Review

Structural bioinformatic analysis of DsbA proteins and their pathogenicity associated substrates

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ABSTRACT

The disulfide bond (DSB) forming system and in particular DsbA, is a key bacterial oxidative folding catalyyst. Due to its role in promoting the correct assembly of a wide range of virulence factors required at different stages of the infection process, DsbA is a master virulence rheostat, making it an attractive target for the development of new virulence blockers. Although DSB systems have been extensively studied across different bacterial species, to date, little is known about how DsbA oxidoreductases are able to recognize and interact with such a wide range of substrates. This review summarizes the current knowledge on the DsbA enzymes, with special attention on their interaction with the partner oxidase DsbB and substrates associated with bacterial virulence. The structurally and functionally diverse set of bacterial proteins that rely on DsbA-mediated disulfide bond formation are summarized. Local sequence and secondary structure elements of these substrates are analyzed to identify common elements recognized by DsbA enzymes. This not only provides information on protein folding systems in bacteria but also offers tools for identifying new DsbA substrates and informs current efforts aimed at developing DsbA targeted anti-microbials.

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1. Introduction

Bacteria rely on the production of a wide range of virulence factors to adapt to different environments, surmount host immune responses and colonize and invade host cells [1–3]. These virulence factors are often secreted or cell surface proteins that include disulfide bonds (DSB) in their three-dimensional structures, which provide stability in the hostile, protease-rich extracellular environment [4,5]. Disulfide bonds are catalyzed by the disulfide bond forming enzyme A (DsbA) of the DSB system in a process known as oxidative protein folding [4,5]. The discovery of DsbA (EcDsbA) within the periplasmic space of Escherichia coli, provided the first experimental basis for a catalytic mechanism of disulfide bond formation in Gram-negative bacteria [6]. The reliance on this disulfide bond formation machinery for the correct functioning of several bacterial proteins, including virulence factors, has been directly shown in a number of in vitro and cell-based assays [7]. Furthermore, several studies have demonstrated that mutants defective in the DSB oxidative folding pathway throughout diverse bacterial pathogens, have reduced virulence phenotypes in animal infection models [6,8–19]. Since the discovery of the DSB system in E. coli K-12, the study of these systems has been expanded to include diverse Gram-negative and Gram-positive bacteria [4,5,20–22]. However, how this important DSB forming enzyme interacts with a great array of structurally diverse protein substrates is still poorly understood. In this review we summarize current knowledge on DsbA systems, particularly focusing on how DsbA proteins interact with partner oxidases and virulence substrates. Through an extensive bioinformatic analysis we define common features present in the highly diverse set of DsbA substrates.

1.1. The archetypal DSB machinery in prokaryotes

The DSB system was first characterized and has been best studied in the Gram-negative bacterium Escherichia coli K-12 [6]. This prototypical DSB system consists of two different pathways: (i) The DSB oxidation pathway (Fig. 1A), where the oxidoreductase enzyme DsbA introduces disulfide bonds between consecutive cysteines in unfolded substrates as they are translocated into the periplasm [6]; and (ii) the DSB isomerization pathway (Fig. 1B), where non-native disulfide bonds are proofread and corrected [23,24] by the isomerase enzyme DsbC [25]. For each one of these pathways, the active oxidized state of DsbA and the active reduced state of DsbC are maintained by the proteins DsbB and DsbD, respectively [7,26–29]. DsbA is an integral membrane protein with two periplasmic loops, each containing two pairs of redox active cysteines required for DsbA re-oxidation. DsbB is a multidomain membrane protein with two periplasmic domains flanking a transmembrane domain. These membrane proteins display high substrate specificity for DsbA and DsbC, respectively, allowing for both opposite redox pathways to function independently in the shared periplasmic compartment.

2. The thiol oxidase DsbA from E. coli K-12

EcDsbA, as revealed by its crystal structure, is composed of two domains: a thioredoxin (TRX) domain, and a helical domain (Fig. 2A) [30]. The TRX domain consists of four β-strands and two α-helices ([β2,α3,β4] and [β1,α2,α4,α5] motif), a structural scaffold characteristic of disulfide oxidoreductases, while the helical domain ([α2–α4]), which is inserted within the TRX domain, is comprised of five α-helices (a three-helical bundle with two additional helices). The active site of EcDsbA sitting within the TRX domain is composed of a conserved CXXC motif (Cys30-Pro31-His32-Cys33). With an unusually low pK_a of 3.5 [31], the N-terminal Cys30 exists as a thiolate ion in the reduced form at physiological pH. This Cys30 thiolate is stabilized by a number of polar interactions such as (i) a hydrogen bond with the thiol of Cys33 (ii) hydrogen bonds with the backbone amide nitrogens of Cys30 and His32; and (iii) electrostatic interactions with the side chain of His32 [32]. Additionally, the partial positive charge from the dipole of the α1 helix, where the active site is located, has also been suggested to stabilize the Cys30 thiolate [32]. This results in superior stability of the reduced form of DsbA and a stronger tendency to oxidize substrates. Another key feature of the TRX domain is a cis-proline loop (Val-cis-Pro), which is spatially close to the active site. This feature is thought to be important for the correct binding of substrates and/or DsbA-substrate complex resolution, along with influencing the redox properties of thioredoxin fold proteins [33–35] (Fig. 2A). Examination of EcDsbA's electrostatic surface shows a hydrophobic groove present in the TRX domain (Fig. 2A), which has been shown to interact with its cognate membrane partner EcDsbB [27,30,36]. Similarly, the surface of the EcDsbA helical domain forms a large hydrophobic patch located immediately above the active site (Fig. 2A). This patch has been shown to interact with substrates and is thought to provide stability and specificity for substrate binding [36]. The hydrophobic nature of the surface surrounding the active site is consistent with its ability to catalyze folding of a broad range of unfolded or partially folded substrates along with interacting with the periplasmic loop of DsbB [4,24,36–38].

