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REDOX BIOLOGY

*The thiol group of the amino acid cysteine is found in vivo under various oxidation states. This versatility allows cysteine residues to be at the heart of numerous cellular processes by stabilizing protein structures or by fine-tuning protein activity. Some cysteine residues play an essential role in catalysis and in thiol-disulfide exchange reactions by cycling between the thiol state and the disulfide bonded state, such as in ribonucleotide reductase. Another class of cysteine residues is found in many extracellular proteins, which do not benefit from the surveillance of the cellular chaperones and other protection mechanisms. Here, cysteines form disulfide bonds that stabilize protein structure and protect the thiol group from over-oxidation. More recently, some cysteine residues have been shown to play a regulatory role and mediate cellular signaling. For instance, oxidation of cysteine residues to sulfenic acid (-SOH) turns off the activity of protein tyrosine phosphatases. It is now clear that complex enzymatic systems control the oxidation state of cysteine residues, either by reducing or oxidizing them depending on the identity of the protein target, the subcellular compartment and the redox properties of the environment. In our group, we study the mechanisms that control the oxidation state of cysteine residues, mostly using the bacterium *Escherichia coli* as experimental model. We have two main areas of investigation. First, we study the pathways of disulfide bond formation in the bacterial periplasm. Second, we study the mechanisms that regulate the formation of sulfenic acids by reactive cysteine residues.*

DISULFIDE BOND FORMATION IN THE PERIPLASM

In *E. coli*, disulfide bonds are introduced in the periplasm by the Dsb (Disulfide bond) protein family (1, 5).

The primary oxidant is the soluble protein DsbA. DsbA has a CXXC catalytic site motif present within a thioredoxin fold (4). The cysteine residues of this motif are found oxidized

in vivo. The disulfide bond of DsbA is very unstable and is rapidly transferred to secreted unfolded proteins. DsbA is then re-oxidized by the inner-membrane protein DsbB that transfers electrons from DsbA to the electron transport chain (Figure 1).

DsbA is a very powerful oxidant that apparently lacks proofreading activity. If the native disulfide bond pattern involves cysteine residues that are non-consecutive in the amino-acid sequence, DsbA can form incorrect

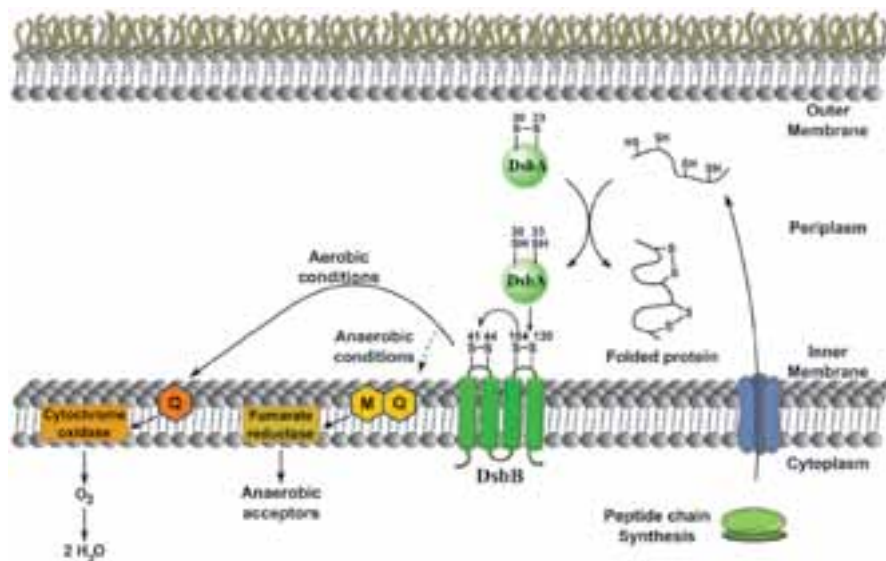


Fig. 1. Disulfide bond formation in the *E. coli* periplasm. Disulfides are introduced into folding proteins by DsbA. DsbA is re-oxidized by DsbB. Electrons flow from the reduced proteins to the CXXC motif of DsbA and then to DsbB. DsbB transfers electrons to the respiratory chain (adapted from (1)).

