Journal of
Synchrotron Radiation

ISSN 0909-0495

Received 13 July 2007
Accepted 3 December 2007

Protein disulfide bond generation in *Escherichia coli* DsbB–DsbA

Kenji Inaba

Medical Institute of Bioregulation, Kyushu University, Fukuoka 812-8582, Japan.
E-mail: inaba-k@bioreg.kyushu-u.ac.jp

Protein disulfide bond formation is catalyzed by a series of Dsb enzymes present in the periplasm of *Escherichia coli*. The crystal structure of the DsbB–DsbA–ubiquinone ternary complex provided important insights into mechanisms of the *de novo* disulfide bond generation cooperated by DsbB and ubiquinone and of the disulfide bond shuttle from DsbB to DsbA. The structural basis for prevention of the crosstalk between the DsbA–DsbB oxidative and the DsbC–DsbD reductive pathways has also been proposed.

Keywords: protein disulfide bond generation; *Escherichia coli*.

Many secretory proteins undergo oxidative folding, in which they acquire intra- or intermolecular disulfide bonds. The periplasmic space of *Escherichia 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.

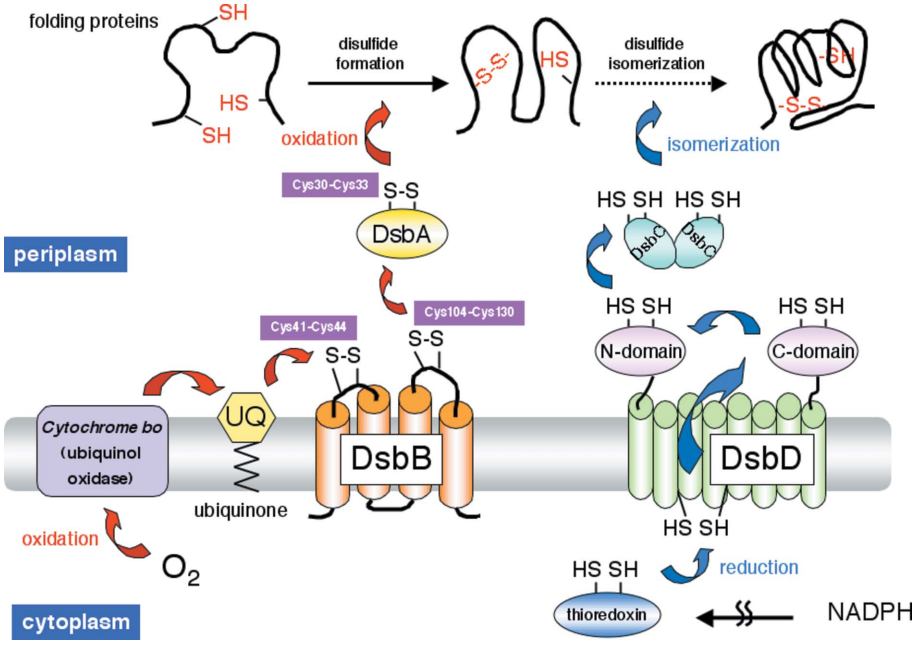

Figure 1
Cellular system responsible for protein disulfide bond formation in *E. coli*.

To achieve a deeper understanding of this disulfide-introducing oxidative system, we determined the crystal structure of the disulfide-linked DsbB–DsbA complex having endogenous UQ (Inaba, Murakami *et al.*, 2006). 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 Inaba, Murakami *et al.* (2006) for more details]. DsbB indeed contains four TM helices with both termini orienting to the cytoplasm (Fig. 2*a*). The four TM segments (TM1–TM4) are arranged into a four-helix bundle configuration. In addition to these TM helices, a short helix with a 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–Phe106 portion of the second periplasmic loop of DsbB is accommodated in this groove, running below the redox active Cys30 residue of DsbA (Fig. 2*b*).

