Flipping the Sidedness of Disulfide Bond Formation

Dirk Jan Slotboom

University of Groningen, Groningen Biomolecular Sciences and Biotechnology Institute and Zernike Institute for advanced Materials, Groningen, The Netherlands

Correspondence to Dirk Jan Slotboom: d.j.slotboom@rug.nl
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Many Bacteria and Archaea catalyze the formation of structural disulfide bonds in exported proteins. In Escherichia coli, the periplasmic protein DsbA is the catalyst required for the oxidation of cysteine pairs in the target proteins (Fig. 1a) [1,2]. DsbA itself contains a disulfide bond in its active site, which becomes reduced when a disulfide bond in the exported protein is formed. The active-site cysteines of DsbA are subsequently re-oxidized by an integral membrane protein. Two types of such membrane proteins are known: DsbB, which is found in many prokaryotes including E. coli, and VKOR, present in some Bacteria and Archaea [3–5]. These membrane proteins transfer the electrons from DsbA to the membrane-bound electron carrier ubiquinone or menaquinone. The reduced quinones subsequently enter the electron transport chain, and a terminal electron acceptor such as molecular oxygen eventually takes up the electrons (Fig. 1a) [6,7].

Crystal structures of DsbB from E. coli and of VKOR from a cyanobacterium revealed a similar architecture, with a core of four transmembrane segments [8,9]. The hydrophobic transmembrane helices anchor the proteins in the lipid bilayer, with the hydrophilic loops and termini protruding into the aqueous environment on either side of the membrane. The orientation of DsbB and VKOR in the membrane follows the well-known positive-inside rule that determines which loops of a membrane protein are located on the cytoplasmic side of the membrane and which parts protrude into the periplasm [10,11]. Cytoplasmic loops are richer in lysines and arginines than the periplasmic loops. In both DsbB and VKOR, the membrane orientation matches the sidedness of the function, as the active sites are located on the periplasmic side of the membrane where disulfide bond formation takes place and where electrons from periplasmic DsbA are taken up (Fig. 1a).

Some (hyperthermophilic) Bacteria and Archaea can also form structural disulfide bonds in the cytoplasm [12], but the catalytic mechanism by which cytoplasmic disulfide bonds are formed in these organisms is not known. Here, Hatahet and Ruddock elegantly explored a mechanism of the disulfide bond formation in the cytoplasm [13]. They engineered DsbB from E. coli so that its orientation in the membrane was flipped (Fig. 1b). To do so, they used engineering principles that had been used for topology inversions in other membrane proteins in the past [14]. The topology inversion of DsbB required the redistribution of lysines and arginines over the loops, in order to obey the positive-inside rule for the opposite orientation, as well as the addition of an extra N-terminal transmembrane helix to target the DsbB N-terminus to the periplasm. E. coli cells producing the engineered DsbB were no longer able to form disulfides in the periplasm, because the active site of DsbB was now located on the cytoplasmic side of the membrane, where it could not take up the electrons from periplasmic DsbA (Fig. 1b). However, quinone-dependent disulfide-forming activity was restored when the E. coli cells also produced an engineered version of DsbA that was located in the cytoplasm rather than the periplasm (by removing the targeting sequence). Excitingly, disulfide bond formation was now catalyzed in the cytoplasm of E. coli (Fig. 1c). The engineering of just two proteins had altered the sidedness of disulfide bond formation. The reason why this experiment worked so well is that quinone, once reduced to quinol and dissociated from the DsbB protein, can spontaneously reorient in the membrane, so that the electron transfer reactions downstream of DsbB do no longer “sense” the sidedness of DsbB.

Hatahet and Ruddock then reasoned that topology inversions like the one they engineered in DsbB...
might have occurred naturally in those organisms that can make intracellular disulfide bonds [12]. In the protein databases, they searched for DsbB or VKOR homologues with inverted topology, as predicted by the positive-inside rule. In five hyperthermophilic Archaea, they indeed found a VKOR homologue predicted to have inverted topology, suggesting a pathway for cytoplasmic disulfide bond formation. Each of these organisms additionally had a second VKOR homologue with standard orientation, indicating that also extracellular disulfide bond formation was catalyzed in these organisms. Hatahet and Ruddock expressed the two VKOR homologues from *Aeropyrum pernix* (VKORi and VKORo, with the active site predicted to be cytoplasmic or extracellular, respectively) in an *E. coli* strain devoid of endogenous DsbB. Fully consistent with the predictions of protein orientation, VKORo allowed periplasmic disulfide bond formation when periplasmic DsbA was present, and VKORi together with cytoplasmic DsbA catalyzed disulfide bond formation in the cytoplasm.

This work is interesting not only from the point of view of membrane topology engineering and evolution. It also shows that disulfide bonds can be formed in the reducing environment of the *E. coli* cytoplasm, using the exact same pathway that normally acts in the periplasm. The formation of cytoplasmic disulfides in *E. coli* required neither the disruption of endogenous disulfide-reducing pathways [15] nor the introduction of exogenous disulfide-bond-forming proteins [16]. Apparently, proteins with structural disulfides have (some) kinetic stability in the reducing *E. coli* cytoplasm, even though they are not stabilized thermodynamically. Whether the cytoplasm of the hyperthermophilic organisms with naturally occurring cytoplasmic disulfides is reducing or oxidizing remains to be found out.

**References**


