

REVIEW ARTICLE

THE DISULFIDE BOND FORMATION AND ITS RELATIONSHIP TO BACTERIAL PATHOGENICITY OF THREE IMPORTANT GRAM-NEGATIVE BACTERIA

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Summary

Disulfide bond formation is necessary for a correct folding and a proper function of many secreted proteins. We know that many of these proteins are involved in bacterial virulence and pathogenesis. The best known pathways of disulfide bond formation and isomerization belong to *Escherichia coli* (*E. coli*). This Gram-negative bacterium is usually used as a model organism. This review is aimed initially at introduction to *E. coli* oxido-reductase enzymatic system. The next part is interested in proteins resembling these from *E. coli* and their relation to virulence and pathogenesis. We have chosen three important Gram-negative pathogens, *Neisseria meningitidis* (*N. meningitidis*), *Yersinia pestis* (*Y. pestis*) and *Francisella tularensis* (*F. tularensis*), because of their high virulence, infectivity, ability to cause severe infections and absence of appropriate vaccines.

Key words: Disulfide bond formation; DsbA; DsbB; DsbC; DsbD; DsbE; CcmG; *Neisseria meningitidis*; *Yersinia pestis*; *Francisella tularensis*.

INTRODUCTION

Secreted proteins play a very important role in bacterial pathogenesis and virulence. For proper function of these proteins there are requirements of correct folding and stabilization of protein structure. The formation of disulfide bonds between

two cysteine residues is vital to correct protein folding. Failure to form right disulfide bonds is probably reason of protein misfolding, which leads to aggregation and degradation.

In Gram-negative bacteria, disulfide bond formation is catalyzed by the Dsb proteins present in the periplasm [22]. In *E. coli*, as in eukaryotes, disulfide bonds are generally found in exported proteins, but are rare in cytoplasmic proteins [15]. The difference in the thiol/disulfide redox state between the cytoplasm and the periplasm may be sufficient for preventing disulfides in the cytoplasm [3]. Dsb proteins belong to thioredoxin-fold proteins which have one or more thioredoxin folds. These folds contain characteristic four α -helices

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that are juxtaposed with five β -strands (β - α - β - α - β - α - β - α) [6]. The next characteristic mark is -CXXC- (X = any amino acid) motif in front of the first helix. Many of them contain other domain insertions with importance in substrate recognition and reactivity [26]. The -CXXC- motif is a major factor determining the function of the protein [52]. The type of the amino acids (X) between active site cysteines varies greatly among members of

thioredoxin family [6]. Depending on their role the -CXXC- is in the reduced form with a dithiol or in the oxidized form with an intramolecular disulfide bond [36]. Each of the Dsb proteins has a specific physiological role in oxidation, reduction or isomerization of disulfides. Moreover, the thioredoxin-fold family includes the eukaryotic protein disulfide isomerase and the ubiquitous reductants, thioredoxin and glutaredoxin [19].

