Human Protein Disulfide Isomerase Functionally Complements a
dsbA Mutation and Enhances the Yield of Pectate Lyase C in
Escherichia coli*

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Human PDI was expressed to the Escherichia coli periplasm, by using a plasmid encoded ompA-PDI fusion
under the control of the trp promoter. Periplasmic extracts
were shown to contain active PDI using the scrambled ribonuclease assay. PDI activity was also demonstrated
by complementation of two phenotypes associated with a dsbA mutation. Alkaline phosphatase activity, which
is reduced in dsbA cells, was restored to wild type levels by PDI. PelC, a pectate lyase from Er
winia carotovora, was shown to be DsbA dependent in E. coli. PDI was able to restore its activity to that seen in
wild type cells. Increased expression of PDI was found to
increase the yield of active PelC above that seen in
wild type cells. PDI also enhanced the yield of PelC in
Dsba cells but only in the presence of exogenous oxidi
dized glutathione. PDI is thus able to functionally sub
stitute for DsbA in the folding of disulfide-bonded pro
teins in the bacterial periplasm and to enhance the yield
of highly expressed protein when the ability of the E.
coli periplasm to fold protein may be saturated. How
ever, our results suggest that the activities of DsbA and
PDI in vivo may be different.

Protein folding in vivo is assisted by two general classes of
proteins. Chaperones, such as GroEL and BiP, are thought
to interact noncovalently with substrates preventing or reversing
interactions that can lead to incorrect folding and aggregation.
Folding enzymes, such as PDI and peptidyl-prolyl-cis/trans-isomerases, catalyze rate-limiting steps in the folding of pro
teins (for a general review see Ref. 1). The effects of chaperones
and folding enzymes on protein folding have been extensively
studied in vitro (for reviews see Refs. 2 and 3). However, study
ning assisted folding of a protein in vivo is more difficult, partly
because of its subcellular location. For example, the major site
for folding, disulfide bonding, and modification of secretory polypeptides in eukaryotes is the endoplasmic reticulum lu
en, the composition of which is not easily manipulable.

PDI is one of the major proteins of the endoplasmic reticulum lumen. It was first shown to oxidatively refold reduced RNase
(4) and was subsequently shown to be the β-subunit of prolyl
4-hydroxylase (5). It is thought to be the main agent for forma
tion and isomerization of disulfide bonds in proteins folding in
the endoplasmic reticulum lumen (for reviews see Refs. 6 and
7). However, this cellular function has largely been demon
strated by indirect methods; PDI has been shown to be physi
cally associated with folding proteins in vivo (8, 9), has restored
defective co-translational disulfide bond formation in PDI-de
deficient microsomes (10), and has been shown to assist the
folding of proteins in vitro (11–13). Here, we have studied
PDI-mediated protein folding by expressing human PDI to the
periplasm of Escherichia coli and assaying its effects on model
proteins. PDI is amenable to study in the E. coli periplasm
because it is highly soluble, is not glycosylated, and does not
require ATP for its function.

The periplasm is an oxidizing compartment and is the site
of disulfide bond formation in E. coli proteins. A number of pro
teins have been identified in E. coli that are involved in disul
fide bond formation, including DsbA, DsbB, and DsbC (14–19).
These proteins possess the motif, NH2-CXX-COOH, containing
cysteines that are important for catalytic activity. PDI also
possesses this motif. Purified DsbA acts as a disulfide oxido
reductase and also acts weakly as a disulfide isomerase (20, 21).
The relative importance of the two activities in vivo is not
known. Strains lacking DsbA show a variety of defects in disul
fide bond formation (see Ref. 22 for a review). We demon
strate here that human PDI can complement two dsbA-depend
ent phenotypes, confirming the idea that PDI and DsbA are func
tionally similar. We also show that when one particular disulfide-bonded protein (PelC) is overexpressed, the folding
capacity of wild type E. coli cells can be saturated. The co
expression of PDI overcome this deficiency resulting in in
creased levels of active PelC. Increased levels of PelC could be
produced from dsbA cells expressing PDI, but only when oxidi
zed glutathione (GSSG) was added to the growth media. This
suggests that although DsbA and PDI are functionally similar
in complementation studies, their activities in vivo have some
significant differences.

EXPERIMENTAL PROCEDURES

Materials—Polygalacturonic acid, indoleacrylic acid (IAA), dithio
treitol, and RNA (Torula yeast) were purchased from Sigma; para
nitrophenyl phosphate was purchased from BDH Ltd. Vent polymerase
was from New England Biolabs, and Sequenase was from U. S. Bio
chemical Corp. RNase and anti-PDI antibodies were generous gifts of
Prof. R. Freedman (Table I). The E. coli strain TG2 was used for all
DNA manipulations.

