Differential in Vivo Roles Played by DsbA and DsbC in the Formation of Protein Disulfide Bonds*

(Received for publication, February 4, 1997)

Michio Sone, Yoshihori Akiyama, and Koreaki Ito†

From the Department of Cell Biology, Institute for Virus Research, Kyoto University, Kyoto 606-01, Japan

Several Escherichia coli proteins participate in protein disulfide bond formation. Among them, DsbA is the primary factor that oxidizes target cysteines. Biochemical evidence indicates that DsbC has disulfide isomerization activity. To study intracellular functions of DsbA and DsbC, we used an alkaline phosphatase mutant, PhoA[SCCC], with the most amino-terminal cysteine replaced by serine. It was found that the remaining 3 cysteines in PhoA[SCCC] form a disulfide bond of incorrect as well as correct combinations. An aberrant disulfide bond was preferentially formed in wild-type cells, which was converted slowly to the normal disulfide bond. This conversion did not occur in the dsbC-disrupted cells. Overproduction of DsbC stimulated the formation of the correct disulfide bond. In contrast, the inefficiently formed disulfide bonds in the dsbA-disrupted cells, and the more efficiently formed disulfide bonds in the same strain in the presence of oxidized glutathione were mostly in the correct form. These results suggest that the DsbA-catalyzed reaction can be too rapid for some proteins. DsbA may simply oxidize available pairs of cysteines, which happen to be in an incorrect combination in the case of PhoA[SCCC]. In contrast, DsbC stimulates the formation of correct disulfide bonds and corrects previously introduced aberrant ones. Thus, DsbC acts to isomerize disulfide bonds in vivo.

Disulfide bonds are found in many extracellular proteins in all organisms and contribute to folding and stability of these proteins. While disulfide bond formation is a simple reaction of oxidation of cysteine residues, and it can be reproduced in vitro under appropriate conditions (1), recent studies established that it does not occur effectively in vivo without the aid of other proteins (2). In Escherichia coli, a periplasmic protein, DsbA, is required for disulfide bond formation in vivo (3, 4). It directly oxidizes cysteines on the target proteins in vitro (5, 6). It has a thioredoxin-like Cys30-X-X-Cys motif characteristically found in disulfide oxidoreductases (7).

DsbB, an integral membrane protein, is also required for the processess (8). The role of DsbB is to reoxidize DsbA to enable its catalytic turnover (8-11). Genes dsbC and dsbD (dipZ) also encode factors involved in disulfide bond metabolism (12-15). DsbD is a membrane protein with a thioredoxin-like motif in the periplasmic domain, and it may have a regulatory role of conferring reducing power to the periplasm. DsbC is a periplasmic protein with 4 cysteines among which Cys101 forms a thioredoxin-like motif. Creighton and his colleagues (16) characterized the redox activity of DsbC using a model substrate. They showed that while DsbA merely oxidized cysteines on the substrate, DsbC efficiently isomerized preformed disulfide bonds.

Bacterial alkaline phosphatase, a periplasmic protein, is a dimer of the phoA gene product (PhoA) with two intramolecular disulfide bonds (Cys168-Cys178 and Cys206-Cys336 (17). Disulfide bond formation is essential for the correct folding of this enzyme (3, 4, 18, 19). We found that, of the two disulfide bonds in PhoA, the carboxyl-terminal one (Cys206-Cys336) is required and sufficient for the active conformation of this enzyme (20). Thus, a mutant form of PhoA, termed PhoA[SSCC], with the two NH2-terminally located cysteines replaced by serine is as active as the wild-type enzyme, although it is no longer resistant to a protease. Interestingly, the presence of an additional cysteine at residue 178 lowered the enzymatic activity significantly (20). We show here that this mutant PhoA, termed PhoA[SCCC], forms an aberrant disulfide bond among Cys178, Cys168, and Cys336.

Using this unique experimental system, we investigated into the in vivo roles played by DsbA and DsbC. It was found that DsbA principally introduced an aberrant disulfide bond into PhoA[SCCC], whereas DsbC stimulated the eventual formation of the correct disulfide bond in vivo. Thus, DsbC functions, in concert with DsbA, as a disulfide isomerase in vivo.