2.1. Diversity of DsbA oxidative proteins across bacteria

DSB systems are widespread across non-pathogenic and pathogenic bacteria [4,5] and the characterization of DSB systems and DsbA enzymes across diverse species has revealed structural and active site differences within the DsbA family of proteins [20]. A combined structural and bioinformatics analysis highlighted that the most notable of these differences is the arrangement of the β-strand ([β1] preceding the TRX domain, which divides DsbAs into
two major classes, class I with β-sheet topology of 3–2–4–5–1 (Fig. 2A and B) and class II with a topology of 1–3–2–4–5 (Fig. 2C and D), throughout most Gram-negative and Gram-positive bacteria [39]. These classes are further split into the la, lb, lla and llb subclasses based on variations of surface features surrounding their active sites [39]. The canonical EcDsBA is grouped into class la (Fig. 2A), which also includes DsBA proteins from other important human pathogens such as Salmonella enterica at the extended hydrophobic groove adjacent to the active site mapping murium (Proteus mirabilis) [39]. These classes are further split into the Ia, Ib, IIa and IIb and D), throughout most Gram-negative and Gram-positive bacteria.

For instance, DsBA1 from divergent EcDsBA can only partially complement EcDsBA due to a deletion in the interaction with DsBB for reoxidation [27]. The same groove is shorter in members of class lb such as Burkholderia pseudomallei (BpsDsBA) (Fig. 2B) [39]. Consistent with the similarity between DsBA proteins from class la, it has been found that other class la DsBAs such as SeDsBA, KpDsBA, VcDsBA and PmDsBA can fully complement EcDsBA in vitro and in vivo [40–44], while DsBA proteins from class lb such as BpsDsBA and Pseudomonas aeruginosa (Pa) DsBA can only partially complement EcDsBA [17,45]. Class II DsBA proteins, such as Staphylococcus aureus (SaDsBA) and Wolbachia pipientis (WpDsBA1), further deviate from the canonical EcDsBA by featuring the most truncated hydrophobic groove and a highly charged electrostatic surface surrounding the active site (Fig. 2C, 2D) [39]. Unsurprisingly, among the four structurally characterized class II DsBAs, only SaDsBA is able to partially restore EcDsBA activity in vivo [46]. A more recent analysis investigating the phylogenetic relationship of 20 structurally characterised DsBA homologues from diverse bacteria, assigned DsBA proteins into three main clades, with the first and second being equivalent to previously defined class la and class lb DsBAs [39], and the third more taxonomically diverse clade consisting of structurally divergent EcDsBA proteins [47].

In addition to being structurally diverse, there is also significant divergence in the redox properties of DsBA-like proteins (Table 1). For instance, DsBA1 from Neisseria meningitidis (Nm) has a redox potential of −79 mV and a \( K_a \) of 3.0 for the N-terminal nucleophic cysteine [48]. In contrast, DsBA1 from W. pipientis (WpDsBA1) is much less oxidizing with a redox potential of −163 mV and a \( K_a \) of 4.7 [48]. Previous studies have demonstrated that the XX dipeptide sequence in the active site CXXC motif and the residue preceding the cis-Pro motif modulate these redox potentials and \( K_a \) values of the nucleophilic cysteine [34,50]. However, these motifs are not the sole determinants of the redox properties. For example, BpsDsBA and PaDsBA share the same CPHC motif and Val-cis-Pro sequence as the EcDsBA, but they are more oxidizing (\( E^{\circ'}=−94 \) mV) than EcDsBA (\( E^{\circ'}=−122 \) mV) and display lower \( K_a \) values of the nucleophilic cysteine. Overall, variations in the active site sequence, three-dimensional structure, surface charge and redox characteristics collectively contribute to the unique features of each DsBA and likely define their substrate specificities [35].

### 2.2. Functional redundancy of DsBA homologues

Genome-wide screening for DSB homologues has revealed that the classical model of bacterial oxidative folding from *E. coli* K-12 is
not universally conserved across bacterial species [4,58]. Bioinformatic and functional studies have revealed that many bacteria encode more than one DsbA [4,5,19]. Examples include uropathogenic E. coli (UPEC) strain CFT073 that possesses a canonical DsbA and an accessory DsbA homologue DsbL [19,54] or the widely distributed facultative anaerobe Shewanella oneidensis, which has a complex DSB machinery with four DsbA (DsbA1–4) and two DsbB (DsbB1–2) homologues [59]. Similarly, Salmonella enterica (Se) encodes four DsbA-like proteins [41], namely DsbA, DsbB, SrgA [42], a virulence plumid-encoded DsbA-like protein, and ScsC [40,61], a DsbA-like protein that protects against copper toxicity [57,62]. These Salmonella DsbA paralogues were found to share low sequence identity along with significant differences in their surface features and redox properties [41]. More recently, a novel DSB-like protein from Salmonella enterica termed BcfH has been identified and characterized. Interestingly, BcfH has been shown to form a trimeric structure, exceptionally uncommon among the thioredoxin superfamily members. Additionally, BcfH has both thiol oxidase and disulfide isomerase activities contributing to Salmonella fimbral biogenesis [57].

Based on current published evidence, it appears that in bacteria with multiple DsbA homologues, there is always one homologue that acts as the primary disulfide donor capable of oxidizing a wide range of substrates, whilst other DsbAs are dedicated for specific substrates [5]. While many bacteria possess an extended collection of accessory DsbA homologues with differing specificities to catalyze the oxidation of selective substrates [4], some DsbA homologues have shown a level of functional redundancy. Such is the case of UPEC DsbL (EcDsbL) that possesses a positively charged surface surrounding the active site that serves as a specific oxidase of the periplasmic protein arylsulfate sulfotransferase (ASST) [54]. The astA gene is a UPEC-associated gene and its expression is upregulated during urinary tract infections. Although less oxidizing in nature than EcDsbL (E°’ ≈ −95 mV), EcDsbA (E°’ ≈ −120 mV) has also been found to oxidize ASST at a similar rate in vitro [63]. Conversely, it has also been observed that EcDsbL can partially complement EcDsbA in vivo [19,54]. Similarly, DsbA homologues from the pathogen Salmonella enterica serovar Typhimurium, SeDsbA, SeSrgA and SeDsbL, are all able to complement each other in restoring bacterial motility and plasmid-encoded fimbriae (Pef) production in vivo [41], irrespective of sharing low sequence identity and having considerably different redox potentials (E°’ of −126 mV, −154 mV and −97 mV, respectively) [41]. Despite this functional redundancy, the flagella system is considered a substrate of SeDsbA, and PefA a substrate of SeSrgA [42]. Although it is still not fully understood why some bacterial genomes encode multiple and functionally redundant DsbA homologues, one possibility could be that under a given set of growth conditions, a single type of DsbA is insufficient to drive efficient oxidative folding. This hypothesis seems to be supported by the co-expression of virulence factors and their specific oxidases as it has been seen with DsbL/ASST and SrgA/PefA [42,54].