disulfides. The correction of these non-native disulfides is the role of a disulfide isomerization system. This system involves a soluble periplasmic protein disulfide isomerase, DsbC (Figure 2). Like DsbA, DsbC presents a thioredoxin fold and a CXXC catalytic site motif. In contrast to DsbA, the CXXC motif of DsbC is kept reduced in the periplasm. This allows DsbC to attack non-native disulfides, a necessary step in the isomerization reaction. The protein that keeps DsbC reduced is the membrane protein DsbD. DsbD transfers reducing equivalents from the cytoplasmic thioredoxin system to the periplasm via a succession of disulfide exchange reactions.

In our lab, we are studying the Dsb proteins of *E. coli*. Over the past few years, we have characterized the disulfide cascade within DsbD, we have identified four proline residues that play an important role in DsbD mechanism and we have identified the proteins that depend on DsbA and DsbC for folding. Moreover, we have engineered a new periplasmic oxidizing system (10) and have characterized the sensitivity of dsbC mutant to copper stress. We have also proposed a revised model for the pathways of disulfide bond formation in the periplasm by showing that DsbC may be

acting as a stand-alone protein folding catalyst that is able to cycle from the reduced to the oxidized state upon substrate oxidation and substrate reduction, respectively (9).

Recently, we have identified two new substrates of the protein disulfide isomerase DsbC. Interestingly, these two substrates, LptD and RcsF, are located in the outer membrane and play important roles in the maintain of envelope integrity. LptD is an essential β -barrel protein that inserts lipopolysaccharides in the outer membrane and whose folding depends on the periplasmic chaperone SurA. We found that deletion of dsbC in strains lacking surA increases *E. coli* sensitivity to hydrophobic antibiotics due to an impaired assembly of LptD. The definitive evidence that DsbC plays a role in the folding of LptD came from the identification of a LptD-DsbC mixed-disulfide complex (3). RcsF is an outer membrane lipoprotein sensor that detects defects in envelope integrity and transduces the signal to the Rcs phosphorelay. The Rcs phosphorelay is a signaling system that allows bacteria to react to a range of envelope stresses by modulating the expression of specific genes. The Rcs system is required for normal biofilm formation, contributes to antibiotic resistance and regu-

