Engineered DsbC chimeras catalyze both protein oxidation and disulfide-bond isomerization in *Escherichia coli*: Reconciling two competing pathways

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In the *Escherichia coli* periplasm, the formation of protein disulfide bonds is catalyzed by DsbA and DsbC. DsbA is a monomer that is maintained in a fully oxidized state by the membrane enzyme DsbB, whereas DsbC is a dimer that is kept reduced by a second membrane protein, DsbD. Although the catalytic regions of DsbA and DsbC are composed of structurally homologous thioredoxin motif domains, DsbA serves only as an oxidase in vivo, whereas DsbC catalyzes disulfide reduction and isomerization and also exhibits significant chaperone activity. To reconcile the distinct catalytic activities of DsbC and DsbA, we constructed a series of chimeras comprising of the dimerization domain of DsbC, with or without the adjacent α-helical linker region, fused either to the first, second, third, or fifth residue of intact DsbA or to thioredoxin. The chimeras fully substituted for DsbC in disulfide-bond rearrangement and also were able to restore protein oxidation in a dsbA background. Remarkably, the chimeras could serve as a single catalyst for both disulfide-bond formation and rearrangement, thus reconciling the kinetically competing DsbB–DsbA and DsbD–DsbC pathways. This property appeared to depend on the orientation of the DsbA active-site cysteines with respect to the DsbC dimerization domain. In vitro, the chimeras had high chaperone activity and significant reductase activity but only 15–22% of the disulfide-isomerization activity of DsbC, suggesting that rearrangement of nonnative disulfides may be mediated primarily by cycles of random reduction and reoxidation.

Disulfide bonds play a key role in the folding process of many secretory and membrane proteins. A series of cysteine thiolsulfide oxidoreductases have evolved in both eukaryotic and prokaryotic systems to catalyze this critical cellular process. In particular, the Dsb family of the *Escherichia coli* periplasm consists of two distinct pathways: DsbA–DsbB and DsbC/DsbG–DsbD. These pathways are involved in the formation of disulfides and the rearrangement of incorrectly formed bonds, respectively (1, 2). The extreme oxidizing nature of DsbA mediates rapid oxidation of substrate cysteines and, therefore, results in the formation of nonnative disulfides, which are in turn rearranged by DsbC and, to a lesser extent, DsbG. Despite the strong oxidizing environment of the periplasmic space, DsbC and DsbG have to be maintained in a reduced state to be able to catalyze the rearrangement of nonnative disulfide bonds (1, 2).

DsbA and DsbC are found in entirely oxidized and reduced states, respectively (3). DsbA is recycled by the membrane protein DsbB, which then passes its electrons to the quinones and the respiratory chain, whereas DsbC is maintained in the reduced state by another membrane protein, DsbD, by means of an electron-transfer relay that involves thioredoxin (TrxA) and TrxA reductase in the cytoplasm (4). Remarkably, the Dsb machinery has evolved to capitalize on a strong thiol oxidant (DsbA) and a strong thiol reductant (DsbC) that do not appear to exchange electrons with each other in the periplasmic space but instead act synergistically in oxidative protein folding. Studies have revealed that the transfer of electrons between the DsbA–DsbB and the DsbC/DsbG–DsbD pathways is strongly disfavored kinetically. Oxidation of DsbC by DsbA is very slow in vitro (5). Compelling evidence shows that the interaction of DsbB with DsbA is highly specific and favored kinetically over the oxidation of DsbC (6, 7). Also, Bader et al. (8) suggest that dimerization protects the DsbC active site from the interaction with DsbB, thus maintaining the segregation of the oxidative and reductive pathways. The recent characterization of the DsbC–DsbD complex crystal structure revealed that the dimerization domain of DsbC is critical for the interaction with DsbD and is, therefore, required for reduction to take place (9). Nonetheless, interactions between enzymes within the same pathway are favored strongly over nonphysiological disulfide-exchange reactions between the two pathways by kinetic differences of 103- to 105-fold (10).