### Table 1

Summary of redox properties of DsbA proteins across bacteria.

| Organism          | Protein name | CXXC | E°’ (mV) | pK_a | Ref |
|-------------------|--------------|------|----------|------|-----|
| E. coli K-12      | DsbA         | CPHC | −122     | 3.4  | [30,31] |
| V. cholerae       | DsbA (TcpG)  | CPHC | −116     | −    | [43,51] |
| S. aureus         | DsbA         | CPYC | −132     | 3.4  | [46,52] |
| X. fastidiosa     | DsbA         | CPFC | −94      | −    | [53]  |
| UPEC CFT073       | DsbL         | CVHC | −163     | 3.7  | [54]  |
| W. pipientis wMel | DsbA1        | CVHC | −87      | −    | [48,55] |
| N. meningitidis MC8 | DsbA1    | CPHC | −89      | 3    | [48,55] |
| Bacillus subtilis 168 | DsbA (DsbD) | CPSC | −80      | −3.5 | [56]  |
| P. aeruginosa      | DsbA         | CPHC | −94      | −    | [45]  |
| S. enterica Typhimurium SL1344 | SrgA | CPFC | −154     | 4.7  | [41]  |
|                   | DsbL         | CPHC | −126     | 3.3  | [41]  |
|                   | BcfH         | CSWC | −101, −151 | − | [57] |
| B. pseudomallei    | DsbA         | CPHC | −92      | 2.83 | [17]  |

### 3. The redox interaction between DsbA and DsbB

The systematic oxidation of substrates in the periplasm by DsbA proteins largely relies on the efficient reoxidation of the enzyme by the membrane embedded protein DsbB [27]. Due to the fast-resolving nature of the mixed disulfide formed between DsbA and DsbB (Fig. 3), it has been particularly challenging to study this interaction at the atomic level. However, a study involving the active site mutant Cys33Ala of EcDsbA was able to successfully trap EcDsbA in a disulfide-linked intermediate form [27]. This hallmark crystal structure of the intermediate complex offered for the first time, molecular insights into the protein–protein interaction between DsbA and DsbB (PDB ID: 2HI7, 2ZUP) [27,64], confirming the formation of a mixed disulfide bond between Cys104 of EcDsbA and the nucleophilic Cys30 of EcDsbB. Additionally, the protein–protein interaction interface was shown to be mediated by the second periplasmic loop of the transmembrane protein EcDsbB (P2). A high shape complementarity is observed between the N-terminal end of the P2 periplasmic loop of EcDsbB (Pro100-Thr103) and the hydrophobic groove of EcDsbA, which is formed by the active site CPHC motif, the cis-Pro motif (L2 loop) and L3 loop (Fig. 3A), while the other end of the P2 periplasmic loop (Asp105-Phe106) of EcDsbB interacts with the hydrophobic patch of EcDsbA via backbone hydrogen bonds with the L2 loop and hydrophobic interactions with the L1 loop. Overall, the DsbA-DsbB binding interface spans the helical domain and the thioredoxin domain of DsbA. The hinge bending motions of the two domains have been suggested to promote the protein–protein interactions and disulfide bond catalysis [32]. Interestingly, binding of EcDsbA to EcDsbB does not seem to induce major conformational changes in EcDsbA with a root mean square deviation (RMSD) value of 0.788 Å over 176 C atoms when overlaid with apo EcDsbA (PDB ID: 1FVK) [65], with most of the structural shifts located in flexible loop regions of the protein.

The binding mode of the EcDsbA-EcDsbB interaction was further validated by a crystal structure of EcDsbA Cys33Ala mutant in complex with an optimized peptidet (PFPATCDS) derived from the P2 loop of EcDsbB [66]. The EcDsbA-peptide complex strongly resembles the interactions observed in the EcDsbA-EcDsbB protein–protein complex (Fig. 3B). Alanine-scaning mutagenesis of the EcDsbB 9-mer P2 based peptide (PSPFPATCDS) showed that the cysteine residue (equivalent to Cys104 in EcDsbB), contributed most to the binding affinity with EcDsbA [66], suggest-
Fig. 3. Interaction between DsbA and DsbB. A. Crystal structure of the EcDsbA–EcDsbB complex (PDB ID: 2ZUJ). Only the DsbA-interacting segment of the DsbB periplasmic loop P2 (purple) is shown for clarity. The dashed line indicates the omitted DsbB protein. Left panel: EcDsbA is shown as colored surface, EcDsbA residues located within 4 Å of EcDsbB are shaded in blue. Residue number of EcDsbB is labelled in the figure. Right panel: EcDsbA residues located within 4 Å of DsbB are shown as sticks. Sulfur atoms of the active site cysteines are shown as yellow spheres. Surface loops L1, L2, L3 and CPHC active site are labelled in each structure. B. Crystal structure of EcDsbA in complex with an optimized heptapeptide (PFATCDS) derived from EcDsbB (PDB ID: 4TKY). C. Crystal structure of PmDsbA C30S mutant in complex with an optimized heptapeptide (PWATCDS) from PmDsbB (PDB ID: 4OD7). D. Crystal structure of BpsDsbA in complex a BpsDsbB-derived peptide (GFSCGF) (PDB ID: 5VYO). E. Crystal structure of XfDsbA in complex with a DsbB-like peptide (PDB ID: 2REM). For B–E, DsbA is shown as colored surface, DsbA residues located within 4 Å of DsbB are shaded in blue. Sulfur atoms of the active site cysteines are shown as yellow spheres. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

ing that the peripheral hydrophobic interactions may only serve to specifically recognize EcDsbB and position the cysteine in the correct orientation for disulfide bond formation.