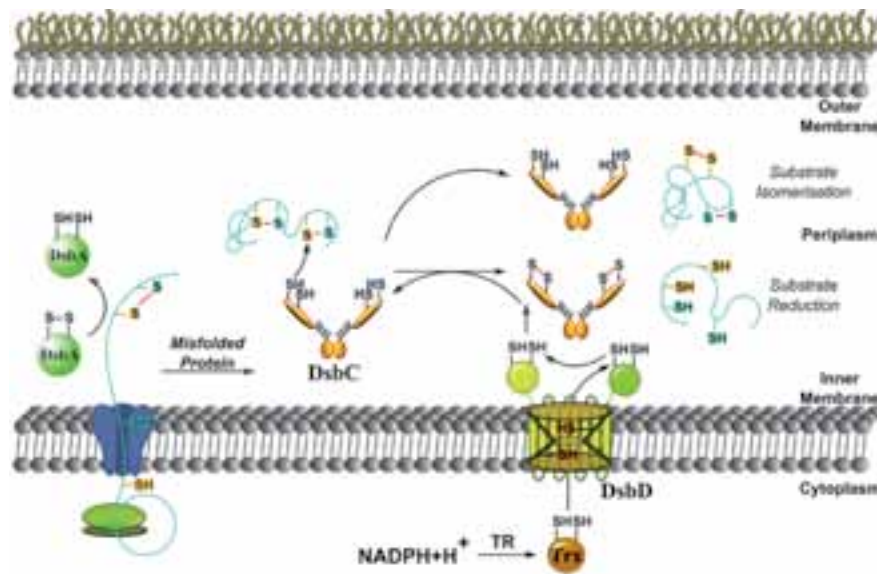


Fig. 2. Disulfide bond reduction/isomerization in the periplasm. DsbA can introduce non-native disulfides when they need to be formed between non-consecutive cysteines. Incorrect disulfides are either isomerized or reduced by DsbC. Oxidized DsbC is regenerated by the membrane protein DsbD that transfers electrons from the cytoplasmic thioredoxin reductase (TR) / thioredoxin (Trx) system to the periplasm (from reference 1).

lates virulence-associated structures involved in motility and host recognition. In collaboration with Prof. Jean-Paul Declercq (UCL), we solved the structure of RcsF (Figure 3). The fold of the protein is characterized by the presence of a central 4-stranded β sheet, which is conserved in several other proteins, including the copper-binding domain of the Amyloid Precursor Protein (APP). RcsF, which contains four conserved cysteine residues, present two non-consecutive disulfides. We showed that formation of the non-consecutive disulfides of RcsF depends on DsbC: we trapped RcsF in a mixed-disulfide complex with DsbC and we showed that deletion of *dsbC* prevents the activation of the Rcs phosphorelay by signals that function through RcsF (2).

We have also characterized the DsbA proteins present in the pathogenic bacteria *Neisseria meningitidis* (8), an invasive bacterial pathogen causing life-threatening infection in children worldwide. Host-pathogen interactions, and therefore virulence, depend on the correct folding of many surface-exposed proteins, which often requires disulfide bond formation.

CONTROL OF CYSTEINE SULFENYLATION IN THE PERIPLASM

The periplasm possesses another soluble Dsb protein, DsbG, but the function of this protein has remained elusive. We sought to find the function of DsbG in the periplasm by trapping it with its substrates. We produced a mutant protein in which the second cysteine residue of the CXXC motif is replaced by a serine. This version of the protein is still able to attack a substrate protein to form a mixed-disulfide intermediate. However, due to the absence of the second cysteine residue of the CXXC motif, this mixed disulfide bond cannot be resolved, allowing the purification of the complexes formed between DsbG and its substrates. We identified three periplasmic proteins, in complex with DsbG. The interaction between DsbG and those proteins was confirmed *in vitro* and *in vivo* (6).

The three periplasmic proteins (YbiS, ErfK and YnhG) are homologous proteins and belong to the same family of L,-D transpeptidases. Unexpectedly, all three enzymes contain only a single cysteine residue. An intriguing