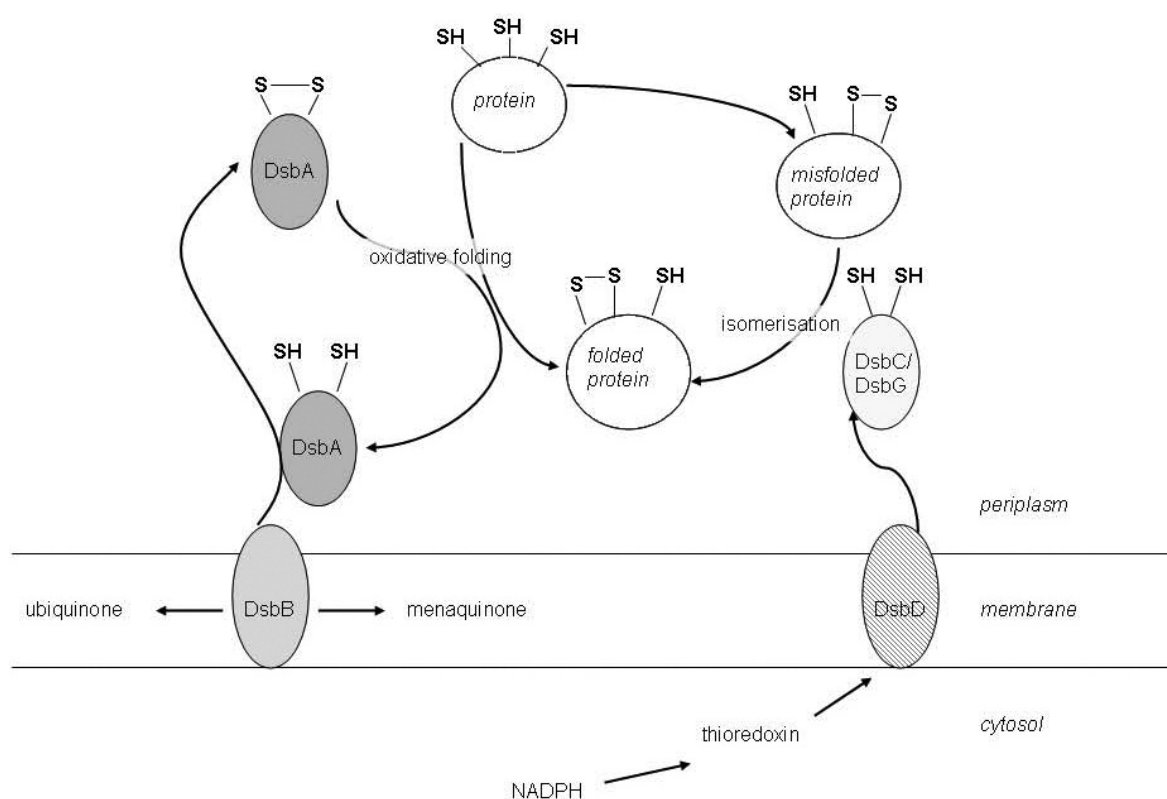


Figure 1. The disulfide bond formation in the *E. coli* periplasm. The direction of electron flow is indicated by the arrows. Inspired by 2, 8, 22, 25, 26.

The formation of disulfide bonds in *E. coli* can be divided into two steps. The first is the oxidation of cysteine residues to form disulfides (DsbA and DsbB proteins), and the second is the isomerization of incorrect disulfide bonds to the correct form (DsbC, DsbG and DsbD proteins) [41] (Figure 1), so the isomerase pathway has a proof-reading role [16]. Formation and breakage of a disulfide bond are chemical reactions, in which two electrons are donated to or taken away from a cysteine pair. This system plays on the oxidizing and the reducing equivalents originating from quinone and NADPH

[26]. Although the oxidation and reduction pathways reside in the same cellular compartment, it was found that their crosstalk should be minimized to avoid invalid consumption of electrons and metabolic energy. *In vitro* kinetic measurements of reactions between various combinations of DsbA, DsbB, DsbC, DsbD α and DsbB γ showed that only 'physiological' combination and direction are formed rapidly [55].

Increasing number of sequenced bacteria genomes shows that formation of disulfide bonds is

diverse and not all bacteria share this type of oxidative folding device. Heras *et al.* carried out a bioinformatical screening for Dsb homologues (predominantly DsbA homologues) and in total they found that of 421 genomes searched, 51 bacteria encoded more than one DsbA and 156 organisms lacked an identifiable DsbA [21].

This review focuses on Dsb proteins in general, describing *E. coli* proteins as a model and their homologues in *N. meningitidis*, *Y. pestis* and *F. tularensis*. We have chosen these Gram-negative bacteria because they possess some similar features. They can be transmitted by aerosol, cause serious diseases to humans, which can end in epidemic. There are also problems with fast diagnostics and with their antibiotic resistance. *Y. pestis* and *F. tularensis* are members of category A of potential biological weapons [31] so they are also important for military health care. We are interested in function of Dsb homologues and their role in bacterial virulence and pathogenesis.