Despite the structural variation amongst DsbA homologues and the low sequence conservation in residues at the DsbB binding interface, the characterized DsbA-DsbB peptide complexes from different bacteria have shown a conserved binding site, which spans across the DsbA hydrophobic patch and hydrophobic groove adjacent to the active site (Fig. 3B–E). Indeed, a study on Proteus mirabilis DsbA (PmDsbA) Cys30Ser mutant co-crystallized with an optimized heptapeptide derived from the P2 periplasmic loop of PmDsbB (PWATCDS) [40] showed that although the peptide does not form the mixed disulfide with PmDsbA, the binding mode is strikingly similar to that of the EcDsbA–EcDsbB complex. This complex also supports the significance of the non-disulfide interactions in DsbB binding (Fig. 3C). Similarly, a complex between BpsDsbA, a distant homologue of EcDsbA with a truncated hydrophobic groove, and a BpsDsbB-derived peptide showed the peptide binding to BpsDsbA in a very similar manner to its E. coli counterpart (Fig. 3D) [20,67]. These findings, in addition to a crystal structure of Xylella fastidiosa (Xf) DsbA crystallized with a DsbB-like peptide [53] (Fig. 3E), highlight the conservation of this binding mode across bacterial species. Notably, in these structures, all DsbB peptides form backbone hydrogen bonds with the L2 loop of DsbA, which seems to facilitate the presentation of the cysteine residue for efficient disulfide bond exchange.

Based on observations of the DsbA-DsbB/DsbB-peptide interactions from different Gram-negative bacteria, it is tempting to propose that the conserved DsbB-interacting interface on DsbA consists of the L1 loop, the active site CXXC motif, the cis-Pro motif (L2 loop), and the hydrophobic groove (L3 loop) (Fig. 3). However, since all DsbAs in these characterized complexes belong to class I DsbAs, it remains to be determined whether class II DsbAs interact with DsbB in a similar manner given their more pronounced structural and surface variations.

4. The DsbA-substrate interaction

As part of the oxidative folding process in Gram-negative bacteria, DsbA introduces disulfide bonds into newly synthesized polypeptides immediately after they are translocated into the periplasm through the Sec channel [68]. The DsbA-catalyzed thiol-disulfide exchange reaction appears to proceed via a biomolecular nucleophilic substitution reaction (SN2) mechanism in two steps (Fig. 4). In the first step, the deprotonated cysteine of the substrate attacks the DsbA Cys30 sulfur atom that is participating in a Cys30-Cys33 disulfide bond. This leads to a DsbA-substrate intermediate complex with a disulfide bond between the DsbA Cys30 and the substrate cysteine. In the second step, a second cysteine in the substrate is deprotonated and attacks the sulfur atom of the substrate cysteine in the mixed disulfide bond, resolving the complex and producing oxidized substrate and reduced DsbA [68–70].

Due to the extremely short-lived nature of the mixed disulfide between DsbA and a substrate, it has been challenging to capture and characterize DsbA-substrate complexes at the atomic level. At present the only two structurally characterized DsbA-substrate peptide complexes offer a glimpse into the molecular determinants underlying the DsbA-substrate interactions. In one
study, a peptide derived from the autotransporter protein SigA of Shigella flexneri was trapped in a complex with EcDsbA by substituting the native cysteine of the peptide with a homoserine (Hse) [36,71]. This modification allowed for the formation of a stable complex through a non-labile covalent bond between the Hse of the peptide and the reactive Cys30 of EcDsbA. The crystal structure of the complex revealed that the SigA peptide mainly interacts with the active site and the hydrophobic patch (L1, L2 loops) of EcDsbA (Fig. 5A). This interaction is comparable with that displayed by the DsbA-DsbB complexes, where the binding site of the substrate also spans the helical domain and thioredoxin domain, and where the backbone hydrogen bonds between the L2 loop of DsbA and the N-terminal segment of SigA peptide (Hse6-Gln7-Lys8) mimic the interactions in the DsbA-DsbB complexes, suggesting a conserved DsbA-substrate recognition mechanism. However, unlike DsbB, the C-terminal segment of the SigA peptide does not extend into the hydrophobic groove below the active site but instead sits on the top of it. Interestingly, the lack of interactions between Cys30 and His32 in the mixed intermediate, allows the sidechain of the His32 to be less constrained where in the case of the SigA peptide, it forms π-π stacking interactions between the Phe4 of the peptide and His32 of DsbA (Fig. 5A).

In another study, the crystal structure of Acinetobacter baumannii DsbA (AbDsbA) in complex with the E. coli elongation factor EF-Tu uncovered a very uncommon substrate binding region that does not involve the disulfide exchange reaction [72]. For this complex, EF-Tu binds tightly to a groove sandwiched between the thioredoxin domain and the helical domain on the non-catalytic face of AbDsbA (Fig. 5B). Although the activity of AbDsbA is shown to be reduced in the presence of EcEF-Tu in vitro (E. coli and A. baumannii EF-Tu share 85% sequence identity), the physiological relevance of this allosteric interaction is still unclear.

5. Structural variability of DsbA substrates

Compared to DsbA proteins, DsbA substrates are highly variable and structurally less conserved [44]. The identification of potential DsbA substrates has been made possible by using (i) two dimensional gel electrophoresis to compare wild type and ΔdsbA strains; (ii) substrate trapping by modification of the C-terminal cysteine present in the active site of DsbA proteins or (iii) mutating the cis-Pro residue (cis-Pro151 in EcDsbA) adjacent to the active site to threonine to trap stable enzyme-substrate intermediate complexes [23,73–75].
To date, the exquisite diversity of the DsbA substrates has not been comprehensively analyzed. Although it is accepted that DsbA enzymes catalyze disulfide bond formation in unfolded proteins as they are transported through the cytoplasmic membrane [68], we sought to understand what types of secondary and tertiary structures require disulfide bonds for stability and are folded by DsbA. With the knowledge that not all cysteine residues participate in disulfide bonds, this observation could allow for a better prediction of which proteins and local structures acquire disulfide bonds. To gain a better understanding of the structural class of substrate proteins folded by DsbA, a protein dataset was constructed containing 43 pathogenicity associated substrates that have been previously functionally characterized (Table S1) [4,5,20]. Among these 43 substrates, 28 have three-dimensional structures available in the Protein Data Bank (PDB) (Table 2). These 28 substrates with known structures, were then classified with the CATH-Gene3D Protein Structure Classification Database v4.2 [76,77] at: (i) class level according to their secondary structure content; (ii) architecture level according to the secondary structure arrangement in three-dimensional space independent of connectivity; (iii) topology/fold level according to their topological connections and number of secondary structures [76,78].