DsbC exhibits significantly higher in vitro isomerase and reductase activity compared with DsbA. The catalytic domains of DsbC and DsbA show a considerable degree of structural homology, and they both contain a CXXC TrxA active-site motif for the catalysis of disulfide-exchange reactions. These similarities in the catalytic domain of the two proteins raise the question whether the disulfide-isomerization activity of DsbC stems simply from the dimerization of a TrxA fold active site. Here, we report the construction, biochemical characterization, and in vivo function of a series of DsbC–DsbA chimeras and a DsbC–TrxA chimera in which the catalytic domains are linked to the dimerization domain of DsbC. Although these chimeras exhibit poor isomerase activity in vitro, they are capable of catalyzing both protein oxidation and disulfide-bond rearrangement in the periplasm of *E. coli*.

**Materials and Methods**

**Strains and Plasmids.** The bacterial strains and plasmids used in this study are listed in Table 2, which is published as supporting information on the PNAS web site. The DsbC chimeras were constructed by overlap extension PCR using the primers listed in Table 2 and cloned into pBAD33 (11). All of the chimeras contain a C-terminal hexahistidine tag. For protein purification, the gene was amplified from an E. coli arabinose-inducible plasmid and transformed into E. coli BL21 cells.

**In Vivo Assays.** To determine alkaline phosphatase activity, overnight cultures were grown in low-phosphate minimal medium containing Mops salts, 0.2% glycerol, 0.2% casamino acids, and 0.5 µg/ml thiamine, supplemented with 50 µg/ml of kanamycin and 25 µg/ml of chloramphenicol, as needed. Cultures were diluted 1:100 in the same media, and arabinose at a final concentration of 0.2% (wt/vol) was added when the cell density reached OD600 = 0.4. The cells were collected 4 h later and mixed with a buffer containing 0.4 M iodacetamide and lysate buffer (B-PER, bacterial protein extraction reagent; Pierce) in a 1:2 ratio. The activity of alkaline phosphatase was determined as described (12).

For cell-motility assays, overnight cultures were grown in M9
chloramphenicol, as needed. When the cells reached late stationary
mM isopropyl
normalized 3-
[52x555]/H11032
[52x695]/H9262
the appearance of active RNase A is proportional to the oxidase
(18). The renaturation of reduced, denatured RNase A was mon-
the ratio of the initial slope of the turbidity curve to the lag time
of reduced insulin was also measured. The activity is expressed as
E. coli
Expression, Purification, and Biochemical Assays.
In Vivo Redox State. The in vivo redox states of the Dsb chimeras
determined by derivatization of free thiols by 4-acetamido-4’-
maleimidyl-stibene-2,2’-disulfonic acid (AMS; Molecular Probes)
in trichloroacetic acid-quenched samples, as described (14), fol-
by Western blotting using mouse anti-His tag polyclonal serum
in trichloroacetic acid-quenched samples, as described (14), fol-
Activity and the initial rate in the RNase A vs. time plot is used to
calculate the rate of disulfide isomerization as described in ref. 19.
Activities are expressed as micromolars of native RNase A formed
per minute per micromolars of enzyme.

The protection of citrate synthase (CS) from thermal inactivation
was monitored according to ref. 20. The rate of thermal inactivation
obtained with or without 4 μM chaperone was determined. Finally,
the aggregation of guanidine hydrochloride-denatured GAPDH
after dilution in the presence of chaperone ranging from 0- to
44.8-μM concentrations was studied according to Cai et al. (21).

Protein Structure Modeling. The secondary structure prediction was
performed by using PREDICTPROTEIN. Manual model building of
the chimeric proteins was done in XFIT (22), based on the infor-
mation obtained for the secondary structure of the region linking
the DsbC dimerization domain to DsbA.

Results
Construction of DsbC Chimeras. The dimerization domain of DsbC
comprises residues 1–59 and is joined to the C-terminal catalytic
domain of a 12 aa-long α-helix linker (amino acids 60–72). We
constructed a series of fusions encoding the dimerization domain
of DsbC with or without the α-helix (DsbCΔd–DsbCd and DsbCd,
respectively). DsbCd was fused to the first, second, or third residue of
mature DbsA [DbsA (1–189), DbsA (2–189), and DsbA (3–189),
respectively] (Fig. 1). DbsA (2–189) and DsbA (3–189) were
similarly fused to DsbCΔd. In addition, a fusion to the fifth residue
of the mature DsbA (5–189) was constructed. In DsbC, the active-site cysteine pairs within each catalytic domain are oriented
facing each other perpendicular to the axis of symmetry along the
dimerization domain. Molecular modeling indicates a similar ori-
entation of the active-site residues in DsbCΔd–DsbA (2–189) and
in DsbCΔd–DsbA (5–189) (see Fig. 5, which is published as
supporting information on the PNAS web site) but not for
DsbCΔd–DsbA (3–189), where the active site is predicted to be
tilted 170° relative to the long axis of symmetry of the molecule.
In other words, in DsbCΔd–DsbA (3–189), the active-site cysteines are

