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Selenite Assimilation into Formate Dehydrogenase H Depends on Thioredoxin Reductase in *Escherichia coli*

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Escherichia coli **growing under anaerobic conditions produce H_2 and CO_2 by the enzymatic cleavage of formate that is produced from pyruvate at the end of glycolysis. Selenium is an integral part of formate dehydrogenase H (FDH_H), which catalyses the first step in the formate hydrogen lyase (FHL) system. The genes of FHL system are transcribed only under anaerobic conditions, in the presence of a σ^{54}-dependent transcriptional activator FhlA that binds formate as an effector molecule. Although the formate addition to the nutrient media has been an established procedure for inducing high FDH_H activity, we have identified a low-salt nutrient medium containing <0.1% NaCl enabled constitutive, high expression of FDH_H even without formate and D-glucose added to the medium. The novel conditions allowed us to study the effects of disrupting genes like *trxB* (thioredoxin reductase) or *gor* (glutathione reductase) on the production of FDH_H activity and also reductive assimilation of selenite (SeO_3^{2-}) into the selenoprotein. Despite the widely accepted hypothesis that selenite is reduced by glutathione reductase-dependent system, it was demonstrated that *trxB* gene was essential for FDH_H production and for labelling the FDH_H polypeptide with ^{75}Se-selenite. Our present study reports for the first time the physiological involvement of thioredoxin reductase in the reductive assimilation of selenite in *E. coli*.**

Key words: formate dehydrogenase H, selenite assimilation, thioredoxin reductase.

Abbreviations: FDH_H, formate dehydrogenase H; FHL, formate hydrogen lyase; SeCys, selenocysteine; SPS, selenophosphate synthetase; *gor*, glutathione reductase gene; *trxB*, thiredoxin reductase gene; GSH, reduced form of glutathione; GSSeSG, selenodiglutathione.

Escherichia coli can use a variety of terminal electron acceptors such as proton and nitrate for anaerobic respiration (*1–3*). In the absence of oxygen, formate is produced from pyruvate and it serves as a major electron donor for the anaerobic respiration (*4*). *Escherichia coli* formate dehydrogenase H (FDH_H) is a component of formate hydrogen-lyase (FHL) complex and delivers electrons from formate to hydrogenase 3, where protons are reduced to hydrogen molecule (*5*). FDH_H is a bacterial selenoprotein in which selenium is covalently bound as a selenocysteine (SeCys) residue that is co-translationally inserted at in-frame opal codon, UGA (*6*, *7*). Formation of SeCys-tRNA^{UGA} requires the highly reactive selenium donor compound, monoselenophosphate (HSe-PO_3^{2-}), which is synthesized from ATP and selenide (HSe:$^-$) by the catalysis of selenophosphate synthetase (SPS).

In contrast to the well-established pathway in which selenide (HSe:$^-$) is converted to monoselenophosphate and subsequently incorporated into SeCys-tRNA^{UGA} (*8*) or 2-selenouridine-containing tRNA (*9*), there is little information concerning the pathway by which selenite (SeO_3^{2-}) is reduced and transported to the bacterial SPS. Because selenium is present at an extremely low concentration compared to that of sulfur, a specific pathway for selenite reduction and transport is essential in the biosynthesis of FDH_H. The first step in the selenite assimilation in *E. coli* would most likely be stepwise reduction by thiols. Previous studies on chemical reduction of selenite proposed that SeO_3^{2-} can be reduced non-enzymatically by GSH to both GSSeSG and GSSeH at acidic conditions (*10*, *11*). In physiological pH, however, GSSeSG is quite unstable and generates a perselenide derivative, glutathioselenolate GSSe$^-$, which rapidly decomposes by further reactions with GSH to yield elemental selenium as the terminal product that is insoluble in aqueous solutions (*12–14*).

Alternatively, it is noteworthy to consider that cysteine residues of proteins are also reactive toward selenite, and they could be involved in reduction and transport of selenium. Thioredoxin is a small (12 kDa) ubiquitous

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protein with a redox-active dithiol/disulfide in the active site, and it operates together with NADPH and thioredoxin reductase (*15*). Selenite has been reported to interact with *E. coli* thioredoxin, causing an oxygen-dependent non-stoichiometric oxidation of NADPH in the presence of thioredoxin reductase (*16*). In anaerobic conditions, thioredoxin system is a good candidate for reducing and transporting selenium to the bacterial SPS.