EXPERIMENTAL PROCEDURES

E. coli Strains and Plasmids—Strain MS3 was a ΔphoA strain, KS272 (21), into which F lacP lacPL8 LacZ Y’ A’ pro’ had been introduced (20). MS4 was a ΔdsbA ΔdsbD (4) transconjugant of MS3. As a dsbC deletion strain we used W3110 tonA ΔdsbC, which was kindly provided by John Joly of Genentech. This strain had been constructed by integration and segregation of a plasmid carrying ΔdsbC, and the dsbC deletion was confirmed by polymerase chain reaction analyses. An isogenic dscB– strain, W3110 tonA, was also provided by J. Joly of Genentech.

PhoA and its Cys/Ser mutant forms were designated by the four letter notations in brackets, with C for Cys and S for Ser, for residues 168, 178, 286, and 336 in this order (20; numbering according to Ref. 22). To whom correspondence should be addressed: Inst. for Virus Research, Kyoto University, Sakyo-ku, Kyoto 606-01, Japan. Tel.: 81-75-751-4015; Fax: 81-75-771-5699 or 81-75-761-5626; E-mail: kito@virus.kyoto-u.ac.jp.涌

† To whom correspondence should be addressed: Inst. for Virus Research, Kyoto University, Sakyo-ku, Kyoto 606-01, Japan. Tel.: 81-75-751-4015; Fax: 81-75-771-5699 or 81-75-761-5626; E-mail: kito@virus.kyoto-u.ac.jp.

‡ To whom correspondence should be addressed: Inst. for Virus Research, Kyoto University, Sakyo-ku, Kyoto 606-01, Japan. Tel.: 81-75-751-4015; Fax: 81-75-771-5699 or 81-75-761-5626; E-mail: kito@virus.kyoto-u.ac.jp.

1 J. Joly, personal communication.

2 The abbreviations used are: IPTG, isopropyl-β-D-thiogalactoside; PAGE, polyacrylamide gel electrophoresis.

This paper is available on line at http://www.jbc.org/Downloaded from http://www.jbc.org/ by guest on July 25, 2018

THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 272, No. 16, Issue of April 18, pp. 10349–10352, 1997
© 1997 by The American Society for Biochemistry and Molecular Biology, Inc.
Printed in U.S.A.
Roles of DsbA and DsbC in Vivo

Fig. 1. PhoA[SCCC] forms an aberrant disulfide bond. Cells of MS3 (lanes 1–4 and 6–9) or MS4 (dsbA::Tn5; lanes 3 and 10) were transformed with pMS002 (PhoA[SCCC]; lanes 1 and 6), pMS015 (PhoA[CCSS]; lanes 2 and 7), pMS004 (PhoA[CCSS]; lanes 3 and 8), or pMS003 (PhoA[SCCC]; lanes 4, 5, 9, and 10). They were grown in L medium with IPTG, and whole cell proteins were separated by SDS-PAGE under nonreducing (lanes 1–5) and reducing (lanes 6–10) conditions. PhoA proteins were visualized by immunoblotting with anti-PhoA serum, red, ox1, and ox2 indicate positions of reduced PhoA, oxidized PhoA with the correct disulfide bond between Cys286-Cys336 and oxidized PhoA with an aberrant disulfide bond, respectively.

RESULTS

PhoA[SCCC] Forms an Aberrant Disulfide Bond—Disulfide-bonded and reduced forms of PhoA can be separated by SDS-PAGE under nonreducing conditions. PhoA[SCCC] migrated identically with wild-type PhoA (Fig. 1, compare lanes 1 and 3) under nonreducing conditions. In contrast, PhoA[CCSS] migrated at the same position as the reduced PhoA (Fig. 1, lane 2). These results indicate that Cys286-Cys336 disulfide bond mainly contributes to the increased electrophoretic mobility of the oxidized PhoA molecule. We designate this electrophoretic mobility “ox1” (Fig. 1). PhoA[SCCC] produced two bands when expressed in wild-type cells (Fig. 1, lane 4). The minor band was at the ox1 position, whereas the major band migrated even faster than ox1. The latter mobility is designated “ox2” (Fig. 1, lane 4). Obviously, the former should represent Cys286-Cys336 disulfide-bonded molecules. The latter species was not due to a proteolytic cleavage, since reduced PhoA[SCCC] migrated as a single band at the position (“red” in Fig. 1) identical to the reduced wild-type PhoA (Fig. 1, lanes 9 and 10). These results indicate that the ox2 form of PhoA[SCCC] contains an aberrant disulfide bond, between Cys178 and either Cys286 or Cys336.