PROTEINS INVOLVED IN THE DISULFIDE BOND FORMATION SYSTEM (Dsb)

DsbA catalyses disulfide bond formation

DsbA is one of the most oxidizing thiol-disulfide oxidoreductases known. It has a redox potential of -121 mV [75]. Its highly oxidizing nature is a result of hydrogen bond, electrostatic and helix-dipole interactions [19]. It is very important that oxidized DsbA is less stable than reduced DsbA even if it is known that disulfides generally stabilize proteins [73]. Because of the great oxidizing potential of DsbA, there is a great possibility of incorrect disulfides formation [16]. These incorrect disulfides have to be refit to prevent protein misfolding and aggregation. This is a commission for two disulfide isomerases, DsbC and DsbG [36].

DsbA has a redox-active site -CXXC- like other redox-active members of the thioredoxin fold family. This motif converts from disulfide to dithiol. Adjacent *cis*-Pro motif is involved in substrate interaction [28]. This *cis*-Pro is conserved in most proteins with thioredoxin fold. It is known that mutation in this *cis*-Pro dramatically influences the stability of the protein [36]. Surface groove near the active site Cys30-Cys33 is considered to be putative substrate binding site [22]. This hydrophobic groove is formed by a helical insertion [26]. DsbA is acting as a thiol-reductase thus -CXXC- motif is found

oxidized *in vivo*. The disulfide bond existing in DsbA is very unstable and can be quickly transferred to newly translocated protein [36]. After this transfer DsbA appears in the reduced form.

The crystal structures of reduced and oxidized DsbA show that hinge bending motions occur between two domains. These motions are not dependent on the redox state but it is a result of interactions at the putative peptide-binding groove of DsbA. This motion could be important for substrate binding and/or for interactions with other proteins as DsbB [19].

It is believed that DsbA creates disulfide bonds indiscriminately and mostly between cysteine residues that are consecutive in the primary sequence. But it was demonstrated that oxidation is not strictly cotranslocational and that it is in kinetic competition with folding [37].

Numerous studies interested in substrates of DsbA suggest that DsbA has a broad substrate specificity. More than 300 periplasmic and membrane proteins contain 2 or more cysteine residues and could be DsbA substrates. But only a small number of putative substrates have been in fact identified. The reason could be that other proteins are not measurable under laboratory conditions [23].

DsbA seems to be very important in bacterial virulence. It was shown to be crucial in biogenesis of toxins and multimeric structures on the bacterial surface (e.g. fimbriae, adhesive molecules, components of secretory machinery). In fact, *dsbA* mutant is severely defective in the formation of disulfide bond *in vivo* [3] and it is observed accumulation of periplasmic proteins in their reduced form [77]. Mutation in *dsbA* usually creates the same phenotypic effect as a mutation in the gene encoding the virulence determinant itself [74].

DsbB keeps DsbA in the active form

Reoxidation of DsbA is performed by the membrane protein DsbB [40]. Once DsbA donates its disulfide to a substrate protein, DsbA is reoxidized to carry out other disulfide catalysis [22]. DsbB creates four-helix-bundle structure passing through the inner membrane. Furthermore it contains a short periplasmic loop and a longer flexible periplasmic region with a short, horizontal α -helix [24]. Each of the periplasmic loops contains one pair of essential cysteines: Cys41-Cys44 and Cys104-Cys130. The Cys104-Cys130 pair is involved in the disulfide exchange with DsbA, the second pair of Cys41-Cys44 is the target of oxidation by ubiquinone [25].

DsbB is reoxidized by components of the respiratory chain. Under aerobic conditions, DsbB transfers the electrons to oxidized ubiquinone. It becomes reoxidized by the terminal cytochrome oxidases, which finally transfer the electrons onto molecular oxygen. There is another situation under anaerobic conditions. DsbB transfers electrons to menaquinone, which passes them to the fumarate or nitrate [2].

