A Bacterial Glutathione Transporter (Escherichia coli CydDC) Exports Reductant to the Periplasm*

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Glutathione (GSH), a major biological antioxidant, maintains redox balance in prokaryotes and eukaryotic cells and forms exportable conjugates with compounds of pharmacological and agronomic importance. However, no GSH transporter has been characterized in a prokaryote. We show here that a heterodimeric ATP-binding cassette-type transporter, CydDC, mediates GSH transport across the Escherichia coli cytoplasmic membrane. In everted membrane vesicles, GSH is imported via an ATP-driven, protonophore-insensitive, orthovanadate-sensitive mechanism, equating with export to the periplasm in intact cells. GSH transport and cytochrome bd quinol oxidase assembly are abolished in the cydD1 mutant. Glutathione disulfide (GSSG) was not transported in either CydD or CydC strains. Exogenous GSH restores defective swarming motility and benzylpenicillin sensitivity in a cydD mutant and also benzylpenicillin sensitivity in a gshA mutant defective in GSH synthesis. Overexpression of the cydDC operon in dsbD mutants defective in disulfide bond formation restores dithiothreitol tolerance and periplasmic cytochrome b assembly, revealing redundant pathways for reductant export to the periplasm. These results identify the first prokaryotic GSH transporter and indicate a key role for GSH in periplasmic redox homeostasis.

All cells must maintain intracellular compartments at appropriate reduction potentials for metabolism, generally via the interconversion of reduced and oxidized forms of a redox molecule such as NAD(P)/H or NAD(P)OX (1). The tripeptide glutathione (L−γ-glutamylcysteinylglycine, GSH) is a major thiol-disulfide reductant buffer, reaching ~10 mm in the cytosol and mitochondrion of eukaryotic cells (2, 3) and the Escherichia coli cytoplasm (4). GSH reduces disulfide bonds in proteins, including those that may form on exposure to oxidative stress, the neutralization of free radicals, and the detoxification of xenobiotics (2). Maintenance and adjustment of the ratio of [GSH]/[GSSG] glutathione disulfide, or “oxidized” glutathione allow individual subcellular compartments to have different, appropriate redox balances. For example, in animal cells, the [GSSG]/[GSH] ratio is 30–100-fold greater in the endoplasmic reticulum than in the cytoplasm. This oxidizing endoplasmic reticulum environment (5, 6) is achieved by the opposing reducing and oxidizing activities of GSH and Ero1, respectively, and is required for formation of disulfide bonds in proteins destined for export (7). Although GSH must enter the endoplasmic reticulum, a transporter for GSH has not been genetically defined.

The extracytoplasmic compartment of Gram-negative bacteria, the periplasm, is also the site of elaborate and finely balanced redox control but, to date, the GSH/GSSG couple has not been considered a major player in that compartment. Redox control is a particularly important prerequisite for heme ligation during cytochrome c assembly (8) in the predominantly oxidizing environment of the periplasm, where formation of disulfide bonds is controlled by thiol-disulfide oxidoreductases (9). The oxidizing pathway comprises DsbA, a periplasmic, strongly oxidizing, 21-kDa protein, E′0 = −89 mV (10), which is responsible for random formation of protein disulfide bonds in a rapid disulfide exchange reaction (11). DsbA is reoxidized by DsbB, an integral membrane protein that transfers electrons via quinones (12) to the terminal oxidases and reductases of the respiratory chain (13). The reducing pathway includes DsbD (14, 15), a membrane protein with a periplasmically oriented CXXC motif. In addition, CcmG and CcmH are specialized oxidoreductases required for the redox pathway of cytochrome c biogenesis (9).