DsbA-dependent Preferential Introduction of an Aberrant Disulfide Bond to PhoA[SCCC]—PhoA[SCCC] was expressed in dsbA+ cells and dsbA-disrupted and dsbA-disrupted cells growing in broth medium, and the electrophoretic mobilities were examined by immunoblotting. Whereas the ox2 isoform was the major product in the wild-type cells, the ox1 isoform became the major accumulated product in the dsbA− strain (Fig. 1, compare lanes 4 and 5). Apparently, the correct disulfide bond was preferred in the absence of DsbA. The enzymatic activity of PhoA[SCCC] was higher when produced in the dsbA-disrupted cells than in the dsbA+ cells.

We then studied the synthesis and conversion of the PhoA[SCCC] isoforms by pulse-chase/immunoprecipitation experiments, using cells growing in minimal salt medium. In wild-type cells, the ox1 and ox2 forms of PhoA[SCCC] were initially labeled in about equal intensities. During chase with unlabeled methionine, intensity of the ox2 form decreased with concomitant increase in the ox1 form (Fig. 2, lanes 1–9). This ox2 to ox1 conversion took place over some 1 h.

When PhoA[SCCC] was expressed in the dsbA-disrupted cells, the majority of the newly synthesized molecules were now in the reduced form, which was slowly degraded (Fig. 2, lanes 10–18). A small amount of oxidized form in this strain was in the ox1 form, and no aberrant form (ox2) was detected (Fig. 2, lanes 10–18). The experiments reported in Fig. 1 (lane 4) and Fig. 2 (lanes 10–18) gave different proportions of PhoA[SCCC] isoforms for the dsbA− strain. This may be explained by the presence of some broth components, such as cysteine, that may have acted as an oxidant in the former experiment (see below for oxidant effects). In addition, instability of the reduced form may have lowered the detection by immunoblotting.

We examined effects of oxidized glutathione (GSSG) added to the minimal medium (Fig. 3, E and G). In the presence of GSSG, PhoA[SCCC] was almost all in the ox2 form in the wild-type cells (Fig. 3E), whereas it was almost all in the ox1 form in the dsbA− mutant (Fig. 3G). Thus, DsbA introduces an abnormal disulfide bond into PhoA[SCCC] molecule in vivo. DsbA-independent disulfide bond formation occurs mostly between the natural combination of cysteines; this was true even when disulfide bond formation was driven by a “nonspecific” oxidant, GSSG (Fig. 3G).

DsbC Is Required for the Conversion of Aberrant to Normal Disulfide Isoforms of PhoA[SCCC]—The results in Fig. 2 (lanes 1–9) demonstrated that the ox2 isoform of PhoA[SCCC] was gradually converted to the ox1 isoform in the wild-type cells. We found that this conversion did not occur in a dsbC deletion strain (Fig. 2, lanes 19–27). The ox2 species in this strain was degraded over the time. These results suggest that the DsbC function is needed for the posttranslational conversion from the aberrant to the correct disulfide isoforms of PhoA[SCCC] in vivo.

DsbC Overproduction Enhances the Production of the Correctly Disulfide-bonded PhoA[SCCC] Molecules—In the presence of a DsbC-overproducing plasmid, even the dsbA− strain produced preferentially the correctly disulfide-bonded PhoA[SCCC] (Fig. 3B). Control cells with the vector produced mainly the ox2 isoform, which was later converted to the ox1 isoform (Fig. 3A). DsbC overproduction in the dsbA-disrupted strain resulted in almost exclusive production of the reduced form (Fig. 3D), whereas the control cells with the vector produced both the reduced and the ox1 forms (Fig. 3C). Thus, excess DsbC cannot substitute for DsbA with respect to the oxidation of PhoA[SCCC]. Neither can it stimulate the correct disulfide bond formation in the absence of DsbA. Excess DsbC in the absence of DsbA seems to be inhibitory against the background.