DsbC corrects the misfolded disulfide bonds

E. coli encodes two disulfide isomerases, DsbC and DsbG. They share just 24% sequence identity, both are homodimers with each monomer comprising a catalytic thioredoxin domain jointed by a linker α -helix to a dimerization domain [20]. Two subunits form a V-shape structure with hydrophobic surface on its inner part. This is probably a binding site of substrates [22]. DsbC is kept in a reduced form by DsbD. DsbC must be in the reduced form in order to have its Cys98 prepared for attack on a wrong disulfide of a substrate to initiate isomerization [26].

There are number of possible mechanisms by which DsbC can isomerize incorrect disulfide bonds. First, DsbC can attack incorrect disulfides forming mixed disulfide between the more N-terminal cysteine of DsbC and the protein substrates. This situation can be immediately transferred back, but with correct disulfide bond pattern. The second way is that the mixed disulfide can be resolved such that DsbC is oxidized and the substrate is reduced. The reduced form of substrate can then be reoxidized by DsbA or DsbC [16]. But there is some evidence against the first model and in favour of the second reduction/reoxidation mechanism [54].

The crystal structure of oxidized DsbC shows two 23 kDa monomers of DsbC which form a V-shaped homodimer. Each monomer consists of two domains: a C-terminal thioredoxin-like domain with active site Cys⁹⁸-Gly-Tyr-Cys¹⁰¹ and an N-terminal dimerization domain connected via a flexible linker helix [35]. Except its isomerase activity, DsbC also has the chaperone activity. *In vitro*, DsbC can assist the refolding of denatured proteins like lysozyme or glyceraldehyde-3-phosphate dehydrogenase [7]. The function of DsbC as isomerase and chaperone is completely dependent on its dimerization because a heterodimer that has had one of the -CXXC- sites inactivated by carboxymethylation has no isomerase activity [61].

DsbG also works as disulfide isomerase

DsbG is a second putative isomerase. Primarily it was described as a disulfide oxidase [1], but nowadays it is considered that DsbG acts as an isomerase in periplasm of *E. coli* [4]. This confirmed the study of crystal structures of the DsbG. The oxidized structure reveals a mixture of reduced and oxidized forms. This suggests that oxidized DsbG is less stable than the reduced form. This information can contribute to DsbG isomerase activity, which requires that the active-site cysteine residues are kept reduced, regardless of the oxidative environment of the periplasm [20].

DsbG forms a dimer and has a two conserved cysteine residues, Cys109 and Cys 112, which are present in a -CXXC- motif [4]. The protein appears as a V-shaped protein with two domains, N-terminal dimerization domain and C-terminal catalytic domain with thioredoxin fold where -CXXC- motif is located [36].

There are some structural similarities but also differences between the isomerase DsbC and DsbG [20]. Actually, DsbG does not catalyze the folding of proteins known as substrates of DsbC [22]. DsbG is spatially larger and has longer linker connecting two domains. Also a V-shaped cleft has negatively charged patches. Another difference is the charged groove formed by residues in a loop connecting two β strands of the N-terminus. These facts can help with detection of DsbG substrates [36].

DsbD is the partner protein of DsbC and DsbG

DsbD is the inner membrane redox-active protein. Its role is maintenance of DsbC, DsbG and DsbE (also called CcmG a specific disulfide reductase involved in *c*-type cytochrome maturation in the periplasm) in their active reduced form [23]. These proteins contain thioredoxin-like domains with characteristic -CXXC- motif. These cysteines are the targets of the DsbD, when they are joined in disulfide bonds [47]. DsbD plays a key role in transferring electrons from the reducing cytoplasm to the oxidative environment of the periplasm. DsbD has two periplasmic domains, an immunoglobulin-like N-terminal domain (nDsbD or α) and a thioredoxin-like C-terminal domain (cDsbD or γ). Either is linked to a central transmembrane domain (tDsbD or β). Each of the domains has two reactive cysteines [22]. Some studies established that electrons flow from NADPH in the cytosol, via TRX-1 (thioredoxin-1), β , γ and α to DsbC [29]. The mechanism by which DsbD β is able to transfer electrons across the

cytoplasmic membrane via its pair of cysteines Cys163 and Cys285 is not completely explained. But Cho *et al.* [8] present a structural model where DsbD β creates two cavities that are inside the membrane portion of the protein, to accommodate the appropriate interacting thioredoxin protein. This structural model could elucidate a mechanism of electron transfer.