The 28 DsbA substrates, which collectively encompass 32 protein domains harboring disulfide bonds (S2 subunit, DppA, LptD and LasB) substrates, each have two domains with disulfides, (Table 2), were found to be organized into three different classes according to the secondary structure content, with slightly more than half having a α-sheet structures (59%), followed by mainly β-sheets (31%) and mainly α-helix (10%) (Fig. 6, Table S1). At the architecture level, 11 different architectures were identified, with ~56% showing a sandwich type architecture (22% 2-layer sandwich, 19% 3-layer (αβα) sandwich, 12% sandwich and 3% 3-layer (ββα) sandwich) (Fig. 6). Finally, this analysis found 25 different folds at the topology level, with the immunoglobulin (Ig-), Rossman and oligonucleotide/oligosaccharide binding (OB-) folds being the most prominent type among the structurally characterized DsbA substrates. The Ig-fold is widely distributed in nature, present in vertebrates, invertebrates, plants, fungi, parasites, bacteria and viruses [79]. This fold is characterized by a pair of β-sheets often linked by a disulfide bond and composed of antiparallel β-strands surrounding a central hydrophobic core [80] (Fig. 7, Caf1M). The wide occurrence of the Ig-fold has been attributed to its functional plasticity and its specific structural characteristics that confer stability and resistance to proteolysis [80]. Our structural classification of DsbA substrates revealed that proteins with an Ig-fold include the periplasmic fimbrial chaperones PapD from E. coli, one of the first examples of Ig-fold proteins identified in bacteria.
and Caf1M from *Y. pestis* [83] involved in the process of adhesion [84]; and ASST from *E. coli* [63] involved in the detoxification of phenolic compounds.

The OB-fold (Fig. 7B, CtxB) varies from between 70 and 150 amino acids in length and is characterized by 5 highly coiled antiparallel β-sheets arranged into β barrels, with an α-helix capped at one end and a binding cleft at the other. Proteins showcasing this type of fold have the ability to establish protein-DNA, -RNA or -protein interactions and so are usually involved in a number of cellular processes such as DNA replication and repair, and activation of the DNA-damage checkpoint pathways [85–87]. DsbA substrates found to have this type of fold include secreted toxins such as CtxB from *V. cholerae* [87–89], EltB from *E. coli* [87,90], and the N-terminal domain of S2 subunit from *B. pertussis* [91,92].

The Rossmann fold (Fig. 6). CATH classification of disulfide bond containing domains from structurally characterised DsbA substrates. Classification of DsbA substrates by class according to: Secondary structure content (inner circle), substrates divided into mainly α-helices (α) (cyan), mainly β-sheets (β) (raspberry) and a combination of both (αβ) (deep teal); Architecture (middle circle), substrates divided into 11 different architectures; and Topology (outer circle), DsbA substrates display 25 different folds at the topology level. EPEC: Enteropathogenic *E. coli*; ETEC: Enterotoxigenic *E. coli*; UPEC: Uropathogenic *E. coli*; NTC: N-terminal domain; CTD: C-terminal domain. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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Overall, although our substrate database only captures a very small fraction of the DsbA interactome, this analysis clearly showcases the large array of structurally diverse substrates folded by DsbA and highlights how DsbA proteins indiscriminately introduce disulfide bonds in a plethora of proteins leading to different types of protein folds.

5.1. Common features of DsbA substrates

Although DsbA introduces disulfide bonds to unfolded substrates, we wondered what structural elements harbor DsbA mediated disulfides. Using PDBsum [118] in combination with PyMol we analyzed the three-dimensional structures of the 28 characterized substrates (Table 2), to identify the secondary structures that contain disulfide bond forming cysteine residues. In particular, we
focused on substrates with a single disulfide or consecutive disulfides, which would be directly formed by DsbA (three substrates, E. coli STb, RcsF and LptD, each have two non-consecutive disulfide bonds and therefore would require the combined action of DsbA and DsbC). From this analysis, it was found that disulfide bonds are formed between cysteine residues irrespective of their position in their primary sequence (Table 2). Additionally, for 80.6% of the total number of disulfide bonds found within these substrates, at least one of the cysteines that forms a disulfide bond is harbored within a loop, with the remaining 19.4% found either in an α-helix or a β-sheet (Table 2) (Fig. 7). The presence of disulfide bonds in loops may ensure the local stability of these structural elements that frequently harbor active site residues required for function [119,120,121–123].

DsbA is thought to recognize the most N-terminal cysteine in each disulfide bond forming cysteine pair, when proteins are delivered unfolded via the Sec system frequently in an N- to C-terminal direction [6,68]. In order to investigate if there is a DsbA recognition motif, we specifically focused on E. coli substrates, as this class Ia DsbA comprises the largest collection of structurally characterized substrates for which we know the disulfide connectivity (Table 2). For each one of these substrates, the first cysteine

