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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 *Escherichia coli*, disulfide bonds are introduced in the periplasm by the Dsb (Disulfide bond) protein family (1, 9).

The primary oxidant is the soluble protein DsbA. DsbA has a CXXC catalytic site motif present within a thioredoxin fold. 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

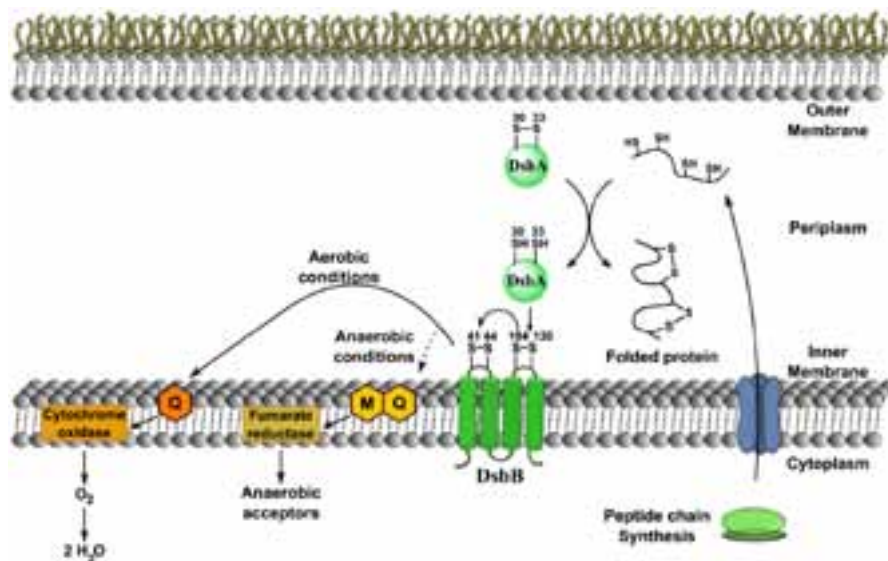


Figure 1. Disulfide bond formation in the *E. coli* periplasm. Disulfides are introduced into folding proteins by DsbA. DsbA is re-oxidized by DsbB. Electrons flow from the reduced proteins to the CXXC motif of DsbA and then to DsbB. DsbB transfers electrons to the respiratory chain (adapted from (1)).

residues that are non-consecutive in the amino-acid sequence, DsbA can form incorrect 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. Recently, we have proposed a revised model for the pa-

thways 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 (7).

We have also characterized the DsbA proteins present in the pathogenic bacteria *Neisseria meningitidis*, 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. Uniquely among bacteria, *N. meningitidis* possesses three genes encoding active DsbAs: NmDsbA1, NmDsbA2 and NmDsbA3. In collaboration with Laurence Serre and Céline Lafaye (Grenoble, France), we have characterized the neisserial enzymes biochemically and structurally. We found that the most striking feature shared by all three is their exceptional oxidizing power. With a redox potential of -80 mV, they are by far the most oxidizing thioredoxin-like enzymes known to date (6).

One of our long-term goal is to understand how disulfide bond formation is coordi-