Homologues of DsbD or of one or more of its domain were found in a wide range of bacteria, including species of Archae. These bacteria also contain DsbC/DsbG homologues [47].

DsbE (CcmG) has specific reductase activity

DsbE, also called CcmG, also belongs into thioredoxin-fold proteins with regard to its sequence and functionality. DsbE has a highly specialized role in electron transport in periplasm. DsbE is involved in cytochrome c maturation (Ccm). Cytochrome c is the protein with covalently bound heme as a cofactor, which is necessary for respiratory and/or photosynthetic electron transport chains. Cytochrome c maturation requires ligation of heme to reduced thiols of the apocytochrome [69]. Maturation of c-type cytochromes in Gram-negative bacteria is done in the periplasm during a series of sequential or coordinated steps [53] and requires redox control. The heme attachment to the apocytochrome cannot come unless the heme binding cysteines are in the thiol, reduced form. But these cysteines can be oxidized by DsbA when apocytochrome is secreted into the periplasm [10]. DsbE selectively donates electrons to apocytochrome c cysteines via another periplasmic cytochrome c maturation factors [11] in order to restore the apocytochrome c cysteines to reduced form. DsbE is maintained in its reduced form by DsbD [59]. DsbE has a highly hydrophobic N-terminus, which is predicted to function as a membrane anchor and a hydrophobic TRX (thioredoxin) domain is presumed to face toward the periplasm [33]. DsbE, as in all redox TRX-like proteins, has a -CXXC- motif at the N-terminus of the first helix in the TRX fold. The crystal structure of DsbE homologue from *Bradyrhizobium japonicum* revealed that the active site is however unusual in its surrounding region with negative charge. This speciality seems to be conserved among DsbE homologues. There is also a groove near the active site. Residues in the insert are conserved in DsbE homologues, and deletion of the central insert restrains cytochrome c maturation [10].

Dsb homologues were also found in Gram-positive bacteria, for instance in *Mycobacterium*

tuberculosis. These were homologues to DsbE and DsbD. But some structural and functional differences between these proteins in Gram-positive and Gram-negative bacteria were observed [17].

In recent studies other important proteins involved in disulfide bond formation in *E. coli* were found. The uropathogenic *E. coli* strain CFT073 contains an additional oxidative system, DsbL and DsbI, which is encoded in a tri-cistronic operon together with a periplasmic, uropathogen-specific arylsulfate sulfotransferase (ASST). It is proposed that the DsbL/DsbI pair was acquired as a specific redox couple which maintains biological activity of ASST [18].

IMPORTANT GRAM-NEGATIVE BACTERIA POSSESSING Dsb SYSTEM

Neisseria meningitidis

The *Neisseria* is a large family of bacteria where the 11 species are able to colonize the humans, but only two can cause a disease. *Neisseria gonorrhoeae* (*N. gonorrhoeae*) is a mucosal pathogen that causes a gonorrhoea, one of the most wide-spread sexual diseases. *N. meningitidis* is generally a commensal bacterium colonizing the nasopharynx of germ carriers. But this bacterium is also a potential pathogen and the causative agent of a life threatening cerebrospinal meningitis and systemic sepsis. The bacteria can be spread by aerosol and huge epidemic meningitis cases are known from sub-Saharan Africa [66]. The disease process is very abrupt so there is a problem with early diagnosis. Another problem is the antibiotic resistance so *N. meningitidis* remains a significant worldwide health danger.