DsbB in isolation usually contains two intramolecular disulfide bonds, between Cys41 and Cys44 and between Cys104 and Cys130. Strikingly, residue 130 in the crystal structure of the DsbB–DsbA complex was separated from Cys104 beyond the range (~2 Å) of disulfide bond formation (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

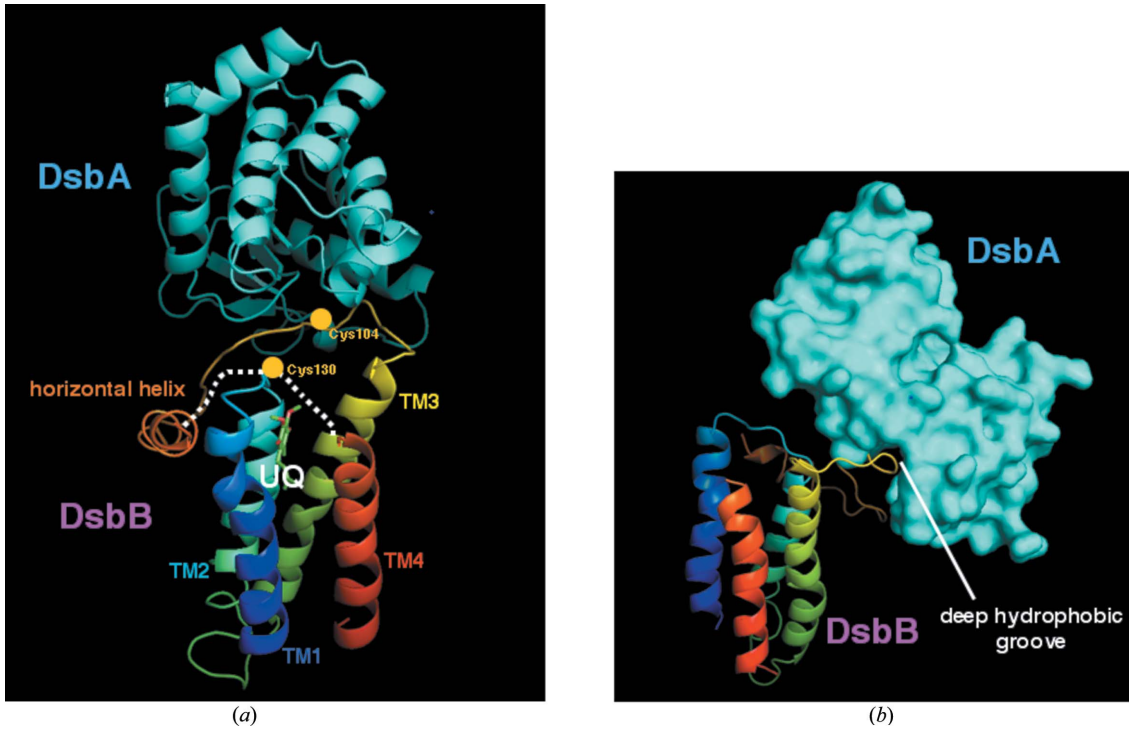

Figure 2
(*a*) Overall structure of the DsbB(UQ)–DsbA complex. (*b*) Another view of (*a*), rotated by 90° around a vertical axis, in which DsbA is shown in a surface model.

gain the ability to oxidize DsbA effectively and exclusively even without the aid of UQ (Inaba & Ito, 2002; Inaba *et al.*, 2005).

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 disc-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 demonstrates a strong UQ-specific peak at the position that coincides 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 (Inaba *et al.*, 2004; Inaba, Takahashi *et al.*, 2006), a residue of an implicated quinone-binding role. The area of DsbB having UQ aligned 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*) (Inaba, Takahashi *et al.*, 2006).

