

CRYSTAL STRUCTURE OF THE DSBB-DSBA COMPLEX REVEALS A MECHANISM OF DISULFIDE BOND GENERATION

Many secretory proteins undergo oxidative folding, in which they acquire intra- or intermolecular disulfide bonds. The periplasmic space of *E. coli* contains a series of Dsb enzymes, which catalyze introduction and isomerization of protein disulfide bonds (Fig. 1). DsbA is the primary disulfide bond donor having the Cys30-Cys33 disulfide at the active site. It is kept in the oxidized and active state by a cytoplasmic membrane protein, DsbB, which transmits electrons from DsbA to either ubiquinone (UQ) or menaquinone, the source of oxidizing power under aerobic or anaerobic conditions, respectively. DsbB has been predicted to have four transmembrane helices and two periplasmic loops. Each of the loops contains one pair of essential cysteines: Cys41-Cys44 and Cys104-Cys130. While the Cys104-Cys130 pair is involved directly in the disulfide exchange with DsbA, the Cys41-Cys44 pair is the target of oxidation by UQ.

TM4) are arranged into a four-helix bundle configuration. In addition to these TM helices, a short helix with horizontal axis exists in the second periplasmic loop of DsbB. DsbA contains a long and deep hydrophobic groove that presumably captures substrate proteins devoid of tight folding. In the structure of the DsbB-DsbA complex, a Pro100 to Phe106 portion of the second periplasmic loop of DsbB is accommodated in this groove running below the redox active Cys30 residue (Fig. 2(b)).

DsbB in isolation usually contains two intramolecular disulfide bonds, between Cys41 and Cys44 and between Cys104 and Cys130. Strikingly, the residue 130 in the crystal structure of the DsbB-DsbA complex was separated from Cys104 beyond the range (~2 Å) of disulfide bond formation (see Fig. 2(a)). This separation is ascribable to the capture of the Cys104-containing DsbB segment by the deep groove of DsbA. We envisage that such DsbA-induced cysteine relocation should prevent the reverse reaction caused by the backward attack by Cys130 against the Cys30 (DsbA)-Cys104 (DsbB) intermolecular disulfide bond. Moreover, Cys130 has proved to reside very close to the Cys41-Cys44 pair, which must be advantageous for the physiological electron flow from the Cys104-Cys130 pair to the Cys41-Cys44 pair. Thus, we propose that DsbB is designed elegantly to undergo an induced-fit and to gain the ability to oxidize DsbA effectively and

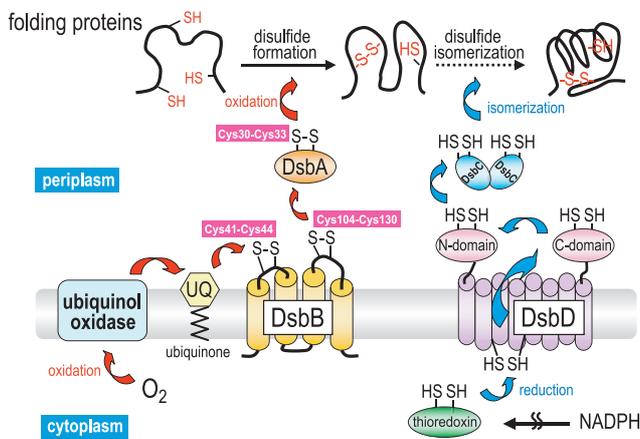


Fig. 1. Dsb enzymes responsible for protein disulfide bond formation in *E. coli*.

To achieve the deep understanding of this disulfide-introducing oxidative system, we determined the crystal structure of the disulfide-linked DsbB-DsbA complex having endogenous UQ, using beamline BL44XU [1]. The 3.7 Å resolution structure was complemented with engineered selenomethionine (SeMet) signals to enable structural refinement and consequent illumination of the reaction mechanisms (see [1] for more details). DsbB indeed contains four TM helices with both termini orienting to the cytoplasm (Fig. 2(a)). The four TM segments (TM1-

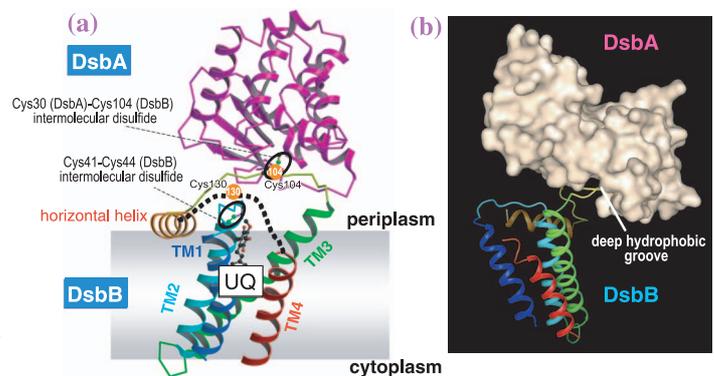


Fig. 2. (a) Overall structure of the DsbB(UQ8)-DsbA complex. The segment that connects the horizontal helix and TM4 lacks any electron density and is shown by dotted curve. (b) Another view of (a) rotated by 90° around a vertical axis.

exclusively even without the aid of UQ [2].