The genome of *N. meningitidis* encodes homologues for DsbD, DsbC and DsbB proteins, but not for DsbE or DsbG proteins [65]. There are also some differences between DsbA homologues across neisserial species. *N. meningitidis* has three genes which encode three active DsbAs (DsbA1, DsbA2, DsbA3). These genes are named *nmb0278*, *nmb0294* and *nmb0407* in the sequenced genome of the serogroup B strain MC58 [32]. *N. gonorrhoeae*, *Neisseria lactamica*, *Neisseria cinerea* and *Neisseria polysaccharea* possess homologues of *nmb0278* (DsbA1) and *nmb0407* (DsbA3) alone. *Neisseria flava*, *Neisseria subflava* and *Neisseria flavescens* possess only homologue of *nmb0294* (DsbA2). The diversity of these proteins can suggest the possibility

of their specialized role in bacterial biology including virulence [58]. Each of DsbAs shows different enzymatic properties. DsbA1 and DsbA2 are lipoproteins associated with the inner membrane, while DsbA3 is a soluble periplasmic protein [70]. These enzymes share about 20% sequence identity with their *E. coli* homologue, but they have more oxidizing power [32]. The primary sequences of the three neisserial proteins DsbA1, DsbA2 and DsbA3 are similar, but some variances in the active-site domain which may underline the distinct properties of each protein were found [70]. The phenotypic differences observed between the three neisserial DsbAs are probably related to differences in substrate specificity and gene regulation [32]. Tinsley *et al.* found that the one or both of membrane-bound DsbA enzymes (DsbA1 and DsbA2) are essential for growth under reducing condition and for the biogenesis of functional type IV pili which are needed for interaction of the bacteria with their human host. The results of their studies were that the presence of at least one of the two membrane-bound enzymes is necessary to obtain wild type phenotype, the third enzyme (DsbA3) being incapable of supporting the required disulfide oxidoreductase activities. The explanation of its different properties is not clear. It might be because DsbA3 is specialized only for narrow group of preferred substrates [70]. Another observation was carried out by Sinha *et al.* Pathogenicity of *Neisseria* is dependent on the expression of membrane proteins complex the type IV pili. One of these membrane proteins is the secretin PilQ. Type IV pili are very important for interactions with the host cell. The absence of both DsbA1 and DsbA2 resulted in reduced levels of PilQ confirming its inefficient folding. Because secretin PilQ is involved in DNA binding and transport as well as pilus biogenesis, the reduced DNA uptake was also found. The lack of DsbA1 and DsbA3 cannot be compensated by overexpression of DsbA3 [57].

Yersinia pestis

The *Yersinia* genus includes 15 species, but only three of them are pathogenic to humans. *Yersinia pseudotuberculosis* and *Yersinia enterocolitica* cause nonfatal gastrointestinal disease and are transmitted by the food-borne route [77]. *Y. pestis* is causative agent of deadly disease plague. Plague is enzootic in rodent, humans are sometimes infected for example by fleabites, through handling infectious animal tissues or by inhalation of aerosolized bacteria. Most cases are bubonic plague. Patients have a fever and

swollen, necrotic lymph node called bubo. Other type of plague is septicemic, in which bacteria circulate in blood and pneumonic with pulmonary infiltrates and cough [5]. We know about three pandemics of plague that were recorded in human history and claimed hundreds of thousands of lives [44]. *Y. pestis* is a member of category A of potential biological weapons, which includes agents that can be easily disseminated or transmitted from person to person resulting in high mortality rates [31].

One of the known proteins associated with virulence of *Y. pestis* is the envelope antigen F1. This antigen is encoded by plasmid-located gene *cafI* [14] and its expression is mediated by periplasmic molecular chaperone Caf1M. The *caf1M* (*cafI* mediator) gene has homology with the chaperone protein PapD of *E. coli* [13] so it is supposed that product of *caf1M* gene is involved in biogenesis of antigen F1. Caf1M is a member of chaperone subfamily where deputies contain two cysteine residues with the potential of forming a disulfide bond in the putative binding pocket. The other characteristic feature is the chaperoning of a very simple structure surface organelles sometimes associated with virulence [68]. It was established that disulfide bond between cysteine residues is not necessary for stabilization of Caf1M structure, but is important for the fine structure of the binding site, where binding of the capsular subunit Caf1 by the Caf1M chaperone takes place, thus for its function. It was uncovered that DsbA can control right folding of Caf1M through its chaperone-like activity but it is also capable of affecting the binding properties of Caf1M through its oxidative activity [76]. The periplasmic disulfide isomerase DsbA is essential for Caf1M activity [46].