In most Gram-positive bacteria, except for some members of the Bacillus-Clostridium group (16), intracellular GSH appears to result from import via an energy-dependent, uncharacterized mechanism (17). In Haemophilus influenzae, GSH is imported from the growth medium and protects cells from organic hydroperoxides and S-nitroso-glutathione (18). Evidence that GSH similarly mediates oxidative stress tolerance in E. coli comes from the finding that a GSH-deficient strain is hypersensitive to hypochlorous acid (19), but GSH appears dispensable for bacterial growth under many conditions (20). In E. coli, five cotranscribed genes have homology to the dpp genes and appear to encode a transporter required for utilization of GSH as sole sulfur source, suggesting a role in GSH transport (21). The possibility that GSH might be secreted or leaked out into the periplasm has also been suggested (22). The mutation of the E. coli porin gene ompL bypassed the DsbA requirement for protein oxidation and led to the view (22) that OmpL might allow passage into the periplasm of reduced low molecular mass agents that are oxidized by DsbB, but no such substrates were identified.

Recently, we demonstrated that an ABC-type transporter, CydDC, originally identified by its requirement for assembly of the cytochrome bd-type terminal oxidase of E. coli (23–25), is a transporter of cysteine outwards across the cytoplasmic membrane (26). Given the established roles of GSH in redox buffering in eukaryotic cells (7), we hypothesized that GSH is also a substrate of CydDC. We now report that CydDC has higher transport activity with GSH than with cysteine as substrate (all-ascorite). Recently, GSH flux from lung airway epithelial cells has been shown to be mediated by the cystic fibrosis transmembrane conductance regulator (27), a protein with which CydDC has structural similarities. This paper, however, is the first report of a bacterial GSH transporter.
EXPERIMENTAL PROCEDURES

Bacterial Strains and Plasmids—E. coli wild-type strain AN2342 (F−) and its isogenic cydD mutant derivative AN2343 (cydD1, G431D, G429E) have been described before (28). The GSH-deficient mutant used was MJF355 (gshA::Tn10, kmR), derived from the isogenic gshA− strain Frag1 (thy rha lacZ gal) (29), both kindly provided by Professor I. R. Booth (University of Aberdeen). The dsbD mutant DM2355 (dsbD::ΩSp) and its isogenic parent K38 (HfrC tonA22 garB10 ompF relA1 phoA6) (30) were kindly provided by Dr. D. Missiakas (University of Chicago). Plasmid pRK1602 contains the cydDC+ operon under the control of its native promoter and complements in trans the phenotype of mutations in these genes (28).

Media and Culture Conditions—Cells were grown in Luria-Bertani (LB) broth (pH 7.0), or in MOPS-buffered minimal medium (pH 7.4) (31) supplemented with 40 mM glucose as the sole carbon source or with 10% (v/v) LB. Benzylpenicillin (Penicillin G; Sigma) was used where shown at a concentration of 20 μg ml−1, and GSH was added to the medium at concentrations given in the text. Aerated cultures were grown with shaking (200 rpm) at 30 or 37 °C. For strains harboring pRK1602, medium contained ampicillin (150 μg ml−1).

Assays of Mutability and Sensitivity to Benzylpenicillin and Dithiothreitol (DTT)—These were conducted essentially as described before (26) except that DTT sensitivity was measured in disk diffusion assays. Filter-sterilized solutions (10 μl) of the stated DTT concentration were pipetted onto sterile filter paper disks (5-mm diameter) that were laid on a lawn of bacteria prepared from an overnight culture. After overnight growth at 37 °C, growth inhibition was measured as the radial distance from the edge of the filter disk to the periphery of the zone of inhibition. Each DTT concentration was assayed three times.

Cytochrome Assays in Cells and Periplasm—Cytochrome bd was assayed spectrophotommetrically in intact cells as described before (26) using an absorption coefficient (622 − 444 nm) of 12.6 μM cm−1 (32). Periplasmic fractions for spectroscopic analysis were isolated using a modified osmotic shock procedure (33). In brief, anaerobic cultures (typically 3.6 liters) were grown in sealed bottles filled to the brim and mixed for 45 s on ice before MgCl2 was added (final concentration 1 mM). After incubation on ice for 10 min, centrifugation (10,000 × g for 10 min) at 4 °C gave a supernatant fraction that was retained as the periplasmic fraction. The clear solution was concentrated 5-fold over a 10 min) at 4 °C gave a supernatant fraction that was retained as the periplasmic fraction. The clear solution was concentrated 5-fold over a YM30 (30 kDa cut-off) membrane in an Amicon Model 8050 stirred cell and assayed immediately in difference spectra (reduced − oxidized). To improve signal:noise ratio for these dilute samples, each sample was scanned >10 times in the dual wavelength scanning mode of a Johnson Foundation SDB4 spectrophotometer, with a reference wavelength of 500 nm and spectral bandwidth of 2 nm (34). Spectral acquisition, summing, and the plotting of difference spectra were performed using SoftSDS (Current Designs, Philadelphia, PA) and Cricket Graph III (Computer Associates Limited) software. Pyridine hemochrome spectra were recorded as described in Ref. 35. Protein was assayed using a modification of the Lowry method (36).

