucleoid condensation in these bacteria, just as in E. coli. In contrast, since the distributions of Fis and PerR never overlap, oxidative stress will induce nucleoid condensation in the species which possess PerR.

The multifarious activity of the fascinating Dps protein family is likely to be exerted also through other interactions and molecular mechanisms that have not been clarified, but will surely be revealed by future studies.

6. ACKNOWLEDGEMENTS

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