The other plasmid-encoded virulence proteins are termed Yops (*Yersinia* outer membrane proteins), which are secreted through the bacterial inner and outer membrane by type III secretion mechanism [39]. The *Y. pestis* type III secretion system is created by several genes. One of the important genes for Yop secretion is *yscC* [38]. YscC contains four carboxyl-terminal cysteine residues and forms two intramolecular disulfide bonds. One disulfide bond is between Cys551 and Cys576 and the second one is between Cys591 and Cys598. Both predicted disulfide bonds are important for stable production of YscC, but only disruption of disulfide bond between Cys551 and Cys576 induces the function of YscC in Yop secretion. DsbA appears to be necessary for formation of two disulfide bonds in YscC because YscC is accessible to proteolytic degradation in the *Y. pestis dsbA* mutant. [27].

Francisella tularensis

Gram-negative facultative intracellular pathogen *F. tularensis* is one of the most infectious organisms known. Following the terrorist attacks in 2001 and subsequent mailing of anthrax there has been a refreshed interest in this organism [43]. *F. tularensis* is a causative agent of zoonotic disease called tularemia. It is a natural focused disease which can be transmitted from animal to human. For its extreme virulence, low infectious dose and ability to cause severe disease, it was classified as a member of category A of potential biological weapon [31]. *F. tularensis* is divided into four subspecies where only two are clinically important. *F. tularensis* subsp. *tularensis* (also called type A) is the most virulent, less than 10 CFU can cause fatal disease without treatment with appropriate antibiotics [12]. *F. tularensis* subsp. *holarctica* is less virulent, the infection dose less than 10³ CFU is able to cause disease which is protracted and very exhausting [63]. A main route of *F. tularensis* transmission to animals and humans is via arthropod vectors such as ticks and biting flies. Infection can also be transmitted through contaminated water and food or through handling infected animals or cadavers [9]. *F. tularensis* also can be aerosolized and very quickly disseminated, which is the most dangerous way in context of terrorist attacks [34]. Nowadays we know certain genes utilized by *F. tularensis* during infection within the host [43], but plenty of information about its pathogenesis and virulence remain unknown.

Thakran et al. in their studies identified a specific *Francisella* product capable of stimulating the toll-like receptors (TLR2/TLR1 heterodimer) [67]. These receptors are expressed on the host cell surface or in the endosomal compartments where they are able to detect products from bacteria, viruses, yeast and protozoans [62]. One of the proteins found was triacylated lipoprotein encoded by the gene *ftt1103* [67]. This protein FTT1103 shares some similarity to DsbA proteins so it should be found in periplasm [49]. FTT1103 contains -CXXC-domain [42] and also has a disulfide-oxidoreductase activity [60]. The deletion mutant for the gene *ftt1103* was attenuated in *in vivo* and *in vitro* models and provides a protection against infection caused by the fully virulent wild type strain. The mutant strain was found to be defective in intracellular growth and replication