GSH Assay—GSH and GSSG in medium were measured using the DTNB-GSSG reductase recycling method (37). The cuvettes were incubated at 30 °C for 15 min followed by the addition of LB (to make a final volume of 1 ml) and 0.27 units of GSSG reductase. The reaction was monitored at 412 nm for 3 min. GSH amounts were calculated by reference to standards (0.1−10 nmol).

Oxidation of Reduced Glutathione to GSSG—Commercially available [35S]GSH contains 10 mM DTT as reductant, which was removed by solvent extraction (38). [35S]GSH (0.132 nmol, 28.1 TBq mmol−1, 760 Ci mmol−1, Amersham Biosciences) was acidified with HCl to pH 2.0, and the DTT was extracted with 10 volumes of ethyl acetate by shaking for 2 min followed by centrifugation for 1 min. The aqueous phase was retained. This was repeated three times, and the aqueous phase was assayed for thiols (37).

Preparation of Everted Membrane Vesicles—Vesicles for transport assays were prepared as described before (26) using a modification of the French pressure cell procedure (39).

RESULTS

GSH Is Transported by CydDC in an ATP-dependent Manner—The E. coli CydDC system is a heterodimeric ABC-type transporter (28) and could be involved in either import or export across the cytoplasmic membrane. Several lines of evidence point to its function as an exporter in vivo. First, mutants defective in cydD or cydD encoding the polypeptides of the heterodimer, exhibit several defects of the periplasm, namely a hyperoxidized redox state and failure to assemble periplasmic cytochromes b and c (40, 41). Second, cydD mutants fail to assemble the cytochrome bd-type oxidase complex (25, 41); in such mutants, the CydA and CydB subunits are detectable but the hemes are not incorporated into the subunits (42). Interestingly, both the hemes and the oxygen−reactive site (43) are thought to be close to the periplasmic side of the cytoplasmic membrane. Third, no cognate periplasmic-binding protein that would be expected for function of an inwardly transporting complex has been discovered. Fourth, the proteins with which CydDC has closest similarity, e.g. the multidrug resistance protein MRP1, the P-glycoprotein Pgp and cystic fibrosis transmembrane conductance regulator (44) are all exporters in vivo. Finally and most importantly, CydDC imports cysteine in everted vesicles, exporting with equal uptake. Recognizing the broad substrate spec-
ificity of ABC transporters (45) and the fact that cysteine and GSH have been reported to exert similar redox effects in the endoplasmic reticulum (7, 46), we attempted to demonstrate GSH transport by CydDC.

Kinetic experiments were conducted using $[^{35}S]$GSH in the presence of 1 mM cold GSH, a concentration significantly lower than that of the anticipated GSH pool in the E. coli cytoplasm but demonstrated to be appropriate for GSH transport assays using cystic fibrosis transmembrane conductance regulator, without having nonspecific effects on cystic fibrosis transmembrane conductance regulator function (27). To demonstrate that the everted membrane vesicles support active transport, their ability to accumulate $[^{14}C]$lactose in response to energization of the membrane was determined first. Everted vesicles prepared from cells grown in MOPS medium supplemented with glucose have a low rate of lactose transport (47). Therefore, everted vesicles were prepared from wild-type strain AN2342 grown in MOPS medium supplemented with 40 mM lactose as described under “Experimental Procedures.” Significant accumulation of $[^{14}C]$lactose occurred only if d-lactate was added as an energy source. Typical rates of $[^{14}C]$lactose transport were 0.2 nmol min$^{-1}$ (mg of protein)$^{-1}$ (data not shown). The addition of the proton ionophore, CCCP, abolished transport, demonstrating that accumulation of $[^{14}C]$lactose was dependent upon $\Delta$p (data not shown). The ability of vesicles from strain AN2343 (cydD) to accumulate lactose could not be investigated, as this strain was unable to grow, for unknown reasons, in MOPS minimal medium when supplied with lactose (40 mM) as the sole carbon source.