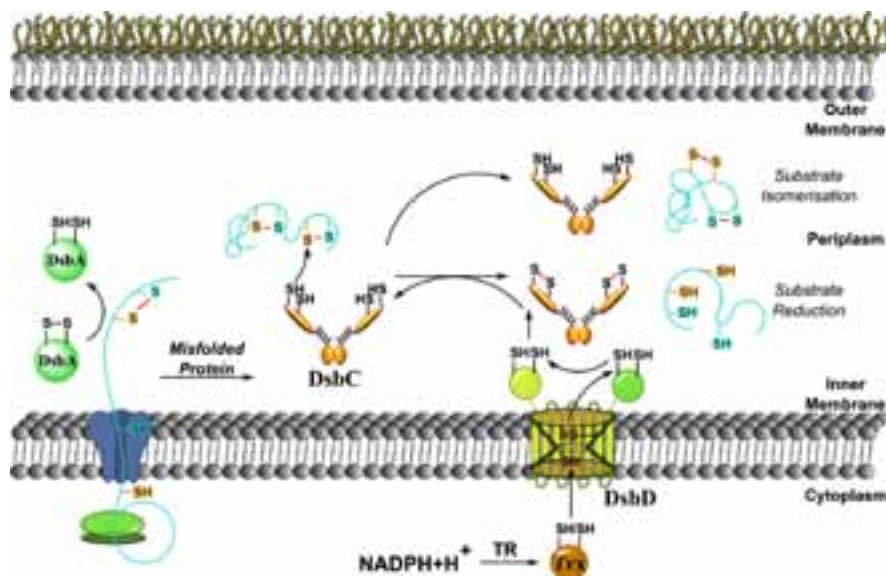


Figure 2. Disulfide bond reduction/isomerization in the periplasm. DsbA can introduce non-native disulfides when they need to be formed between non-consecutive cysteines. Incorrect disulfides are either isomerized or reduced by DsbC. Oxidized DsbC is regenerated by the membrane protein DsbD that transfers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

nated with the protein secretion and protein folding processes. In particular, we would like to understand how the Dsb proteins cooperate with the chaperones present in the periplasm. However, our understanding of the global folding process is hampered by the poor characterization of the roles of the periplasmic chaperones. Therefore, we have set out to clearly determine the specific role of the periplasmic chaperones involved in envelope biogenesis. We have developed a proteomic technique based on 2D-LC-MS/MS to better characterize the various periplasmic chaperones (2). In particular, we have characterized the function of the *E. coli* periplasmic chaperone SurA. SurA had been proposed to escort β -barrel proteins en route to the OM. However, our understanding of SurA's function was based on studies performed on a small number of OM proteins and its role on the global OM proteome had never been determined. We compared the relative abundance of 64 OM proteins, including 23 β -barrel proteins, in wild-type and *surA* strains. We found that the loss of *SurA* affects the abundance of 8 β -barrel proteins. Using qPCR, we showed that for 6 of them, the decreased protein abundance could be attributed,

at least in part, to decreased mRNA levels in the *surA* strain. In the case of LptD, an essential protein involved in OM biogenesis, our data support a role for SurA in the assembly of this protein and suggest that LptD is a true SurA substrate. Based on our results, we propose a revised model in which only a subset of OM proteins depends on SurA for proper folding and insertion in the OM (5). We are currently using our powerful proteomic technique to characterize other periplasmic chaperones.

CONTROL OF CYSTEINE SULFENYLATION IN THE PERIPLASM

The periplasm possesses another soluble Dsb protein, DsbG, but the function of this protein has remained elusive (1). 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* (3).

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 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, University of Michigan), 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. On the basis of these results, we proposed the following model (3). 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. Be-

cause 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 (Fig. 3).

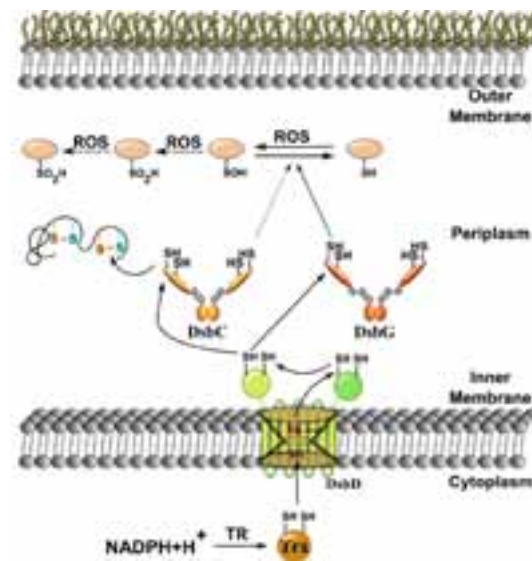


Figure 3. Rescue of sulfenylated cysteine residues in the periplasm. Proteins with single cysteine residues are easy prey for reactive oxygen species present in the periplasm. They form sulfenic acids (-SOH) which are susceptible to irreversible oxidation to sulfinic (-SO₂H) and sulfonic acids (-SO₃H). DsbG rescues sulfenylated cysteine residues. DsbC could serve as a backup for DsbG. Both DsbC and DsbG are kept reduced by DsbD. (Adapted from (1)).

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 (3).

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