DNA Manipulations—Standard methods were used (23).

Growth Conditions— Cultures grown in Luria broth supplemented
with antibiotics were shaken (200 r.p.m.) at 37 °C. Expression of PDI
from pDPH5 was either uninduced or induced by including IAA at
concentrations from 1 to 120 µg ml−1. Expression of PelC from pDPH9
was induced by inclusion of IPTG at one or 0.2 or 0.4 mg in cultures
grown at 33 °C.

Construction of PDI Expression Plasmids pDPH5 and pDPH13—
DNA coding for the mature PDI peptide was generated as a HindII-

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‡The abbreviations used are: PDI, protein disulfide isomerase; PelC,
pectate lyase C; IAA, 3-β-indoleacrylic acid; IPTG, isopropyl β-D-thio
galactopyranoside; GSSG, oxidized glutathione; sRNase, scrambled RNase; BTP1, bovine pancreatic trypsin inhibitor.
TABLE I

| Bacterial strains and plasmids | Reference |
|-------------------------------|-----------|
| TG2 supE hsdD15 thi1 (lac-proAB) Δ (srl-recA)306::Tn10 (tet') F [traD36 proAB' lacZΔM15] | 23 |
| JCB570 MC 1000 phoR zil12::Tn10 | 14 |
| JCB571 J CBT 70A badA::kan1 | 14 |
| JCB502 MG 1655 LacZ::Tn10 tet' Δ69 | 14 |
| JCB517 J CBT 52A badA::kan1 | 14 |
| pUC118 Multi-purpose cloning vector containing lacZ gene, colE1 ori, Amp' | 24 |
| pCT54 Expression vector containing trp promoter, colE1 ori, Amp' | 24 |
| pSU18 Multi-purpose cloning vector containing lac promoter, pACYC ori, Cm' | 26 |
| pUK151 C DNA encoding human PDI | Prof. R. Freedman |
| pl S6197 DNA encoding PelC | 27 |
| pSKOMP OmpA signal peptide coding region with Shine-Dalgamo sequence | Celltech Ltd. |
| pDPH5 Plasmid expressing periplasmic PDI from the trp promoter, pCT54 based | This study |
| pDPH6 Control plasmid, ompA-PDI gene in reverse orientation to trp promoter, pCT54 based | This study |
| pDPH9 Plasmid expressing periplasmic PelC from the lac promoter, pSU18 based | This study |
| pDPH13 Plasmid expressing periplasmic PDI from the lac promoter, pSU18 based | This study |

PDI Complements dsbA Mutation and Enhances Yield of PelC

**Assay of PDI Activity from E. coli Periplasmic Extracts**

Periplasmic extracts of A. polare strains JCB571 and JCB570 (Fig. 3, compare lanes 1 and 5) and the E. coli strain TG2 showed that large amounts of PDI activity could be performed by the lysozyme/EDTA method. Fig. 3 shows that large amounts of PDI activity could be measured in such extracts. This activity was proportional to the concentration of IAA and therefore to the final concentration of PDI. Periplasmic extracts from cells bearing pCT54 gave no measurable activity, showing that the activity was due to PDI alone.

**Complementation of dsbA Phenotypes by PDI**

Alkaline Phosphatase—This protein is known to be DsbA dependent in its folding (14, 15) and was used as a model protein. We assayed the alkaline phosphatase activity from overnight cultures of the phoR strains JCB570 and JCB571, which express alkaline phosphatase constitutively.

Fig. 3 shows that dsbA cells are deficient in alkaline phosphatase activity measured relative to the wild type (compare columns 1 and 2). Inclusion of the PDI expression plasmid, pDPH5, under noninduced conditions gave nearly full restoration of alkaline phosphatase activity. The control plasmid pDPH6 had no effect on alkaline phosphatase activity. This
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The degradation of polygalacturonic acid spectrophotometrically. The results are shown in Fig. 4. There was a significant reduction in the PeC activity recovered from dsbA cells, as shown by comparing columns 1 and 2. When PDI was supplied by including the compatible plasmid pDPH5, the activity of PeC recovered was restored to near wild type levels.

This demonstrates clearly that PeC activity, and therefore presumably PeC folding, is dsbA-dependent in vivo. E. coli PDI is able to act on PeC protein and restore its activity to near wild type levels in a dsbA background. pDPH5 was introduced for PDI expression in this experiment. Under these conditions, there is only a very small amount of PDI in the periplasm, which is only easily visualized by immunoblotting of such periplasmic extracts separated on SDS-polyacrylamide gel electrophoresis gels (data not shown). However, the level of PeC activity recovered from the JCB571 + pDPH9 + pDPH5 cells appeared to be slightly greater than that from wild type cells. This led us to investigate whether increased PDI expression could give enhanced PeC yield.