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Fig. 7. Examples of DsbA substrates with different folds grouped by class. A. Cartoon representation of mainly α-helix class for substrates LasB (CTD) [100], STb [101] and PulS [102]. Fold is highlighted in cyan, sulfur atoms from disulfide bonds shown as yellow spheres and the rest of the protein shown in white. B. Cartoon representation of mainly β-sheets class for substrates Pmc [103], CelZ [104], Caf1M [105], CtxB [88] and YodA [106]. Folds are highlighted in raspberry, sulfur atoms from disulfide bonds shown as yellow spheres and the rest of the protein shown in white. C. Cartoon representation of αβ class for substrates S1 subunit [92], RcsF [107], Ppp4 [108], S2 subunit (CTD) [92], DppA (CTD) [109], LasB (NTD) [110], Lpa [99], Bla [111], DppA (NTD) [109], BfpA [112], PhoA [113], OmpA [114], EscC [115], FtsN [116], LptD (NTD) [100] and PilA [117]. Folds are highlighted in deep teal, sulfur atoms from disulfide bonds shown as yellow spheres and the rest of the protein shown in white. For each structure, substrate name, organism of origin, fold and PDB codes are provided. NTD: N-terminal domain; CTD: C-terminal domain. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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involved in the formation of a disulfide bond together with its flanking 10 amino acids were extracted for analysis. These sequences were then aligned using the software Jalview (V 2.10.5) (Fig. 8A). Additionally, the Shannon sequence logo generated with the online server Seq2Logo [124] was used to visualize the frequency of amino acids preceding and succeeding the cysteine residues (Fig. 8C). No significant amino acid conservation was observed among EcDsbA substrates, although a predominance of small-medium uncharged amino acids were enriched surrounding the substrate target cysteines. The diversity of DsbA substrates made the identification of a consensus motif challenging, however this analysis informed about the chemical properties that underline EcDsbA-substrate interactions, which may be beneficial for the development of inhibitors targeting the DsbA substrate recognition site. It is important to note that this analysis may not be applicable to substrates of other DsbA proteins, particularly those interacting with DsbAs from classes other than la [39].

For comparison, we also analyzed the recognition site surrounding the DsbB cysteine known to interact with DsbA (first cysteine residue in the second periplasmic loop). For this analysis, we selected DsbB proteins from nine organisms containing class I DsbAs, since previous work has shown that these DsbBs interact with DsbA proteins in a similar mode (Fig. 3). The amino acid sequences surrounding the DsbB cysteine residue directly interacting with DsbA were retrieved from Uniprot (Table S2). An alignment of these sequences was performed using Jalview (Fig. 8B) followed by a position specific amino acid enrichment analysis using Seq2Logo (Fig. 8D) [124]. These analyses revealed a somewhat more conserved consensus sequence surrounding the cysteine residue consisting of S(56%)–P(67%)–F(56%)–A(33%)–T(56%)–C(100%)–D(56%)–F(56%). Although DsbB homologues are expected to share higher sequence homology than a diverse set of substrates, this finding reflects the somewhat different binding mode of the cognate oxidases compared to protein substrates, whereby the presence of aromatic residues neighboring the DsbB cysteine would facilitate the interaction with the hydrophobic groove [27] characteristic of class I DsbA proteins [39].

6. Summary and outlook

As the threat of antibiotic resistance continues to grow, it has become increasingly important to understand the molecular mechanism of bacterial pathogenesis. The reliance of virulence factors on DSB systems for their assembly, has indisputably linked these proteins, primarily DsbA thiol oxidases to the bacterial pathogenic potential. This, in addition to the wide distribution of DSBs across bacteria, make these systems attractive targets to disrupt bacterial...
pathogenesis. In this review we have summarized the current knowledge on DsbA proteins including their structural diversity as well as their biochemical and functional properties. We have particularly focused on the DsbA interactome; collating the current understanding of how this enzyme interacts with cognate DsbB oxidases and explored the sequence and structural diversity of DsbB substrates. This detailed examination, has allowed us to identify that cysteine residues interacting with DsbA are primarily present in loop regions of both substrates and cognate oxidases. Furthermore, we have proposed a consensus motif recognized by class I DsbA proteins in DsbB oxidases. Overall, a detailed understanding of how these important redox enzymes interact with partner proteins, not only provides fundamental understanding on the bacterial oxidative protein folding machinery but may offer tools to allow the identification of so far unexplored DsbB substrates.

As DsbA plays a pivotal role in bacterial virulence, inhibitors of DsbA are being actively pursued as anti-microbial agents [125]. There are a number of advantages to targeting these redox enzymes; firstly, in conditions where DsbAs are not essential for viability or growth, their inhibition would reduce virulence and likely impose low selection pressure for resistance development [126]. Secondly, DsbA is present in the periplasm, which is more accessible relative to cytoplasmic targets. Furthermore, all DsbA inhibitors described so far target the hydrophobic groove of DsbA [47,66,125,127–130], which is not present in human thioredoxin or PDI. Therefore, despite DsbA belonging to the widespread thioredoxin superfamily, DsbA-tailored inhibitors are less likely to inhibit TRX-like proteins in humans. The detailed analysis of the DsbA-substrate and DsbA-DsbB interactions described in this work could therefore inform a number of international campaigns in their efforts to designing specific inhibitors against DsbA enzymes [47,66,125,127–130].

CRediT authorship contribution statement

Carlos Santos-Martin: Conceptualization, Writing – original draft, Data curation, Formal analysis. Geqing Wang: Conceptualization, Writing – original draft, Data curation, Formal analysis. Pramod Subedi: Writing – original draft. Lilian Hor: Writing – review & editing. Makrina Totsika: Writing – review & editing. Jason John Paxman: Formal analysis, Writing – review & editing. Begona Heras: Conceptualization, Funding acquisition, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.csbj.2021.08.018.