GSH transport into everted membrane vesicles was assayed by pre-equilibrating components at 30 °C before initiating transport by adding 10 mM ATP. No significant uptake of $[^{35}S]$GSH was observed in the 5 min prior to the addition of ATP in vesicles of wild-type strain AN2342 (Fig. 1A). Upon addition of ATP, however, $[^{35}S]$GSH uptake was rapid (Fig. 1A). The maximum rate of $[^{35}S]$GSH uptake observed in the conditions tested was 3.8 nmol min$^{-1}$ (mg of protein)$^{-1}$, and uptake reached a maximal level 5 min after the addition of ATP (Fig. 1A). Although fluctuations in uptake rates were observed among different vesicle preparations (2–4 nmol min$^{-1}$ (mg of protein)$^{-1}$), the pattern of these assays was highly reproducible. Significantly, no $[^{35}S]$GSH uptake was observed in vesicles of the cydD mutant AN2343 before or after the addition of ATP (Fig. 1A).

The CydDC transporter is an ABC-type transporter and is expected to derive the energy for transport solely from ATP hydrolysis; indeed, mutation of the ABC domain abolishes function (28). CCCP is a protonophore that dissipates the bacterial proton motive force without effect on ATP-driven reactions, and, as predicted, its addition (2 $\mu$M) had no discernible effect upon the uptake of $[^{35}S]$GSH by everted vesicles of either the wild-type strain or the cydD mutant (Fig. 1A). However, incubation of everted membrane vesicles from the wild-type strain in the presence of sodium orthovanadate (an inhibitor of ABC-type transport systems) (48) abolished transport completely such that the rate was indistinguishable from that observed with everted vesicles of AN2343 (cydD) (Fig. 1C). Collectively, the data demonstrate

![Figure 1. Glutathione uptake by E. coli vesicles.](image-url)
that \(^{35}\text{S}\)GSH is a substrate of the CydDC transporter and that accumulation into everted membrane vesicles is ATP-dependent and protonophore-insensitive. The rates of transport of GSH were dependent on added GSH concentrations up to at least 3 mM (Fig. 1), and, at all substrate concentrations tested, the rates of GSH uptake were ~5 times higher than that observed with L-cysteine (Fig. 1D). The data did not allow accurate determinations of \(V_{\text{max}}\) and \(K_{m}\) values.

GSH Complements Bacterial Motility Defects in a cydD Mutant—We have previously reported several phenotypes of a cydD mutant that are indicative of periplasmic redox stress and their alleviation by exogenous cysteine (26). Because, in the present work, GSH is also shown to be exported to the periplasm and might thereby influence periplasmic functions including motility as cysteine does, we tested the effects of exogenous GSH on bacterial swarming. *E. coli* wild-type and cydD mutant strains were grown to stationary phase at 30 °C in MOPS minimal glucose media, and culture aliquots (5 μl) were plated onto LB (semisolid Difco agar, 0.3%) without or with (1 or 2 mM) GSH. After incubation at 30 °C for 2–3 days, the wild-type strain exhibited a zone of swarming with a mean diameter of 58 mm (Fig. 2). In marked contrast, the cydD

\[ \text{FIGURE 2. Glutathione restores the swarming ability of a cydD mutant.} \]

Strains AN2342 (wild type, black bars) and AN2343 (cydD, white bars) were grown to stationary phase in MOPS minimal medium (pH 7.0) supplemented with 40 mM glucose at 30 °C. A 5-μl aliquot of each strain was plated onto LB (0.3% Difco agar) and incubated at 30 °C for up to 2 days. The diameter of the swarm was measured, and the average of three experiments was taken. At 2 mM GSH, the observed effect of added GSH was not significant at the 95% confidence level.