Although such *in vitro* studies demonstrate possible biochemical mechanisms in detail, we cannot rule out the possibility that the physiological reactions might require unidentified factors and conditions, and accordingly *in vitro* experiments might be showing some artificial biochemical reactions. A resolution of this intriguing question may be approached by gene disruption technique, by which we can discern physiological significance of certain biochemical factor. Glutathione reductase and thioredoxin reductase constitute two of the major flows of reducing equivalents in *E. coli*. We constructed a glutathione reductase knockout mutant (*gor522::Tn10*) and a thioredoxin reductase knockout strain (*trxB::kan*) of *E. coli* MC4100. The wild-type strain and the *gor* mutant produced FDH_H activity but *trxB*-knockout strain clearly diminished the FDH_H activity. ^{75}Se-labelled selenite was specifically incorporated into FDH_H in wild-type and *gor*-mutant strains, whereas ^{75}Se -labelled FDH_H was not produced in the *trxB* mutant strain. Another line of supporting evidence was also obtained by another set of experiments; *E. coli* WL400 strain (*selD::crm*), in which the bacterial SPS gene (*selD*) is disrupted by chloramphenicol resistant gene, was complemented by a recombinant human lung *Sps2Cys* gene. The selenite assimilation study was carried out in a novel low-salt nutrient medium by which *E. coli* produces high activity of FDH_H in the absence of formate and D-glucose, which are normally added to the medium to facilitate FDH_H induction (*17*, *18*).

MATERIALS AND METHODS

Bacterail Strains, Media and Plasmids—Escherichia coli MC4100 and WL400 were used as the parent strains for disrupting *gor* and *trxB* genes. Peptone medium contained 2% (w/v) polypeptone, 2% D-glucose, 30 mM sodium formate, 0.5% NaCl, 100 mM potassium phosphate at pH7.5, supplemented with 10 μM Na_2MoO_4 and 1 μM Na_2SeO_3. A low-salt medium containing 1.6% (w/v) polypeptone, 1% yeast extract and 0.1% NaCl was termed as Stadtman medium in the present study. All the media used in the present study was prepared in Milli Q water, and pH was adjusted before sterilization as stated for each experiment. Human *Sps2* was cloned from the lung adenocarcinoma cells NCI-H441, and genetically modified to change the opal codon encoding SeCys60 (TGA) to Cys (TGT) (*19*). The pTrcHis2-TOPO expression vector (Invitrogen) was used to construct expression vectors for the human lung *Sps2Cys* gene and *E. coli trxB*. ^{75}Se-Selenite was purchased from the University of Missouri Research Reactor Facility; Columbia, MO, USA. General DNA manipulation was performed as described by Sambrook and Russel (*20*).

Construction of gor522::Tn10 *and* trxB::kan *Mutants—* The *gor* gene in MC4100 and WL400 strains was disrupted by inserting tetracycline resistance gene *Tn10*(tet^R) by P1 transduction (*21*) using a temperature sensitive bacteriophage P1-cmc that carries *gor522::Tn10*(tet^R) gene derived from *E. coli Origami* (Novagen). The *trxB* gene in MC4100 and WL400 was disrupted by kanamycin resistance marker using the temperature sensitive P1 bacteriophage that carries t*rxB::kan* gene derived from *E. coli* A304 (*E. coli* Genetic Resource Center, New Haven, CT, USA).

Anaerobic Growth and Assay for FDH_H Activity— Escherichia coli strains were grown overnight in 5 ml of LB medium under aeration at 37°C, and 100 μl broth (300 μl for *trxB* mutant) was inoculated in 5 ml of Stadtman medium in glass vials. The silicon-lined screw cap was screwed on tightly, and the anaerobic culture was carried out at 30°C for 20 h. Then, cells in 1 ml of the culture were harvested by centrifugation at 19,000 xg for 1 min, washed and suspended in 100 mM tris–borate buffer, pH 7.5, containing 5 mM $MgSO_4$. The cell suspension was transferred to a 1.5 ml sample tube, and added with 2 mM benzyl viologen and 20 mM sodium formate in 100 mM potassium phosphate buffer, pH 7.0. The FDH_H activity was assayed by the increase in the absorbance at 600 nm, which was observed within 10–15 min. One unit of FDH_H activity is defined as the amount of protein that reduces 1 μmol of benzyl viologen ($\varepsilon = 7{,}400\ M^{-1}\ cm^{-1}$) in 1 min. Total protein was assayed by Lowry method after the cell suspension was thoroughly dissolved in 5% SDS. The FDH_H activity was also detected by the benzyl viologen agar overlay method (*22*).