The UQ-binding site on DsbB has not unequivocally been determined in the past. Our crystallographic data indicate the presence of a prominent area of electron density near the N-terminal end of TM2, whose dimension fits that of the quinone ring (Fig. 3(a)). Although the electron density of the isoprenoid chain of UQ was invisible, the disk-like electron density could represent the head group of endogenous UQ8. The difference-Fourier map calculated from the UQ8-bound and the UQ-free crystals demonstrated a strong UQ-specific peak at the position that coincided with the electron-dense area addressed above (Fig. 3(b)). The quinone-binding site we specified here is consistent with the formation of the Cys44-UQ charge transfer complex and its enhancement by Arg48 [3,4], a residue of an implicated quinone-binding role. The area of DsbB having UQ lined up with Cys41, Cys44 and Arg48 can be regarded as the reaction center, where disulfide bonds are generated *de novo* along the chemical scheme shown in Fig. 3(c) [4].

In addition to the disulfide-introducing oxidative pathway, *E. coli* has a disulfide-isomerizing reductive pathway composed of DsbC, DsbD and cytoplasmic thioredoxin (Fig. 1). While DsbC, a disulfide isomerase, resides in the same cellular compartment as DsbA and possesses a thioredoxin fold similar to that of DsbA, it is kept in the reduced state by the action of DsbD. DsbC must not be oxidized by DsbB

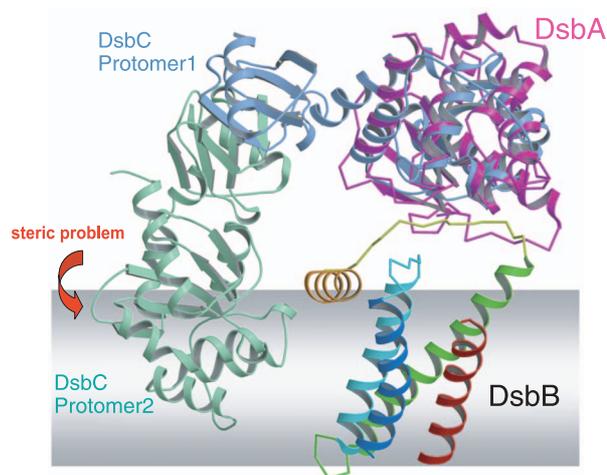


Fig. 4. A hypothetical DsbB-DsbC complex model.

to avoid futile cycles of electron transfer from DsbD to DsbB via DsbC. Unlike DsbA, DsbC exists as a homo-dimer and superimposition of one of its thioredoxin domains on the DsbB-DsbA complex in a way to minimize RMSD resulted in a clash of the other protomer onto the membrane surface (Fig. 4). Thus, DsbC would have a serious steric problem if it were to bind DsbB. This explanation is supported experimentally by the observation that a monomerized mutant of DsbC can function as a DsbB-dependent oxidase in place of DsbA [5]. Our structure provides a basis for the prevention of the crosstalk between the DsbA-DsbB oxidative and the DsbC-DsbD reductive pathways.

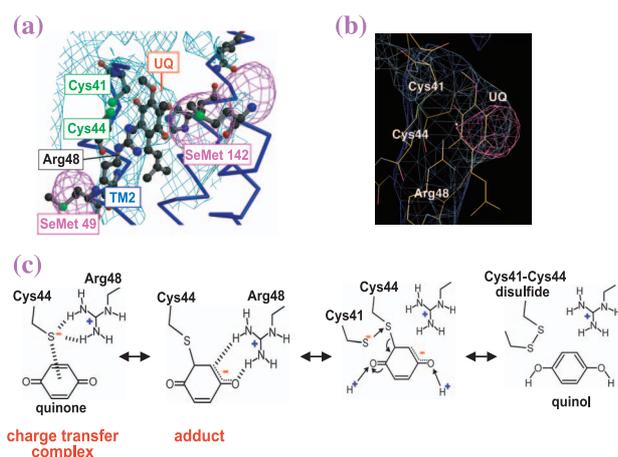


Fig. 3. (a) A UQ-binding site on DsbB. (b) Difference-Fourier map constructed from UQ8-bound and UQ-free crystals. Electron density that is assigned as representing the UQ8 ring is shown in magenta. (c) A chemical scheme for the generation of a disulfide bond conducted by DsbB and UQ.

Kenji Inaba^{a,*} and Koreaki Ito^{b,c}

^a Medical Institute of Bioregulation, Kyushu University

^b Institute for Virus Research, Kyoto University

^c CREST, JST

*E-mail: inaba-k@bioreg.kyushu-u.ac.jp

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