Facing away from each other (Fig. 1B). Finally, a gene fusion encoding a chimera, consisting of the DsbC dimerization domain followed by the linker α-helix (amino acids 60–72 in DsbC), and the N-terminal residues of the catalytic domain fused to the complete sequence of TrxA, was constructed also. The redox potential of TrxA fold proteins involved in disulfide transfer is modulated by the identity of the two amino acids within the C—X—X—C catalytic motif (23). In DsbCd–TrxA, the G—P dipeptide in the active site of TrxA was substituted with the G—H sequence found in the active site of protein disulfide isomerase (PDI). TrxA (CGHC) protein exhibits a higher redox potential ($E_0 = -235$ mV), making it more similar to PDI and conferring higher isomerase activity (24, 25).

These fusions were placed downstream from the arabinose promoter in the medium copy number plasmid pBAD33 (11). After induction of protein expression with arabinose, the wild-type DsbC and all of the DsbC–DsbA chimeras accumulated to nearly identical levels, as determined by Western blotting with a polyclonal antibody that recognizes the C-terminal His tag. In contrast, DsbCd–TrxA “PDI-like” was expressed at a 4- to 5-fold higher level (Fig. 2A).

**Disulfide-Bond Formation and Isomerization in Vivo.** In *E. coli*, the folding yield of eukaryotic proteins with multiple disulfide bonds is limited by the isomerization activity afforded by DsbC (1, 2, 26, 27). In particular, the yield of proteolytically active vtPA, containing a total of nine disulfide bonds, depends on the DsbC expression level (28). In cells containing pBADdsbC and grown with 2% (wt/vol) arabinose, active vtPA accumulates at a 25-fold higher level (13) than in the absence of overexpressed DsbC. In contrast, expression of DsbA from a similar pBAD vector in cells grown under identical conditions confers essentially no increase in the yield of vtPA relative to the control (26). However, expression of the DsbC–DsbA chimeras afforded vtPA yields comparable with those provided by pBADdsbC, irrespective of the nature of the fusion residue in DsbA or the presence or absence of the DsbC linker α-helix. DsbCd–N–TrxA also supported the folding of vtPA in a DsbD-dependent manner suggests that these chimeras function by facilitating disulfide-bond rearrangement.

DsbC cannot normally serve as an oxidant to complement the phenotypes of *dsbA* mutants, such as low PhoA activity or loss of cell motility. In contrast to DsbC, however, the DsbC–DsbA chimeras all were able to support protein oxidation to various degrees. In *E. coli* MC1000 *dsbA* grown in low-phosphate media, the PhoA activity is 30-fold lower than that in its isogenic parent. Coexpression of the DsbC–DsbA chimeras restored PhoA activity to 45–100% of the values obtained in the parental strain MC1000, whereas DsbCd–N–TrxA was a somewhat weaker oxidant (Fig. 2B). As expected, neither the DsbC–DsbA chimeras nor DsbCd–N–TrxA could restore PhoA activity in *dsbB* cells (Fig. 2B).

The size of the motility halo in *dsbA* cells plated on soft agar plates represents an additional, more stringent measure of the ability of proteins to catalyze periplasmic oxidation (43). MC1000 *dsbA* cells expressing DsbC from pBAD33 were completely non-motile, whereas the expression of the DsbC–DsbA chimeras restored cell motility to various degrees (Fig. 2C). Proteins containing the DsbC α-helix linker gave larger diameter motility halos compared with identical fusions lacking the 12-aa linker region [Fig. 2C; compare DdsbCd–DsbA (3–189) and DdsbCd–DsbA (5–189) with DdsbCd–DsbA (1–189) and DdsbCd–DsbA (3–189)]. In contrast, even though the DsbCd–N–TrxA fusion was capable of oxidizing PhoA partially, it could not restore cell motility, suggesting that this is a weaker catalyst of disulfide-folding than the chimera in terms of its activity or with respect to its substrate specificity.