3 M. Sone and K. Ito, unpublished data.
disulfide bond formation.

**Dsbc Exhibits Different Properties in the Presence of an Excess Oxidant**—We repeated these experiments in the presence of supplemented GSSG (Fig. 3, E–H). As already discussed, GSSG stimulated the correct disulfide bond formation in the absence of DsbA (Fig. 3G). Overproduction of DsbC in the presence of both GSSG and DsbA gave only a small stimulation of the formation of the ox1 isoform (Fig. 3, compare E and F). Overproduction of DsbC in the presence of GSSG and in the absence of DsbA resulted in the production of both ox1 and ox2 forms (Fig. 3H). Since GSSG alone (in the absence of DsbA) supported the formation of only the ox1 form (Fig. 3G), the results of Fig. 3H indicate that excess DsbC gained the ability to introduce the incorrect disulfide bond in the presence of GSSG. This is in marked contrast to the oxidation inhibition observed in the dsbA+ cells in which DsbC was overproduced in the absence of added GSSG (Fig. 3D).

Thus, in the presence of GSSG, DsbC is transformed to have a DsbA-like ability to introduce disulfide bonds that are not necessarily in the correct combination. Under oxidative conditions, the isomerization activity of DsbC may be suppressed.

**DISCUSSION**

Artifacts in the determination of in vivo redox states of proteins can be minimized by examining them after acid denaturation (29, 27), the method employed in this study. Of the two disulfide bonds of PhoA, the amino-terminal disulfide (Cys178-Cys286) constrains a loop of only 9 amino acids, while the carboxy-terminal located disulfide (Cys178-Cys336) constrains a loop of 49 amino acids. The fast migration of the oxidized PhoA in SDS-PAGE can essentially be ascribed to the Cys286-Cys336 disulfide bond (Fig. 1). Since any incorrect disulfide bonds that can be formed in PhoA should connect cysteines that flank at least 107 amino acids (in the case of Cys178-Cys286, Cys286), they are expected to confer even more increased mobility in gel electrophoresis. The ox2 species we observed in PhoA[SCCC] meets this expectation, although we have not determined whether it contains Cys178-Cys286 or Cys178-Cys336 disulfide bond.

Why does PhoA[SCCC] form an aberrant disulfide bond in the presence of DsbA? Disulfide bond formation is essential for the folding of alkaline phosphatase in vivo (3, 4) and in vitro (19), triggering the subsequent folding and dimerization reactions (19). The fact that PhoA[SCCC] has almost 100% enzymatic activity (20) indicates that the PhoA molecule without the NH2-terminal disulfide bond retains the ability to position the active site residues in proper geometry, but this event is incomplete until the carboxyl-terminal disulfide bond has been formed. In PhoA[SCCC], kinetic competition may occur between different combinations of the 3 cysteines for disulfide bond formation, and it should be modulated by ongoing folding reactions (28). Thus, extremely rapid disulfide bond formation may be more random than slower disulfide bond formation. Furthermore, DsbA may be so potent and efficient that it introduces or initiates to introduce a disulfide while a substrate polypeptide chain is still in the process of membrane translocation; the first translocating cysteine, Cys178, will be committed for disulfide bond formation when it reaches the periplasm and forms a transient disulfide with the reactive Cys30 residue (29–31) of DsbA. In contrast, “spontaneous” or glutathione-driven disulfide bond formation in the absence of DsbA will be less efficient and slower such that the correct combination is preferred due to the local folding properties of the polypeptide chain. DsbA-dependent formation of aberrant disulfide bonds was implicated previously (but not demonstrated) in a system where a foreign protein was expressed in *E. coli* (32, 33).

We obtained two kinds of results that suggest that DsbC functions to stimulate the formation of the correct disulfide bond. First, slow conversion occurs from the aberrant to the correct disulfide bonds of pulse-labeled PhoA[SCCC] in wild-type cells, but not in the dsbA deletion strain. This observation clearly indicates that DsbC-dependent isomerization of disulfide bond occurs in the cell. Second, overproduction of DsbC in the dsbA+ cells enhanced the rapid formation of the correct disulfide bond. This observation could be interpreted in terms of either very rapid isomerization in the presence of excess DsbC or de novo introduction of the correct disulfide bond in the presence of excess DsbC. In view of the biochemical demonstration of isomerization activity of DsbC (16), we think it reasonable to assume the rapid isomerization. This is consistent with the observation (Fig. 3, D and H) that DsbA is required for DsbC to exhibit the correct disulfide-introducing function.