Bacterial Glutathione Transporter

GSH completely abrogated the bactericidal activity of the antibiotic against the cydD mutant and furthermore allowed formation of normal colonies (1–2-mm diameter) in numbers similar to the LB-only control (Fig. 3A), presumably as a result of extra provision of reductant. However, GSH completely abrogated the bacterial activity of the antibiotic against the cydD mutant and furthermore allowed formation of normal colonies (1–2-mm diameter) in numbers similar to the LB-only control (Fig. 3A), presumably as a result of extra provision of reductant.

A bacterial glutathione transporter

If GSH were the substrate of the CydDC transporter, then a gshA mutant, which is unable to catalyze GSH synthesis, would be expected to display some phenotypes in common with a cydD mutant. To assess swarming motility, strain MJF355 (gshA) and its isogenic wild-type parent FragI were grown to stationary phase in MOPS minimal medium.
supplemented with 40 mM glucose, and 5-μl aliquots of each were inoculated onto semisolid agar. After 2 days of incubation at 30 °C, the wild-type strain displayed a zone of swarming ~45 mm in diameter, whereas MJF355 (gshA) produced a swarm diameter of 15 mm (results not shown). Thus, a gshA mutant, like a cydD mutant (Fig. 2) is defective in swarming motility. These results suggest that GSH export to the periplasm is critical for proper assembly of the apparatus for taxis and/or motility.

When tested on agar plates lacking both benzylpenicillin and GSH, the gshA mutant showed a decrease in viability compared with that of the isogenic wild-type strain (1.1 × 10^7 and 1.9 × 10^7 (ml of undiluted culture)^−1, respectively) (Fig. 3B). In the presence of 2 mM GSH, however, the viability of the gshA strain increased to that of the wild type grown on LB alone (1.7 × 10^9 (ml of culture)^−1 (Fig. 3B)). There was no growth of either strain in the presence of benzylpenicillin (20 μg ml^−1) (Fig. 3B), as expected. However, when challenged with benzylpenicillin in the presence of GSH, both strains were viable, giving counts equivalent to 2.5 × 10^8 and 1.5 × 10^8 (ml of culture)^−1 for the wild-type and gshA strains, respectively (Fig. 3B). The patterns of growth under these conditions are strikingly similar to those observed for strains AN2342 (wild type) and AN2343 (cydD) (Fig. 3A). The morphology of the gshA mutant colonies after 16 h when grown under these conditions was comparable with when grown on LB alone (1–2 mm), whereas colonies of the wild-type strain appeared as pin pricks (see above). Collectively, these data point to the requirement for an optimal intracellular GSH status for motility and benzylpenicillin resistance, which is not achieved in cydD or gshA mutants unless provided with exogenous reductant.

Oxidized Glutathione Is Not a Substrate of CydDC—Motility defects in a dsbB mutant can be corrected by the exogenous addition of cysteine, i.e. an oxidant (50), in apparent contradiction to the present results demonstrating the corrective effects of GSH, a reductant. We therefore sought evidence that a substrate of CydDC might be GSSG that forms during the transport assays. [35S]GSH from Amersham Biosciences is provided as an aqueous solution containing 10 mM DTT to maintain GSH in a reduced state. DTT was therefore first removed by solvent extraction with ethyl acetate, and the GSH was then oxidized to GSSG by exposure to air for 24 h. To determine whether the oxidation was complete, we employed DTNB, which undergoes oxidation in the presence of sulphydryl groups. The transport assay conditions used to determine [35S]GSSG uptake into everted membrane vesicles were identical to those employed for [35S]GSH, yet no uptake of GSSG into everted membrane vesicles of AN2342 was observed, even after the addition of 10 mM ATP (results not shown). We conclude that in everted vesicles CydDC supports uptake of [35S]GSH but not [35S]GSSG.