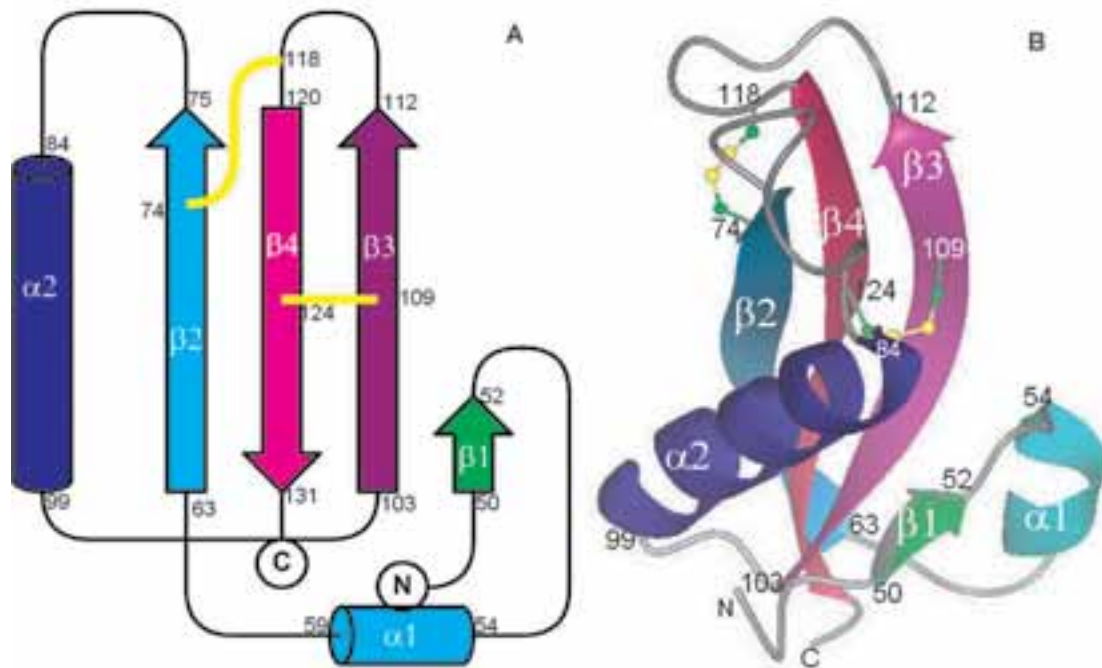


Fig. 3. Overall fold of the RcsF molecule. The protein is colored from green (N-terminal) to pink (C-terminal). A. Topological diagram on which the disulfide bonds are represented by a yellow line. The limits of the secondary structure elements are indicated. B. Ribbon diagram in a more or less similar orientation. The side chains of the cysteine residues taking part in the disulfide bonds are drawn as balls and sticks (from ref. 2).

question pertained therefore to the nature of the oxidation that affects the single cysteine residue of DsbG substrates. We considered the possibility that the cysteine residue might be oxidized to a sulfenic acid (Cys-SOH) by biological oxidants present in the periplasm. Sulfenic acids are highly reactive groups that tend to either rapidly react with other cysteine residues present in the vicinity to form a disulfide bond or to be further oxidized by reactive oxygen species (ROS) to irreversible sulfinic or sulfonic acids.

To test whether the cysteine residue of one of those proteins, YbiS, is indeed able to form a stable sulfenic acid, we used the dimedone-based DAz-1 probe (in collaboration with K. Carroll's lab, The Scripps Institute), which is chemically selective for sulfenic acids. We found that the cysteine residue of YbiS can form a sulfenic acid *in vitro* and *in vivo* and that the reduction of this cysteine depends on the presence of DsbG. In the course of our

experiments, we observed that, in addition to YbiS, several other periplasmic proteins were also labeled by the probe and that the level of sulfenylation in this compartment is controlled by DsbG and DsbC. We are currently working on the identification of the proteins that were labeled by the DAz probe. On the basis of these results, we proposed the following model. In the oxidizing periplasm, most proteins contain an even number of cysteine residues. These residues form disulfide bonds and are therefore protected from further cysteine oxidation. However, there is a significant number of proteins that contain a single cysteine residue. Because they are not involved in disulfide bonds, these cysteines are vulnerable to oxidation and form sulfenic acids which are susceptible to reaction with small molecule thiols present in the periplasm to form mixed disulfides or to further oxidation to sulfinic and sulfonic acids. DsbG appears to be a key player in a reducing system that protects those single cysteine residues from oxidation. DsbC

could serve as a backup for DsbG and could even have its own subset of favorite sulfenic acid modified substrates to reduce. Both DsbC and DsbG are kept reduced in the periplasm by DsbD, which transfers reducing equivalents from the thioredoxin system across the inner membrane. Thus, the electron flux originating from the cytoplasmic pool of NADPH provides the reducing equivalents required for both the correction of incorrect disulfides and the rescue of sulfenylated orphan cysteines.

Proteins from the thioredoxin superfamily are very widespread and have been identified in the majority of the genomes sequenced so far, making it tempting to speculate that some of these thioredoxin superfamily members, particularly those that are closely related to DsbC and DsbG, play similar roles in controlling the global sulfenic acid content of eukaryotic cellular compartments (6).

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