Recently, Rietsh et al. (34) reported in vivo results that a dsbC disruption had no effect on OmpA (normally with a single disulfide bond), that it resulted in accumulation of a small amount of reduced PhoA4, and that it resulted in greatly diminished production of active urokinase (normally with 12 disulfide bonds). Although these results are consistent with their interpretation that DsbC has a disulfide isomerizing role in vivo, the evidence for the aberrant disulfide bond formation in the absence of DsbC was inconclusive.

Our results provide some additional information about the DsbC functions in vivo. Excess DsbC was inhibitory against the...
background disulfide bond formation that occurs inefficiently in the absence of DsbA. Probably, the reducing character of DsbC might dominate the air oxidation. It was also found, however, that excess DsbC can exhibit DsbA-like function when GSSG was supplemented to the medium. This is consistent with another finding of Rietsch et al. (34) that the suppression of dsbA null mutation by loss-of-function dsbD ( dipZ) mutations requires the functional DsbC. They proposed that DsbD (DipZ) together with thioredoxin normally keeps DsbC in the partially reduced state, and the loss of reducing factor leads to the production of oxidized DsbC, which in turn substitutes for DsbA. This model is consistent with our results; excess DsbC is oxidized by GSSG and then it substitutes for DsbA.

We have now demonstrated that the principal role of DsbC is to facilitate disulfide bonds that are formed between correct pairs of cysteines. This demonstration was made possible by using a unique model protein, PhoA.[SCCC]. The results of Rietsch et al. (34) that the formation of active urokinase, a natural (but still foreign to E. coli) protein with multiple disulfide bonds, depends absolutely on DsbC also supports this notion. These in vivo results establish that the cellular role of DsbC lies in the isomerization of disulfide bonds until the protein has been folded into a stable conformation.

Acknowledgments—We thank Dr. John Joly for generously providing the dsbC deletion strain, Dr. Guy Condemine for pDS30, and Kyiko Mochizuki for laboratory supplies.

REFERENCES
1. Anfinsen, C. B. (1973) Science 181, 223–230
2. Bardwell, J. C. A. (1994) Mol. Microbiol. 14, 199–205
3. Bardwell, J. C. A., McGovern, K., and Beckwith, J. (1991) Cell 67, 581–589
4. Kamitani, S., Akiyama, Y., and Ito, K. (1992) EMBO J. 11, 57–62
5. Zapun, A., and Creighton, T. E. (1994) Biochemistry 33, 5202–5211
6. Akiyama, Y., Kamitani, S., Kusukawa, N., and Ito, K. (1992) J. Biol. Chem. 267, 22440–22445
7. Holmgren, A. (1989) J. Biol. Chem. 264, 13963–13966
8. Bardwell, J. C. A., Lee, J.-O., Jander, G., Martin, N., Belin, D., and Beckwith, J. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 1038–1042
9. Kishigami, S., Kanaya, E., Kikuchi, M., and Ito, K. (1995) J. Biol. Chem. 270, 17072–17074
10. Guillot, C., Jander, G., Martin, L. N., and Beckwith, J. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 9895–9899
11. Kishigami, S., and Ito, K. (1996) Genes Cells 1, 201–208
12. Missiakas, D., Georgopoulos, C., and Raina, S. (1994) EMBO J. 13, 2013–2020
13. Missiakas, D., Schwager, F., and Raina, S. (1995) EMBO J. 14, 3415–3424
14. Shevchik, V. E., Condemine, G., and Robert-Baudouy, J. (1994) EMBO J. 13, 2007–2012
15. Crooke, H., and Cole, J. (1995) Mol. Microbiol. 15, 1139–1150
16. Zapun, A., Missiakas, D., Raina, S., and Creighton, T. E. (1995) Biochemistry 34, 5075–5089
17. Bradshaw, R. A., Cancedda, F., Ericsson, L. H., Neumann, P. E., Piccoli, S. P., Schlesinger, M. J., Shriefe, K., and Walsh, K. A. (1994) Proc. Natl. Acad. Sci. U. S. A. 78, 3473–3477
18. Derman, A. I., and Beckwith, J. (1991) J. Bacteriol. 173, 7719–7722
19. Akiyama, Y., and Ito, K. (1993) J. Biol. Chem. 268, 8146–8150
20. Sone, M., Kishigami, S., Yoshihisa, T., and Ito, K. (1997) J. Biol. Chem. 272, 6174–6178
21. Strauch, R. L., and Beckwith, J. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 1576–1580
22. Shimazaki, T., Taura, T., Kihara, A., Yoshihisa, T., Akiyama, Y., Cannon, K., and Ito, K. (1995) J. Biol. Chem. 270, 5519–5526
23. Pollitt, S., and Zalkin, H. (1983) J. Bacteriol. 153, 27–32
24. Laemmli, U. K. (1970) Nature 227, 680–685
25. Akiyama, Y., Ogora, T., and Ito, K. (1994) J. Biol. Chem. 269, 5218–5224
26. Miller, J. H. (1972) Experiments in Molecular Genetics, p. 431, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
27. Kishigami, S., Akiyama, Y., and Ito, K. (1995) FEBS Lett. 364, 55–58
28. Frech, C., Wunderlich, M., Glockshuber, R., and Schmid, F. X. (1996) Biochemistry 35, 11386–11395
29. Nelson, J. W., and Creighton, T. E. (1994) Biochemistry 33, 5974–5983
30. Zapun, A., Cooper, L., and Creighton, T. E. (1994) Biochemistry 33, 1907–1914
31. Grauschopf, U., Winther, J. R., Korber, P., Zander, T., Dallinger, P., and Bardwell, J. C. A. (1995) Cell 83, 947–955
32. Alksne, L. E., Keenney, D., and Rasmussen, B. A. (1995) J. Bacteriol. 177, 462–464
33. Alksne, L. E., and Rasmussen, B. A. (1996) J. Bacteriol. 178, 4306–4309
34. Rietsch, A., Belin, D., Martin, N., and Beckwith, J. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 13048–13053
Differential in Vivo Roles Played by DsbA and DsbC in the Formation of Protein Disulfide Bonds
Michio Sone, Yoshinori Akiyama and Koreaki Ito