A gshA Mutant Assembles Cytochrome bd and Exogenous GSH Cannot Restore a Functional bd-type Oxidase in a cydD Mutant—A cydD mutant fails to synthesize cytochrome bd (25) and periplasmic cytochromes c (41). Because GSH is a substrate of the CydDC transporter, it may be directly required in cytochrome bd assembly. To test this, cytochrome bd assays were performed on strains MJF355 (gshA) and Frag1 (wild-type) grown aerobically as described previously (24). CO difference spectra (i.e. the spectrum of the carbonmonoxy ferrous form minus the reduced form) of intact cells showed a band at 644 nm in both the wild-type and gsh mutants, corresponding to the carbonmonoxy form of cytochrome d, at levels of ~0.05 nmol mg of protein−1 in both strains (not shown). Thus, even complete GSH deficiency does not cause loss of cytochrome bd, perhaps because the periplasmic requirement for reductant continues to be satisfied by the CydDC-dependent export of cysteine. To investigate the role of GSH in cytochrome bd assembly further, we determined whether addition of exogenous GSH could restore oxidase synthesis in a cydD mutant. The wild-type and cydD mutant strains were grown aerobically at 37 °C to stationary phase in media supplemented with GSH (0.1, 0.25, 0.5, 1, or 2 mM, final concentrations). The use of DTT to maintain reduced GSH was avoided, as a cydC mutant is sensitive to this reducing agent (40). At GSH concentrations above 0.1 mM, strain AN2343 (cydD) grew very poorly, and at 2 mM GSH growth was inhibited such that 2.5 liters of culture were required to gain enough cells to perform the cytochrome d assays. These mutant cells grown with 2 mM GSH revealed no cytochrome d.

This may be because the added GSH solutions, even though they were made up fresh prior to addition to the culture to minimize oxidation, are readily oxidized in the medium or periplasm. As a control, CO difference spectra recorded for the wild-type strain in the absence or presence of 2 mM GSH revealed a band at 644 nm, corresponding to the carbonmonoxy form of cytochrome d.

CydDC Expression Rescues DTT Sensitivity and Periplasmic Cytochrome Synthesis in a dsb Mutant Defective in Provision of Periplasmic Reductant—The multidomain transmembrane protein DsbD transports reductant to the predominantly oxidizing environment of the periplasm. We hypothesized that enhanced outward transport of GSH and/or cysteine might provide another route for reductant necessary for soluble cytochrome maturation in the periplasm and for restoration of DTT sensitivity, as do certain Ccm proteins (51). Both cydD and dsbD mutants are hypersensitive to DTT as reported before (14, 40), but the DTT sensitivity of the dsbD mutant (Fig. 4) and cydD mutant (not shown) was rescued by overexpression of wild-type cydDC genes on plasmid pRK1602 (28). Furthermore, although a dsbD mutant failed to synthesize spectrally detectable periplasmic cytochromes, as described before (52), transformation with pRK1602 resulted in the assembly of a periplasmic heme protein (Fig. 5) with γ- and α-bands at 427.5 and 561 nm, respectively, indicating the presence of cytochrome b. Presumably the periplasmic cytochrome bγδ of unknown function in E. coli (53). To determine the heme type present, the pyridine hemochromes of periplasmic fractions were made, which clearly revealed the presence of heme B (Fig. 5C, α-band at 556 nm). Although we cannot exclude the possibility of a minor heme C component in the α-region (54), the results clearly demonstrate a restoration of heme protein assembly in the periplasm.

DISCUSSION
Low molecular mass thiol-containing compounds play essential roles in many biochemical reactions (55). Together with thioredoxin, GSH is
one of the most important of such redox-active molecules, but its role in bacteria has not been studied in depth. In E. coli, GSH is dispensable for resistance to hydrogen peroxide and radiation (20), yet a deficiency can result in surprising consequences such as thiamine auxotrophy in Salmonella (56). In some GSH-dependent reactions, the GSH is known to be recycled in the cytoplasm, as in the GSH-dependent detoxification of N-ethylmaleimide (57). In other bacterial cases, GSH is "known to be secreted or leaked out into the periplasm" (22), but a pathway has not been identified and a molecule "probably larger than glutathione" is proposed to be the substrate of the OmpL channel (22). In a gshA mutant, defective in GSH synthesis, several periplasmic proteins were overproduced when cytoplasmic, membrane, and periplasmic proteins were labeled in vivo with monochlorobimane (58). Thus, a disruption of the cytoplasmic GSH biosynthetic pathway influences the periplasmic protein profile, and in the light of the present data this may be attributable to a loss of a periplasmic GSH pool.

