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Disulfide Formation in Gram Positive Bacteria: Structure of a Redox Catalyst

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Disulfide oxidoreductases are ubiquitous proteins in eukaryotes and prokaryotes. They catalyze the *in vivo* formation of disulfide bonds, which is critical for the stability and activity of many proteins. In *E. coli*, disulfide bond formation occurs in the periplasm and is primarily catalyzed through the actions of the highly oxidizing soluble protein DsbA [1]. Homologues of DsbA are found in many Gram-negative bacteria and it has been shown that this protein plays an essential role in the biogenesis of virulence factors and toxins secreted by numerous pathogenic organisms [2]. The process of oxidative protein folding in Gram-positive bacteria is poorly understood. These organisms do not have a periplasmic compartment, yet genomic analysis shows that they do encode DsbA like proteins. Moreover, secreted virulence factors produced by Gram-positive bacteria, such as the human pathogen *Staphylococcus aureus*, are known to contain disulfide bonds in their active forms but the mechanism by which these disulfide bonds are formed is unknown [3]. To elucidate the catalysis of disulfide bonds in Gram-positive bacteria we have undertaken a structural and biochemical analysis of *Staphylococcus aureus* DsbA (SaDsbA) [4]. We have determined the 1.8 Å resolution crystal structure of SaDsbA by MAD analysis using data measured at the Advanced Light Source Synchrotron in Berkeley. This represents the first Gram-positive DsbA to be structurally characterized. Like *E. coli* DsbA, the structure of SaDsbA incorporates a thioredoxin fold with an inserted 3-helix bundle domain. However, other embellishments to the classic DsbA structure of SaDsbA suggest that it has a more narrow substrate specificity than the *E. coli* protein. SaDsbA lacks the characteristic hydrophobic patches and grooves surrounding the active sites of Gram-negative *E. coli* and *V. cholerae* DsbAs, and instead has a loop covering the active site that is apparently conserved in other Gram-positive DsbAs. Our biochemical characterization of SaDsbA also suggests divergent activities; thus unlike *E. coli* DsbA, the SaDsbA is inactive in the classic insulin reduction assay.

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S. solfataricus DPS-like Protein Contains a Ferritin-Like Di-Iron Binding Site

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The superfamily of ferritin-like proteins has recently expanded to include a phylogenetically distinct subclass of proteins, termed ″DPS-like″ (DPSL). Despite their distinct genetic signatures, members of this subclass share considerable similarity to previously recognized DPS proteins. Like DPS, these proteins are expressed in response to oxidative stress, form dodecameric cage-like particles, preferentially utilize H_2O_2 in the controlled oxidation of Fe^{2+}, and possess a short N-terminal extension implicated in stabilizing cellular DNA. Given these extensive similarities, the functional properties responsible for preservation of these distinct protein signatures in the genomes of diverse prokaryotes has been unclear. Here we describe the first high-resolution structure of a DPSL protein, that from the hyperthermophilic archaeon *Sulfolobus solfataricus*. Although the overall fold of the polypeptide chain and the oligomeric state of this protein are indistinguishable from that of authentic DPS proteins, several important differences are observed. First, rather than a ferroxidase site at the subunit interface, as is observed in all other DPS proteins, the ferroxidase site in SsDPSL is buried within the four-helix bundle, similar to bacterioferritin. Second, the structure reveals a channel leading from the exterior surface of SsDPSL to the bacterioferritin-like di-iron binding site, possibly allowing divalent cations and/or H_2O_2 to access the active site. Third, a pair of cysteine residues unique to DPSL proteins is found adjacent to the di-iron binding site, they are juxtaposed between the exterior surface of the protein and the active site channel. These cysteine residues of this ″thioferritin″ motif may play a redox active role, possibly serving to recycle iron at the ferroxidase center.