The data described above reveal that the DsbC chimeras can catalyze disulfide-bond rearrangement in *dsbC* cells and, separately, protein oxidation in *dsbA* mutants. These findings raised the possibility that the chimeras may be able to satisfy the role of a protein thiol oxidant and a disulfide-isomerization catalyst in *dsbA* cells simultaneously. The folding of vtPA, in addition to requiring a high level of DsbC activity as discussed above, also critically depends on the presence of DsbA. For this reason, in strain SF100 *dsbA*, overexpression of DsbC results in background levels of active vtPA (Fig. 2A). In contrast, expression of the DsbC–DsbA chimeras afforded a high yield of active vtPA, with the notable exception of
the two fusions containing the DsbA (3–189) domain. Whereas fusions beginning with the first, second, or fifth residue of the DsbA domain were fully active in terms of their ability to support the formation of active vTPA, fusions beginning at the third amino acid in DsbA were completely inactive in this assay. Thus, DsbA (3–189) fusions exhibit the following properties: They (i) support the formation of active vTPA in wt cells; (ii) are capable of catalyzing protein oxidation in a dshb background; and nonetheless, (iii) fail to allow folding of vTPA in the dshb mutant. The surprising inability of the DsbA (3–189) fusions to support disulfide rearrangement in a dshb background is analyzed in some detail in Discussion.

In order for the DsbC chimeras to catalyze cysteine thiol oxidation and disulfide rearrangement simultaneously, they have to be maintained in the periplasm as a mixture of oxidized and reduced species. The in vivo redox state of the chimeras was determined by harvesting cells into trichloroacetic acid precipitation and by omitting AMS, respectively. (ox) standards obtained by incubating the cells with DTT before trichloroacetic acid precipitation and by omitting AMS, respectively.

**Biochemical Characterization.** Four DsbC–DsbA chimeras, DsbCdαN–TrxA and DsbC as a control were purified by immobilized metal ion affinity chromatography, and oligomerization status was analyzed by gel-filtration FPLC. All of the proteins eluted exclusively as dimers (see Fig. 7, which is published as supporting information on the PNAS web site).

The insulin reduction activity (30) of the DsbC–DsbA chimeras was 20–62% of the insulin reduction activity of DsbC (Table 1). DsbCdαN–TrxA exhibited a slightly higher reduceacta activity. For comparison, DsbA has only ~10% of the activity of DsbC. All of the chimeras exhibited low disulfide isomerase activity in the refolding of reduced RNase A. DsbCd–DsbA (1–189), DsbCdαN–DshbA (5–189), DsbCdαN–DshbA (3–189), and DsbCdαN–TrxA displayed 10–22% of the isomerase activity of DsbC, which was 8-fold less active than PDI. The low values of isomerase activity obtained were nonetheless higher (P < 0.05) relative to the background rate of RNase A refolding in the absence of catalyst or in the presence of DsbA.

The chaperone activity of the chimeras was evaluated based on the protection of CS from thermal inactivation and from the prevention of protein aggregation during the refolding of Gdn-HCl-denatured GAPDH (20). At a 2.7-fold stoichiometric excess, DsbCdαN–TrxA delayed the inactivation of CS to the same extent as DshbA. However, whereas DsbA did not have any effect on CS inactivation, dimerization by virtue of its fusion to DsbCd or to DsbCdαN gave rise to proteins that were 3-fold more efficient relative to DsbC (or 10-fold better, compared with DsbA) in this assay (Table 1). Fig. 4 shows the effect of protein activities on the suppression of GAPDH aggregation during refolding. Even when added at a 15-fold stoichiometric excess, TrxA had no effect on GAPDH aggregation, whereas a large excess of DsbA suppressed aggregation by ~20%. In contrast, fusion to the DsbC dimerization domain markedly enhanced the chaperone activity of TrxA and DsbA. All of the tested chimeras could suppress the aggregation of GAPDH to a significant extent. Collectively, the data presented in Table 1 and in Fig. 4 reveal that fusion of DsbA or TrxA to the dimerization domain gives rise to proteins with appreciable chaperone activity, the exact magnitude of which depends on the substrate and the assay conditions.