Overexpression of PeC in the Presence of PDI

PeC expression was induced with 0.4 mM IPTG in cultures with increasing concentrations of IAA to give increased PeC activity. Fig. 5 shows that increasing co-expression of PDI gave increased recovery of active PeC relative to wild type cells. The activity recovered increased proportionally with IAA concentration. The increase was not due to the effect of IAA or pCT54 (expression vector) on the cells (Fig. 5, compare columns 7 and 8). The ability of E. coli to oxidatively fold PeC under these conditions appears to have been limited. This limitation can be overcome by additional PDI. We wondered if this overproduction of PeC could also be achieved in the dsbA cells JCB571. Fig. 6 shows that even with induction of PeC expression with 80 𝜇g ml⁻¹ IAA, there is no increased recovery of PeC activity above that seen in wild type cells. This suggested that PDI on its own in the periplasm is unable to increase the yield of active PeC in this strain.

We tested if this limitation in DsbA− cells could be reversed by supplying GSSG in the media. It is thought that the relatively oxidizing environment of the endoplasmic reticulum lumen is maintained by a mixed glutathione buffer (34), and it has been shown that use of glutathione buffers in bacterial media affects the redox state of the periplasm (35–38). Fig. 7 shows clearly that PDI can functionally substitute for DsbA.

Further investigation showed that the level of alkaline phosphatase activity in the DsbA− cells (JCB570) was not enhanced by overexpression of PDI from either pDPH5 or pDPH13 (data not shown).

PeC as a Model Folding Protein in Vivo—We wished to study the expression of an assayable protein that was not native to E. coli but that was likely to require the action of disulfide bond-forming proteins to reach its active form in vivo. PeC, a pectate lyase from Erwinia carotovora, was found to be an ideal model protein. It is a secreted enzyme known to have two disulfide bonds (33). Erwinia has Dsb proteins (19), so it seemed possible that PeC folding would be DsbA-dependent in E. coli. PeC is assayable by a plate and turbidometric assay and can be expressed in E. coli, where it is retained in the periplasm (27). Use of a plasmid-encoded model protein would also facilitate investigation of overexpression phenomena.

Induction of PeC expression from pDPH9 with 0.2 mM IPTG resulted in a protein with a molecular mass of 39.8 kDa present in the periplasmic fraction, in agreement with the predicted mass (27). PeC activity was shown to be dsbA-dependent in E. coli by assaying crude periplasmic extracts in vitro by following
shows that in the presence of GSSG, PDI can enhance the yield of PelC above that seen in wild-type cells even in a \( \text{dsbA} \) background. The yield of PelC seen with 5 mM GSSG was approximately half that seen with an equivalent level of PDI induction in wild-type cells as seen in Fig. 5 but is still approximately twice the yield seen in wild-type cells without PDI.

**DISCUSSION**

In this paper we present direct evidence for the ability of human PDI to assist the oxidative folding of proteins in vivo in E. coli. We show for two different proteins that PDI can functionally substitute for DsbA. We also show that high levels of co-expression of PDI result in increased yields of active PelC above that achieved by wild-type cells. This overproduction is itself DsbA-dependent, but the provision of GSSG in media overcame this dependence.

The rate of folding of alkaline phosphatase in \( \text{dsbA} \) strains is lower than that of wild-type cells (14), resulting in lower overall alkaline phosphatase activity in \( \text{dsbA} \) cells. Co-expression of low levels of PDI restores the measured alkaline phosphatase activity to near wild-type levels, suggesting that PDI is influencing the rate of folding of alkaline phosphatase.

We used PelC as a model folding protein and by assaying crude periplasmic extracts in vitro showed that the amount of active PelC recovered from \( \text{dsbA}^{-} \) cells is significantly reduced compared with that recovered from \( \text{dsbA}^{+} \) cells. We interpret this as demonstrating that the folding of PelC is DsbA-dependent in vivo. Because E. carotovora is known to contain Dsb proteins similar to those in E. coli, we would expect PelC folding to be DsbA-dependent in its native host also, as suggested previously (19). Co-expression of low levels of PDI from a plasmid compatible to the one carrying the PelC gene re-
sulted in a restoration of the recovered PelC activity to near wild type levels, demonstrating again that PDI can functionally substitute for DsbA.

While doing PelC assay experiments on the phoR− strains JCB570 and JCB571, we observed that the total protein concentration of periplasmic extracts from dsbA− JCB571 was consistently about 30% lower than that from dsbA+ JCB570. Low levels of PDI expression restored protein concentrations in dsbA− cells to near normal levels.2 PhoR− strains constitutively express several periplasmic proteins (for example, alkaline phosphatase, phosphate-binding protein, and glyceral 3-phosphate-binding protein) (for reviews see Refs. 39 and 40). The lower rate of folding in the periplasm of dsbA cells probably results in a greater proteolytic susceptibility for these proteins, similar to that seen with alkaline phosphatase, β-lactamase, OmpA, and BPT1 (14, 36), and hence a lower final protein concentration. This suggests that PDI can act on different E. coli proteins.