J. Biol. Chem. 1997, 272:10349-10352.
doi: 10.1074/jbc.272.16.10349

Access the most updated version of this article at http://www.jbc.org/content/272/16/10349

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 33 references, 16 of which can be accessed free at http://www.jbc.org/content/272/16/10349.full.html#ref-list-1
Additions and Corrections

Vol. 272 (1997) 6174–6178

Roles of disulfide bonds in bacterial alkaline phosphatase.

Michio Sone, Satoshi Kishigami, Tohru Yoshihisa, and Koreaki Ito

Vol. 272 (1997) 10349–10352

Differential in vivo roles played by DsbA and DsbC in the formation of protein disulfide bonds.

Michio Sone, Yoshinori Akiyama, and Koreaki Ito

Plasmid pMS002 and its derivatives used in the above two publications proved to contain an additional mutation for a Ser-401 → Cys substitution within PhoA. We traced this mutation back to the phoA plasmid (provided by others) that was used to substitute the amplified segment, as described in the first publication (page 6174, “Experimental Procedures”). Given this fact, we eliminated this unwanted mutation from most of the plasmid constructions and repeated key experiments presented in both publications. Mutant forms of PhoA are shown by the abbreviations described in both articles; however, the previous constructions are indicated by C* attached to the end, and its absence indicates new constructions without the Ser-401 → Cys mutation. Relative specific activity of PhoA[CCCC*] was about 80% of that of PhoA[CCCC]. PhoA[SSCC] was as active as PhoA[CCCC], whereas PhoA[SCCC] was about 50% as active as PhoA[CCCC]. We also observed a lowered cellular accumulation of PhoA[SSCC], consistent with its instability. Thus, the essential conclusions of the first publication hold. Although PhoA[SCCC] has a lower than normal enzyme activity, the abnormally migrating protein species reported in the second publication was not observed for PhoA[SCCC]. Thus, although the essential conclusion that DsbA introduces a disulfide bond of an aberrant combination (possibly involving residue 401 in PhoA[SCCC]) and that DsbC isomerizes that bond holds, the interpretation that the abnormal disulfide bond observed was between intrinsic PhoA residues was incorrect. We regret that we overlooked the mutation that existed in one of the starting materials. We thank Dr. George Georgiou for communicating their sequencing results of pMS003 and Yuki Takahashi for eliminating the mutation and characterizing the new PhoA derivatives.

We suggest that subscribers photocopy these corrections and insert the photocopies at the appropriate places where the article to be corrected originally appeared. Authors are urged to introduce these corrections into any reprints they distribute. Secondary (abstract) services are urged to carry notice of these corrections as prominently as they carried the original abstracts.