**Discussion**

Oxidative protein folding involves two complementary but also competing processes: cysteine thiol oxidation and isomerization...
of nonnative disulfide bonds. Throughout nature, enzymes that catalyze thiol-disulfide exchange reactions (with the exception of certain enzymes with narrow substrate specificity, such as DsbB) employ structurally homologous TrxA domains for catalysis (31). Relatively subtle changes, such as the presence of different amino acids within the dipeptide sequence in the TrxA CXXC active site, the insertion of α-helical domains within the TrxA fold, and fusion to additional domains (23, 32–34), have been used during evolution to modulate the function of thiol-cysteine oxidoreductases. DsbA and DsbC provide an illustration of how two TrxA fold enzymes have evolved to perform different reactions in the cell. DsbA is a powerful oxidant ($E_0 = -130$ mV), but it exhibits marginal reductase, chaperone, or disulfide isomerase activity (Table 1) (3, 30). DsbC has an active-site cysteine pair with a redox potential almost as low as that of DsbA (35–37). However, DsbC not only catalyzes disulfide-bond oxidation in vivo, but, in contrast to DsbA, it also displays disulfide isomerase, reductase, and chaperone activities (5, 35).

In principle at least, DsbC should be sufficient to catalyze both disulfide-bond formation and rearrangement in the cell without the need for a specialized oxidant. PDI, the eukaryotic analog of DsbC, catalyzes protein oxidation in the endoplasmic reticulum in an Ero1p-dependent process (38), and recent data suggest that it also serves as an isomerase in the ER (H.F.G., unpublished data). However, this is not the case with DsbC; even though it has high oxidation activity per se, in the periplasmic space it is maintained exclusively in the reduced state and, therefore, can only catalyze disulfide-bond rearrangement. The presence of the DsbD–DsbC and the DsbB–DsbA systems enables the coexistence of a fully reduced and a fully oxidized catalyst in close proximity to each other, without the establishment of an energy-consuming futile cycle that would be draining and ultimately detrimental to the cell (8). The kinetic isolation of the cysteine thiol oxidation and disulfide-rearrangement pathways in prokaryotes partially stems from the fact that DsbC is not readily oxidized by either DsbB or DsbA (5, 7). Bader et al. (8) presented genetic and biochemical evidence indicating that the dimeric nature of DsbC represents the main barrier to its oxidation by DsbB. Also, Rozhkova et al. have measured the kinetics of disulfide-exchange reactions among periplasmic components of the DsbA–DsbB and the DsbC–DsbD pathways and compared them with nonfunctional reactions between redox active sites of periplasmic oxidoreductases. This analysis further highlights how prevention of nonphysiological interactions between the proteins involved in disulfide-bond formation guarantees the separation of the oxidative and reductive pathways in the same cellular environment in vivo (10).

We now show that DsbC chimeras, which consist of the dimerization domain fused to a TrxA catalytic module in close analogy to the architecture of the authentic DsbC, become oxidized by DsbB and are able to substitute for the lack of DsbA. The ability of the chimeras to interact with DsbB was independent of the presence or absence of the α-helix linker joining the two domains. In vitro, gel-filtration analysis showed that the DsbC chimeras are present exclusively as dimers (see Fig. 7), which is a conclusion that is consistent with the recent finding of Zhao et al. (30). Although we cannot rule out the possibility that the DsbC chimeras exist in equilibrium with a very small amount of monomer that interacts with DsbB and serves as an oxidant in vivo, we think that the presence of such monomeric species is unlikely. Our findings suggest that dimerization alone cannot account for the kinetic isolation of DsbC from DsbB, which must be dictated by additional structural features that presumably modulate the accessibility of the active-site cysteines within the catalytic domain.

Although the six DsbC–DsbA chimeras and DsbCΔN–TrxA can be oxidized by DsbB, in the periplasm these proteins are maintained mostly in the reduced form by the action of DsbD. In vitro, DsbD can readily reduce TrxA and DsbB but not the monomeric DsbA (39). Recent evidence shows that DsbA is reduced by DsbB extremely slowly in vitro (10); it is likely that the reduction of the DsbC chimeras by DsbD is due to the increase in the effective concentration of DsbA active sites that results from dimerization or by more favorable steric interactions originated by the fusion of DsbA with the DsbC dimerization domain.