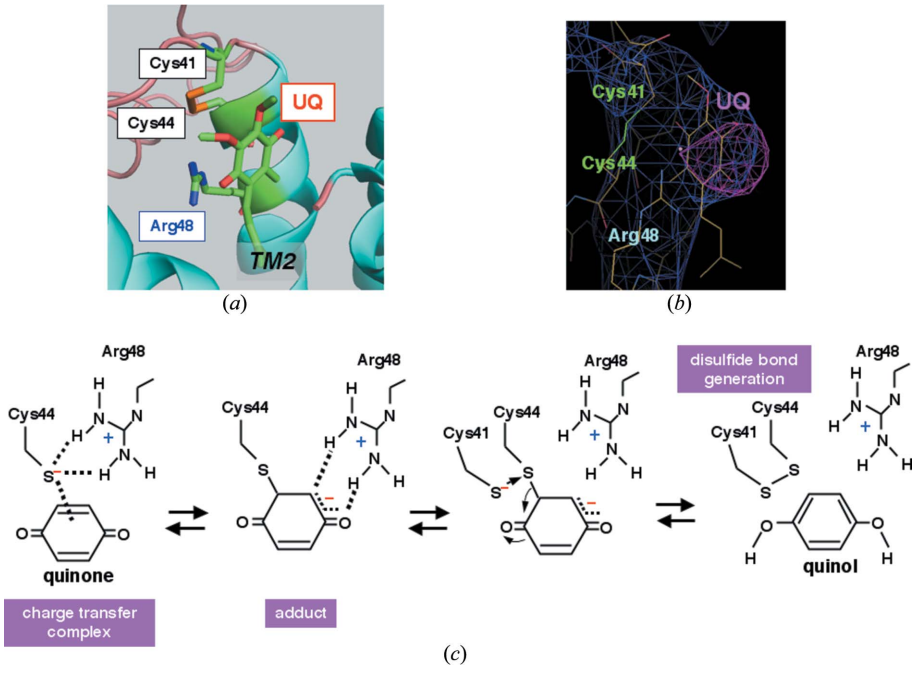

Figure 3
(*a*) UQ-binding site on DsbB. (*b*) Difference Fourier map constructed from UQ-bound and UQ-free forms of crystals. (*c*) Chemical scheme for disulfide bond generation cooperated by DsbB and UQ.

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 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 the r.m.s.d. 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

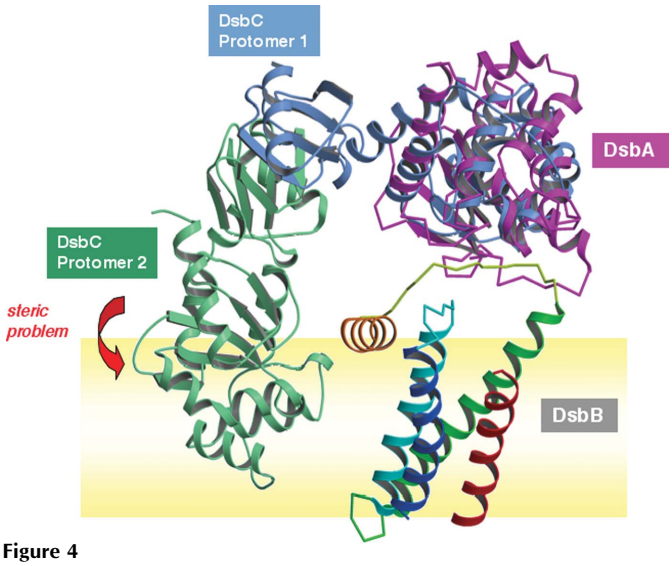

Figure 4
A hypothetical DsbB–DsbC complex model, in which the thioredoxin domain of a DsbC protomer is superimposed on that of DsbA such that the r.m.s.d. between these two domains is minimized.

the observation that a monomerized mutant of DsbC can function as a DsbB-dependent oxidase in place of DsbA (Bader *et al.*, 2001). Our structure provides a basis for the prevention of the crosstalk between the DsbA–DsbB oxidative and the DsbC–DsbD reductive pathways.

In conclusion, the crystal structure of the DsbA–DsbB–UQ ternary complex revealed how protein disulfide bonds are generated *de novo* by the cooperation of DsbB and UQ and how the disulfide bonds generated here are relayed selectively to DsbA, a primary disulfide-introducing catalyst of downstream substrate proteins.

I thank especially Dr Koreaki Ito for his continuous contributions and support in this work. I am also grateful to Drs Satoshi Murakami, Mamoru Suzuki, Atsushi Nakagawa, Kengo Okada, Eiki Yamashita and Tomitake Tsukihara for their great help in the crystallographic studies.

References

Bader, M. W., Hiniker, A., Regeimbal, J., Goldstone, D., Haebel, P. W., Riemer, J., Metcalf, P. & Bardwell, J. C. A. (2001). *EMBO J.* **20**, 1555–1562.
Inaba, K. & Ito, K. (2002). *EMBO J.* **21**, 2646–2654.
Inaba, K., Murakami, S., Suzuki, M., Nakagawa, A., Yamashita, E., Okada, K. & Ito, K. (2006). *Cell*, **127**, 789–801.
Inaba, K., Takahashi, Y.-H., Fujieda, N., Kano, K., Miyoshi, H. & Ito, K. (2004). *J. Biol. Chem.* **279**, 6761–6768.
Inaba, K., Takahashi, Y.-H. & Ito, K. (2005). *J. Biol. Chem.* **280**, 33035–33044.
Inaba, K., Takahashi, Y.-H., Ito, K. & Hayashi, S. (2006). *Proc. Natl Acad. Sci. USA*, **103**, 287–292.