In the complementation experiments PDI was produced from uninduced pDPh5, so the level of expression of PDI is low; rich media are known to repress the trp promoter (41). This demonstrates that only small amounts of PDI are necessary to complement dsbA phenotypes, suggesting that PDI is acting catalytically and not just as an extra source of a periplasmic redox buffer. The fact that PDI appears to act on many periplasmic proteins suggests that PDI has a relaxed peptide binding specificity in vivo, which is consistent with experiments done in vitro (42, 43).

We tested if increased production of PDI would result in increased yields of PelC. The results in Fig. 5 show that indeed higher levels of induction of PDI gave greatly increased yield of PelC activity above that seen in wild type cells. The yield of PelC activity increased with increasing levels of PDI expression, although it reached a plateau in the range of 1AA concentrations tested. This suggests that the yield of overexpressed PelC in DsbA− E. coli is limited by the cellular folding machinery. This may be due to saturation of the Dsb system, resulting in increased proteolytic susceptibility for some molecules of PelC in the periplasm.

We showed that higher levels of periplasmic PDI only achieved overproduction of PelC in the presence of DsbA. 1 and 5 mM GSSG partially restored the ability of PDI to increase the yield of active PelC in a dsbA− background. Others have used redox buffers supplied in culture media while trying to influence folding of periplasmic proteins directly but with mixed success (36–39). In our experiments, the addition of 1 and 5 mM GSSG had no effect on the yield of active PelC in DsbA− cells containing no PDI. Therefore, GSSG alone lacks the ability to increase the yield of oxidatively folding proteins in the periplasm, which is consistent with previous work. However, the same concentrations of GSSG in DsbA− cells in the presence of PDI has a marked positive effect.

The precise mechanism of complementation of dsbA by PDI is not known. It could simply be a result of PDI’s dithiol oxidase activity substituting directly for that of DsbA. If this is true, an unknown oxidant(s) must be responsible for regenerating active PDI. Alternatively, PDI acting as an isomerase may increase the flux through a DsbA-independent pathway, for example by isomerization of disulfide-bonded intermediates that have spontaneously formed in the incorrect conformation. Such a pathway is probably normally catalyzed by DsbC, and it will be of great interest to investigate the ability of PDI to complement dsbC mutants. The fact that overproduction of PelC is only seen in wild type cells but not in dsbA− cells suggests that a DsbA-dependent step is rate-limiting, at least for PelC. This can be overcome by provision of oxidized glutathione. GSSG may act either as an oxidant, thereby improving the regeneration of oxidized PDI, or by forming a mixed adducts with PelC and PDI, which may accelerate formation of the active protein, by the mechanisms demonstrated by Darby et al. (44).

Understanding the exact actions of PDI and DsbA on alkaline phosphatase and PelC folding are complicated in vivo by the dynamic nature of the periplasm, which may have changing needs for oxidation/oxidation activities during cell growth. Co-expressed PDI was able to effect increases in the yield of PelC in the absence of any supplied redox buffers. In contrast, co-expression of DsbA only gave increased yield of native α-amylase/trypsin inhibitor from Ragi (RBI) in the presence of media redox buffers (36). This suggests that PDI and DsbA may have different activities in vivo. We also note that overexpression of PelC in DsbA− cells in the presence of PDI and GSSG resulted in only approximately half the yield of PelC compared with that in the DsbA− cells with PDI (compare column 7 in Fig. 7 with column 5 in Fig. 5). This suggests that the enhancement by GSSG (in DsbA− cells) was not due to direct replacement of the DsbA activity missing.

The phoR− cells used for these experiments constitutively translocate at least six periplasmic proteins, alkaline phosphatase, phosphate-binding protein, glycerol 3-phosphate-binding protein, PelC, PDI, and β-lactamase (from pDPh5), without apparent difficulty. However, overexpression of PelC may have saturated the Dsb machinery. Perhaps PDI only has a beneficial role to play in E. coli under Dsb saturated conditions?

Bacterial proteins generally have fewer disulfide bonds than secreted eukaryotic proteins (20). The ability of PDI to catalyze the folding of more complex proteins in a range of genetic backgrounds is currently being evaluated. Co-expression systems such as this provide a powerful investigative tool. Individual proteins such as PDI can be investigated in an environment closer to that in the cell than can be generated using reconstructed systems in vitro, thereby enabling investigation of other factors that affect the function of PDI, such as redox conditions and PDI-protein interactions.

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