We are unaware of any previous molecular and functional identification of a bacterial GSH transporter. Here, we report the transport of GSH by CydDC, an ABC-type transporter originally discovered because of its essential role in the assembly of cytochrome bd, a terminal respiratory oxidase in E. coli. Given the prevalence of ABC transporters in bacteria and the fact that some are homologues of the human multidrug transporter P-glycoprotein (59), it would be surprising if CydDC were the only GSH transporter in bacteria. Unlike the mitochondrial GSH transporter (60) or GSH transport by renal brush-border membranes (61), GSH export to the periplasm is unaffected by protonophores but is driven directly by ATP. Thus, GSH transport by CydDC is mechanistically similar to that of cystic fibrosis transmembrane conductance reg-

FIGURE 5. Hemeproteins assembled in the periplasm of a dsbD mutant transformed with the cydDC genes. A and B, difference spectra (reduced minus oxidized, Soret and α-bands, respectively) of periplasmic cytochrome(s) in a dsbD mutant transformed by pRKP1602 (4.8 mg of protein ml−1 after concentration). C, pyridine heme-chrome (reduced minus oxidized) spectrum of the periplasmic fraction in B. Spectra shown are each the sums of 12 replicate scans; ΔA bars denote values for an individual spectrum. Spectra were corrected for the baseline drift observed on adding dithionite and were smoothed using the 5-point (−2.5 nm) moving average feature of Cricket Graph III.
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Cytochrome $b_d$ genes, encoding cytochrome $b_d$ $\alpha$, in a cydC mutant results in significant restoration of cytochrome $b_d$ assembly (65). This might be explained by proposing that high levels of this oxidase facilitate the DsbB-mediated reoxidation of DsbA by electron transfer to this oxidase, which is remarkable for its high affinity for oxygen (67). In other words, the failure to assemble cytochrome $b_d$ is attributable solely to the inappropriate redox poise of the periplasm.

That the GSH/GSSG redox couple can directly influence disulfide bonding in proteins, presumably in the periplasm too, is illustrated by direct measurements of the redox state of the endoplasmic reticulum (5), where the GSH/GSSG ratios for the secretory pathway are in agreement with the redox optimum for protein-disulfide isomerase catalysis of oxidative folding of RNase, i.e. 1 mM GSH and 0.2 mM GSSG, equivalent to an optimum reduction potential of $-165$ mV. Similar analyses explain why the cytoplast of E. coli cannot properly fold recombinant proteins with disulfide bonds, where the typical GSH/GSSG ratio is in the range of 50:1–200:1 (5). The mechanism by which GSSG formed in the periplasm from the GSH that CydDC exports is not clear, but GSH reductase is not required in the case of the total E. coli cellular pool (68).

Acknowledgments—We thank I. Booth and D. Missiakas for strains and M. Johnson for technical support.