In the absence of a kinetic barrier between DsbD and DsbB, how does the cell avoid the establishment of a draining futile cycle that would require energy to maintain and would ultimately be detrimental (8)? Such a futile cycle would ultimately result in the consumption of NADPH by TrxB, leading to the transfer of electrons from the TrxA–DsbD relay to DsbB–DsbA and, ultimately, to the respiratory chain. The finding that the chimeras are predominantly reduced in vivo suggests that oxidation by DsbB must be relatively slow and kinetically disfavored, compared with the transfer of the active-site disulfide to substrate proteins or to DsbD. Therefore, it appears that only a small fraction of the DsbC–DsbA and DsbC–TrxA chimeras ends up being shuttled between DsbB and DsbD, and hence, the energy expenditure due to the establishment of a futile cycle may be minimal. Also, we note that, as has been observed in other studies, the establishment of an artificial futile cycle affects the yield on the carbon source but not necessarily the growth rate (40).

The folding of vtPA in the periplasm normally requires the action of DsbA and also a high level of DsbC activity. However, expression of most, but not all, of the chimeric DsbC enzymes in a dshB1 strain, afforded a high yield of vtPA, revealing that a single catalyst is perfectly capable of catalyzing both cysteine thiol oxidation and disulfide rearrangement in vivo. The genomes of several bacteria, including Helicobacter pylori (41) and Clostridium acetobutylicum, encode two DsbC homologues but have no DsbA homologue, indicating that a single catalyst for disulfide-bond formation may have been adapted during evolution. Interestingly, neither organism has a $dshB$ gene, which suggests that the reoxidation of DsbC in these organisms is accomplished by a mechanism that has yet to be determined. Why then do E. coli and other γ-proteobacteria employ DsbA–DsbB for protein oxidation and a separate system comprising of two (DsbC and DsbG) catalysts of disulfide rearrangement? A simple explanation is that the evolution of separate, kinetically isolated, catalysts allows the exocellular environment to be maintained in a highly oxidizing state and, as noted above, may provide an increase in the growth yield by avoiding the establishment of a futile cycle.

Structural and biochemical data indicate that the cleft formed by the dimerization domain is responsible for peptide binding and for the chaperone activity of DsbC (30, 42, 43). It is, therefore, not surprising that the fusion of TrxA domain proteins, such as DsbA and TrxA, which have little or no chaperone activity on their own, gives rise to chimeras that are able to prevent protein aggregation and inactivation. The dimerization of DsbA also conferred a 2-to
7-fold increase in the rate of insulin reduction. Dimerization of TrxA-“PDI-like” decreased the reductase activity of the molecule compared with TrxA or TrxA-“PDI-like” (24).

The isomerase and oxidase activity of the chimeras were determined by using RNase A as a substrate (19). In this assay, the reactivation of reduced, denatured RNase A is monitored as a function of time in the presence of a catalyst. The lag time for the appearance of active RNase A depends on the rate of protein oxidation by the catalyst, whereas the initial slope in the RNase A activity vs. time plot is proportional to the rate of disulfide isomerization. Analysis of the lag times in the refolding of RNase A indicated that the chimeras are much more effective in catalyzing disulfide-bond formation (oxidase activity, ∼50% of PDI), compared with DsbC, which is a very weak oxidant (oxidase activity, 4% of PDI, data not shown). However, the chimeras displayed very low isomerase activity, which was only 10–22% of DsbCs, or 2–3% relative to PDI (Table 1).

On the basis of the analysis described above, we propose that it is the reduction of incorrect disulfide bonds, rather than the isomerase activity per se, that mediates the rearrangement of incorrect disulfides. TrxA and, to a much lesser extent, DsbA can catalyze the net reduction of disulfides in vitro, but both proteins become oxidized by DsbB in the periplasmic space (44) and are, therefore, unavailable for the reduction of nonnative disulfide bonds. Fusion to the DsbC dimerization domain allows the active-site cysteines in the chimeras to be partially maintained in the thiol state, as needed for the catalysis of disulfide-bond reduction. Recently, H.F.G. and coworkers (45) proposed that the isomerization state, as needed for the catalysis of disulfide-bond reduction.

Recently, H.F.G. and coworkers (45) proposed that the isomerization of disulfides by PDI proceeds by means of trial-and-error cycles (45), which may be responsible for the inability of DsbCd (1–189) and in DSbCd–TrxA( wt) fusion. Consistent with our previous observations that these proteins exhibited chaperone activity and are low isomerase activity, which was only 10–22% of DsbCs, or 2–3% relative to PDI (Table 1).

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