in host cell/macrophages and in J774A.1 cells (mouse BALB/c monocyte macrophages) there was a decreased ability to escape the phagosome. Therefore approximately 25% of *ftt1103* mutant appeared to be able to escape to the cytoplasm, which can facilitate a better antigen presentation and a more intense adaptive immunity response. These studies were conducted with *F. tularensis* subsp. *tularensis* strain Schu S4 [49] and with subsp. *holarctica* strain FSC155 and strain FSC200 [60]. Effect of protein FTT1103 seems to be indirect through its substrates. Searching for putative substrates of FTT1103 has recently been conducted. As a putative substrates of protein FTT1103 several proteins including five hypothetical proteins, the serine-type D-alanyl-D-alanine carboxypeptidase and chitinase family 18 protein were found. These proteins were determined in studies which used comparative proteome analysis of enriched membrane protein fractions isolated from parental strain FSC200 and strain with deletion in *ftt1103* gene [60]. The hypothetical proteins do not show any homology to known bacterial proteins found in accessible databases so it is difficult to predict their function. D-alanyl-D-alanine carboxypeptidase family includes bacterial enzymes bound in membrane. These enzymes catalyzed final reactions in biosynthesis of bacterial surface and are inhibited by beta-lactame antibiotics [48]. This type of serine carboxypeptidase was found to be *Brucella abortus* virulence factor [30]. Chitinases play an important role for vector-host transmission of arthropod-borne parasites [71] and it might play a role in *F. tularensis* pathogenesis and virulence because *F. tularensis* is an arthropod-borne bacterium [45].

Other Dsb-like protein was identified to be involved in *F. tularensis* virulence [63]. The *F. tularensis* strain Schu S4 protein encoded by *ftt0107c* shares about 55% similarity to DsbB proteins. DsbB-like activity of protein FTT0107c was confirmed by complementation of *E. coli* *dsbB* mutant phenotype [51]. Previously it was described that Schu S4 transposon insertion mutant in *ftt0107c* is defective in intracellular survival in the hepatic cell line HepG2 [50]. Another effect of mutation of *ftt0107c* gene was a high attenuation of mutant by intranasal route of inoculation in a mice model of infection. It means that protein FTT0107c is required for *in vivo* virulence. Therefore intranasal immunization with *ftt0107c* mutant does not protect C57BL/6 mice against intranasal wild-

type challenge [51], which is in contrast with results obtained in a study provided by Tempel *et al.* with *Francisella novicida* [64]. And finally, the locus *ftt0107c* is transcribed in an operon with genes encoding a RND-type efflux pump, but deletion of the genes which encode this efflux pump did not affect intracellular growth and *in vivo* virulence [49].

CONCLUSION

It has been known for years that formation of disulfide bonds is essential for correct function of many proteins and especially for proteins occurring

in membrane or for proteins determined for secretion. The *E. coli* has the best explored disulfide formation mechanism and that is why it is usually used as a model organism. But with onward research it is clear that the disulfide formation is not identical for all bacteria. It seems that this part of bacterial equipment varies significantly.

One of the reasons why the mechanism of disulfide bond formation is studied lies in its connection with pathogenesis and virulence of many known pathogens. The majority of important antigens contains disulfide bonds and their proper formation is necessary for protein function. On the other hand we know homologues for Dsb proteins but we are not familiarized with their substrates, potential virulence factors.

	DsbA	DsbB	DsbC	DsbG	DsbD	DsbE
<i>Neisseria meningitidis</i>	+	*	*	-	*	-
<i>Yersinia pestis</i>	+	n/a	n/a	n/a	n/a	n/a
<i>Francisella tularensis</i>	+	+	n/a	n/a	n/a	n/a

Table 1. Summary of presence and role of Dsb homologues in *N. meningitidis*, *Y. pestis*, *F. tularensis*

- * present in bacteria
- + present in bacteria and involved in its virulence/pathogenicity
- not present in bacteria
- n/a data not known

This review is focused on three pathogens, *N. meningitidis*, *Y. pestis* and *F. tularensis*, possessing Dsb system (Table 1). Infections caused by these human pathogens can be very serious and can lead to death. So far we do not know all about their virulence factors, but this knowledge is absolutely crucial for development of new antibiotic drugs or vaccines. The studies of Dsb-like proteins and their substrates have contributed to identification of important proteins and putative virulence factors in recent years. Some of the detected proteins play a role in intracellular survival for example, while others are involved in forming of protein complexes required for host-pathogen interaction and so on. Plenty of new information about bacterial virulence are still waiting to be discovered and a investigation of Dsb-like proteins and their substrates appears to be the step in the right direction.

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