REFERENCES

1. Krebs, H. A. (1967) Adv. Enzyme Regul. 5, 409–434
2. Meister, A. (1988) J. Biol. Chem. 263, 17205–17208
3. Schafer, F. Q., and Buettner, G. R. (2001) Free Radic. Biol. Med. 30, 1191–1212
4. Fahey, R. C., Brown, W. C., Adams, W. B., and Worsham, M. B. (1978) J. Bacteriol. 133, 1126–1129
5. Wang, C., Sinskey, A. J., and Lodish, H. F. (1992) Science 257, 1496–1502
6. Hoober, K. L., Jones, B., White, H. B. L., and Thorpe, C. (1996) J. Biol. Chem. 271, 30510–30516
7. Cuzzoc, J. W., and Kaiser, C. A. (1999) Nat. Cell Biol. 1, 130–135
8. O’Brian, M. R., and Thony-Meyer, L. (2002) in Advances in Microbial Physiology (Poole, R. K., ed) pp. 257–318, Academic Press Ltd., London, England
9. Fabianek, R. A., Hennecke, H., and Thony-Meyer, L. (2000) FEMS Microbiol. Rev. 24, 303–316
10. Wunderlich, M., and Gloeckshuber, R. (1993) Protein Sci. 2, 717–726
11. Bardwell, J. C. A., McGovern, K., and Beckwith, J. (1991) Cell 67, 581–589
12. Kobayashi, T., Kishigami, S., Sone, M., Inokuchi, H., Mogi, T., and Ito, K. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 11857–11862
13. Kobayashi, T., and Ito, K. (1999) EMBO J. 18, 1192–1198
14. Missiakas, D., Schwager, F., and Raina, S. (1995) EMBO J. 14, 3415–3424
15. Raina, S., and Missiakas, D. (1997) Annu. Rev. Microbiol. 51, 179–202
16. Thomas, E. L. (1984) J. Bacteriol. 157, 240–246
17. Sherrill, C., and Fahey, R. C. (1998) J. Bacteriol. 180, 1454–1459
18. Vergauwen, B., Paswols, F., and Van Beeumen, J. J. (2003) J. Bacteriol. 185, 5555–5562
19. Chesney, J. A., Eaton, J. W., and Mahoney, J. R. (1996) J. Bacteriol. 178, 2131–2135
20. Greenberg, T. J., and Demple, B. (1986) J. Bacteriol. 168, 1026–1029
21. Parry, J., and Clark, D. P. (2002) FEMS Microbiol. Lett. 209, 81–85
22. Dartigalongue, C., Nkaido, H., and Raina, S. (2000) EMBO J. 19, 5990–5998
23. Georgiou, C. D., Fang, H., and Genin, R. B. (1987) J. Bacteriol. 169, 2107–2112
24. Poole, R. K., Williams, H. D., Downie, J. A., and Gibson, F. (1989) Gen. Microbiol. 135, 1865–1874
25. Poole, R. K., Hatch, L., Cleeer, M. W. J., Gibson, F., Cox, G. B., and Wu, G. (1993) Mol. Microbiol. 10, 421–430
26. Pittman, M. S., Corker, H., Wu, G. H., Binet, M. B., Moir, A. J. G., and Poole, R. K. (2002) J. Biol. Chem. 277, 49841–49849
27. Kogan, I., Ramjee Singh, M. L., Kidd, J. F., Wang, Y. C., Leslie, E. M., Cole, S. P. C., and Bear, C. E. (2003) EMBO J. 22, 1981–1989
28. Cruz-Ramos, H., Cook, G. M., Wu, G., Cleeer, M., and Poole, R. K. (2004) Microbiology 150, 3415–3427
29. Ferguson, G. P., Nikolaeff, Y., Mcllagan, D., Maclean, M., and Booth, I. R. (1997) J. Bacteriol. 179, 1007–1012
30. Krupp, R., Chan, C., and Missiakas, D. (2001) J. Biol. Chem. 276, 3696–3701
31. Stewart, V., and Parales, J. (1988) J. Bacteriol. 170, 1589–1597
32. Kita, K., Onishi, K., and Anraku, Y. (1984) J. Bacteriol. 159, 3375–3381
33. Willis, R. C., Morris, R. G., Giralagou, C., Schellenberg, G. D., Gerber, N. H., and Furlong, C. E. (1974) Arch. Biochem. Biophys. 161, 64–75
34. Kalninieks, U., Galinina, N., Bringer-Meyer, S., and Poole, R. K. (1998) FEMS Micro-

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VOLUME 280 • NUMBER 37 • SEPTEMBER 16, 2005
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J. Biol. Chem. 2005, 280:32254-32261.
doi: 10.1074/jbc.M503075200 originally published online July 22, 2005

Access the most updated version of this article at doi: 10.1074/jbc.M503075200

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