References

[1] Beeceiro A, Tomás M, Bou G. Antimicrobial Resistance and Virulence: a Successful or Deleterious Association in the Bacterial World? Clin Microbiol Rev. 2013;26(2):185.
[2] Diard M, Haridi WD. Evolution of bacterial virulence. FEMS Microbiol Rev. 2017;41(5):679–97.
[3] Sharma AK, Dhasmana N, Dubey N, Kumar N, Gangwal A, Gupta M, et al. Bacterial Virulence Factors: Secreted for Survival. Indian J Microbiol. 2017;57(1):1–10.
[4] Heras B et al. DsbB proteins and bacterial pathogenicity. Nat Rev Microbiol. 2009;7:215.
[5] Landeta C, Boyd D, Beckwith J. Disulfide bond formation in prokaryotes. Nat Microbiol. 2018;3(3):270–80.
[6] Bardwell JCA, McGovern K, Beckwith J. Identification of a protein required for disulfide bond formation in vivo. Cell 1991;67(3):581–9.
[7] Altschul F, Karim J, Bessant N, McInerney-O’Hara J, Roks K. In vitro catalysis of oxidative folding of disulfide-bonded proteins by the Escherichia coli DsbA (ppA) gene product. J Biol Chem 1992;267(31):22440–5.
[8] Bringer M-A, Rohlhin N, Glasser A-L, Darfeuille-Michaud A. The oxidoreductase DsbA plays a key role in the ability of the Cronobacter-disease-associated adherent-invasive Escherichia coli strain L822 to resist macrophage killing. J Bacteriol 2007;189(13):4860–71.
[9] Burall LS, Harro JM, Li X, Lockatell CV, Himpsl SD, Hebel JR, et al. Proteins mirabilis genes that contribute to pathogenesis of urinary tract infection: identification of 25 signature-tagged mutants attenuated at least 100-fold. Infect Immun 2004;72(5):2292–38.
[10] Coulombe SJ, Lilley KS, Hedley PE, Liu H, Torh IK, Slauch JM. DsbA plays a critical and multifaceted role in the production of secreted virulence factors by the phytopathogen Erwinia carotovora subsp. atroseptica. J Bacteriol 2008;280(35):23739–53.
[11] Lee SH, Butler SM, Carnull A. Selection for in vivo regulators of bacterial virulence. Proc Natl Acad Sci U S A 2001;98(12):6889–94.
[12] Lin D, Rao CV, Slauch JM. The Salmonella SPI1 type three secretion system responds to periplasmic disulfide bond status via the flagellar apparatus and the Rcs/DB system. J Bacteriol 2008;190(1):87–97.
[13] Meina R, Eschevins C, Fililnger S, Bolhuis A, Hamoen LW, Dorenbos R, et al. The bbdBC operon of Bacillus subtilis encodes thiol-disulfide oxidoreductases required for competence development. J Biol Chem 2002;277(9):6994–7001.
[14] Poon JA, Taylor BK. Characterization of a periplasmic thiol-disulfide interchange protein required for the functional maturation of secreted virulence factors of Vibrio choloreae. Proc Natl Acad Sci U S A 1992;89(13):6210–4.
[15] Tomim JF. A periplasmic protein disulfide oxidoreductase is required for transformation of Haemophilus influenzae Rd. Proc Natl Acad Sci U S A 1992;89(21):10252–6.
[16] Yu J. Inactivation of DsbA, but not DsbC and DsbD, affects the intracellular survival and virulence of Shigella flexneri. Infect Immun 1998;66(8):3909–17.
[17] Ireland PM, McMahon RM, Marshall LE, Halili M, Furlong E, Tay S, et al. Disgrading BuchnerDemiae pseudomallei:structural and functional characterization of a disulfide oxidoreductase (DsbA) required for virulence in vivo. Antioxid Redox Signal 2014;20(4):606–17.
[18] Basharav MA. Protein folding. J Cell Mol Med 2003;7(3):223–37.
[19] Totsika M, Heras Begon–a, Wurpel DJ, Schembri MA. Characterization of two homologous disulfide bond systems involved in virulence factor biogenesis in uropathogenic Escherichia coli CFT073. J Bacteriol 2009;191(12):3901–8.
[20] Shouldice SR, Heras B, Walden PM, Totsika M, Schembri MA, Martin JL. Structure and function of DsbA, a key bacterial oxidative folding catalyst. Antioxid Redox Signal 2011;14(9):1729–60.
[21] Sevier CS, Kaiser CA. Formation and transfer of disulfide bonds in living cells. Nat Rev Mol Cell Biol 2002;3(11):836–47.
[22] Wiedemann C et al. Cysteines and Disulfide Bonds as Structure-Forming Units: Insights From Different Domains of Life and the Potential for Characterization by NMR. Front Chem 2020;8:280.
[23] Himiker A, Bardwell JCA. In vivo substrate specificity of periplasmic disulfide oxidoreductases. J Biol Chem 2004;279(13):12867–73.
[24] Zapun A, Missiaakas D, Raina S, Creighton TE. Structural and Functional Characterization of DsbC, a Protein Involves in Disulfide Bond Formation in Escherichia coli. Biochem 1995;34(15):5075–89.
[25] Missiaakas D, Georgopoulos C, Raina S, The Escherichia coli dsbC (dxcA) gene encodes a periplasmic protein involved in disulfide bond formation. EMBO J 1994;13(8):2013–20.
[26] Bardwell JC, Lee JO, Jander G, Martin N, Belin D, Beckwith J. A pathway for disulfide bond formation in vivo. Proc Natl Acad Sci U S A 1993;90(3):1038–42.
[27] Inaba K, Murakami S, Suzuki M, Nakagawa A, Yamashita E, Okada K, et al. Crystal structure of the DisdB-DisS complex reveals a mechanism of disulfide bond generation. Cell 2002;107(4):789–801.
[28] Missiaakas D, Georgopoulos C, Raina S. Identification and characterization of the Escherichia coli gene dsbB, whose product is involved in the formation of disulfide bonds in vivo. Proc Natl Acad Sci U S A 1995;92(15):7084–8.
[29] Retsch G, Bessette P, Georgiou G, Beckwith J. Reduction of the periplasmic disulfide bond isomerase, DsbC, occurs by passage of electrons from cytoplasmic thioredoxin. J Bacteriol 1997;179(21):6602–8.
[30] Martin JL, Bardwell JCA, Kuriyan J. Crystal structure of the DsbA protein required for disulphide bond formation in vivo. Nature 1993;365 (6445):464–6.

[31] Grauschopf U et al. Why is DsbA such an oxidizing disulfide catalyst? Cell 1995;83(6):947–55.

[32] Guddat LW, Bardwell JCA, Martin JL. Crystal structures of reduced and oxidised DsbA: Implications for domain motion and thiolate stabilization. Structure 1998;6(6):757–67.

[33] Charbonnier J-B, Belin P, Moutiez M, Stura EA, Quéméneur E. On the role of the cis-proline residue in the active site of DsbA. Protein Sci 1999;8(10):2139–45.

[34] Ren G, Stephan D, Xu Z, Zheng Y, Tang D, Harrison RS, et al. Properties of the thiooxidoreductase DsbA: a highly oxidizing enzyme that catalyzes protein disulfide bond formation. J Biol Chem 2009;284(15):10150–9.

[35] Guddat LW, Bardwell JCA, Zander T. The uncharged surface features surrounding the active site of Escherichia coli DsbA are conserved and are implicated in peptide binding. Protein Sci 1997;6(6):1148–56.

[36] Poxman JJ, Borg NA, Hone J, Thompson PE, Chin Y, Sharma P, et al. The structure of the bacterial oxidoreductase enzyme DsbA in complex with a peptide reveals a basis for substrate specificity in the catalytic cycle of DsbA enzymes. J Biol Chem 2009;284(26):17835–43.

[37] Darby MJ, Creighton TE. Catalytic mechanism of DsbA and its comparison with that of protein disulfide isomerase. Biochem 1995;34(13):5376–81.

[38] Skróko-Glonek J, Sobiecka-Szatulka A, Lipińska B. Characterization of disulfide exchange between DsbA and HtrA proteins from Escherichia coli. Acta Biochim Pol 2006;53(3):585–94.

[39] McMahon RM, Premkumar L, Ichimura S, Gavranich J, Martin JL. Four structural subclasses of the antiviral drug target disulphoxide oxidoreductase DsbA provide a platform for the design of subclass-specific inhibitors. Biochim Biophys Acta 2014;1844(7):1391–401.

[40] Kurth F, Duprez W, Premkumar L, Schermbrüker MA, Fairlie DP, Martin JL. Crystal structure of the dithiol oxidase DsbA enzyme from proteins mirabilis bound non-covalently to an active site peptide ligand. J Biol Chem 2014;289(28):19810–22.

[41] Heras B, Totsika M, Jarrott R, Shouldice SR, Gunc ˇar G, Achard MES, et al. Structural and functional characterization of three DsbA paralogues from Salmonella enterica serovar Typhimurium. J Biol Chem 2010;285(24):18423–32.

[42] Bouwman CW, Kohli M, Kililan A, Touchie GA, Kadner RJ, Martin NL. Characterization of SrgA, a Salmonella enterica serovar Typhimurium virulence plasmid-encoded enzyme required for pilus and cholera-toxin production. Acta Crystallogr Sect C Cryst Struct Commun 2012;68(Pt 10):1290–202.

[43] Lenfant PA, Ramos MJ. Theoretical insights into the mechanism for thiol- disulfide exchange. Chemistry 2004;10(1):257–66.

[44] Kurth F et al. Comparative sequence, structure and redox analyses of Klebsiella pneumoniae DsbA show that anti-virulence target DsbA enzymes fall into distinct classes. PLoS ONE 2013;8(11):e80210.

[45] Shouldice SR, Heras B, Jarrott R, Sharma P, Scanlon MJ, Martin JL. Characterization of the DsbA oxidative folding catalyst from Pseudomonas aeruginosa reveals a highly oxidizing protein that binds small molecules. Antioxid Redox Signal 2010;12(3):291–311.

[46] Heras B, Kurz M et al. Inhibition of Diverse Dsb Enzymes in Multi-DsbA Encoding Pathogens. Antioxid Redox Signal 2013;19(13):1494–506.

[47] Kudalkar H, Beckwith J. Detecting folding intermediates of a protein as it passes through the bacterial translocation channel. Cell 2009;138(6):1164–73.

[48] McEwen AL, Ramesh PA, Ramos MJ. Theoretical insights into the mechanism for thiol-dissulfide exchange. Chemistry 2004;10(1):257–66.

[49] Freund CH, Wunderlich M, Glickshubler R, Schmid FX. Preferential binding of an unfolded protein to DsbA. EMBO J 1996;15(2):392–8.

[50] Coupré J, Vinci F, Dugue C, Quéméneur E, Moutiez M. Investigation of the DsbA Mechanism through the Synthesis and Analysis of an Irreversible Enzyme–Ligand Complex. Biochemistry 2000;39(22):6732–42.

[51] Premkumar L, Kurth F, Duprez W, Grafehaug MK, King GJ, Halli MA, et al. Structural characterization of the Shigella dysenteriae DsbA oxidase DsbA: Bifunctional Enzyme with Both Thiol Oxidase and Disulfide Isomerase Activities. J Biol Chem 2018;293(18):204–50.

[52] Dawson NL et al. CATH: an expanded resource to predict protein function through structure and sequence. Nucleic Acids Res 2016;45(D1):D289–95.

[53] Lewis TE et al. Gene3D: Extensive prediction of globular domains in proteins. Nucleic Acids Res 2017;46(D1):D345–9.

[54] Greene LH et al. The CATH domain structure database: new protocols and classification levels give a more comprehensive resource for exploring evolution. Nucleic Acids Res 2007;35(Database issue):D291–7.

[55] Halaby DM, Morrison JF. The immunoglobulin superfamily: An insight on its tissue-specific, species, and functional diversity. J Mol Evol 1998;46(4):389–400.

[56] Bodeñón G, Palomino C, Fernández IÁ. Immunoglobulin domains in Escherichia coli and other enterobacteria: from pathogenesis to applications in antibody technologies. FEMS Microbiol Rev 2013;37(2):204–50.

[57] Waksman G, Hultgren SJ. Structural biology of the chaperone-usher pathway of pilus biogenesis. Nat Rev Microbiol 2009;7(11):765–74.

[58] Holmgren A, Brändén C-L. Structure and mechanism of bacterial protein disulfide exchange enzymes. In: Structure. Current Science 1989;58(2):153–64.

[59] Vincent-Sealy LV et al. Erwinia carotovora DsbA mutants: evidence for a periplasmic stress signal transduction system affecting transcription of genes encoding secreted proteins. Microbiology 1999;145(8):1945–58.

[60] Mann RL, Zou L, Olgardtsov SI, Golovchenko G. Glycosylation-binding fold proteins: a growing family of genome guardians. Crit Rev Biochem Mol Biol 2010;45(4):266–75.

[61] Arcus V. OB-fold domains: a snapshot of the evolution of sequence, structure and function. Curr Opin Struct Biol 2002;12(6):794–801.