Incorporation of ^{75}Se-labelled Selenite into E. coli *FDH_H—Escherichia coli* strains were grown anaerobically at 30°C for 24 h in 5 ml of Stadtman medium supplemented with 1 μM Na_2SeO_3 that included 20 μCi of ^{75}Se-labelled selenite. The cells were harvested by centrifugation (10,000 × g), suspended in 50 μl of 10 mM Tris–HCl buffer, pH 8.0, and lysed with an equal volume of BugBuster (Novagen). Aliquots of the cell extract containing 12–18 μg of protein were combined with the sample buffer for SDS-PAGE and heated at 95°C for 3 min, and analysed by 12% SDS-PAGE and PhosphorImager.

Detection of FDH_H Transcript in gor-, trxB- *and WT Strains*—The total RNA fractions were prepared from the wild-type, *gor* and *trxB* strains using a RNeasy Mini kit (Quiagen). A cDNA corresponding to FDH_H gene was synthesized from these RNA samples using Moloney murine leukaemia virus reverse transcriptase (Superscript II, Invitrogen), and the cDNA was quantitated by real-time PCR using SYBR green and iCycler (BioRad).

RESULTS

Low-salt Medium for Constitutive FDH_H Expression— In previous studies, FDH_H activity is normally expressed in *E. coli* growing in nutrient media with carbon sources such as formate and D-glucose added (*23*). We first confirmed the effect of D-glucose and formate on FDH_H production at 30 and 37°C. The FDH_H activity produced in *E. coli* MC4100, which was grown anaerobically in the peptone medium, was 0.110 ± 0.011 U/mg at 30°C and 0.095 ± 0.014 U/mg at 37°C. However, their activity was undetectable when sodium formate or D-glucose was

Table 1. **Effects of pH, temperature and carbon source on FDH_H production in *E. coli* MC4100.**

Growth conditions		FDH_H activity (U/mg protein)
pH 7.5		
30°C		0.060 ± 0.005
30°C	+30 mM formate	0.055 ± 0.007
37°C		0.046 ± 0.005
37°C	+30 mM formate	0.034 ± 0.003
pH 6.5		
30°C		0.102 ± 0.014
30°C	+0.5% D-glucose	0.062 ± 0.003
37°C		0.049 ± 0.004
37°C	+0.5% D-glucose	0.059 ± 0.007

Stadtman medium supplemented with 100 mM potassium phosphate at pH 7.5 or 6.5.

omitted from the peptone medium. The results were consistent with the previous report that the production of FDH_H activity in *E. coli* requires formate and D-glucose added in the basal nutrient media (*23*). We then examined various culture conditions including medium components, temperature, salt concentration and pH to develop a medium that allows constitutive expression of FDH_H without D-glucose and formate. We identified that lower salt concentration, NaCl <0.1%, most significantly affected production of FDH_H activity in the absence of D-glucose and formate (Table 1). Under these conditions, 30 mM formate rather decreased the FDH_H activity when the cells were grown either at 30 or 37°C. Furthermore, the initial pH at 6.5 was another important factor that increased the FDH_H activity, which was consistent with a previous report (*24*). The highest FDH_H activity was thus obtained when *E. coli* was grown anaerobically in the low-salt medium at pH 6.5 without formate and D-glucose at 30°C (Table 1). Despite the high FDH_H activity assayed by BV-reduction, vigorous gas formation was not observed in the low-salt cultures. The increased pressure due to the gas evolution had frequently cracked the glass vials during anaerobic culture. Therefore, suppression of gas formation has provided us an experimental convenience by which radioactive selenite can be added to the anaerobic culture and safely incubated until the late stationary phase by 24 h incubation.

Growth and FDH_H Activity of gor- *or* trxB- *Mutants*—To elucidate the chemical nature of reducing equivalents utilized in the reductive selenite assimilation, we disrupted the glutathione reductase gene (*gor*) or thioredoxin reductase gene (*trxB*) by P1 transduction. Gene disruption was confirmed by undetectable enzyme activities in the cell-free extract and PCR amplification using sets of primers for *gor* and *trxB* genes; disrupted gene in the chromosomal DNA has increased the length of the sequence due to the insertion of resistance genes (Fig. 1A). The growth of wild-type, *gor::Tn10* and *trxB::kan* strains were examined by the time course study using the Stadtman medium. The wild-type strain and the *gor::Tn10* mutant reached OD_{600} 0.5 by 5 and 4 h culture, respectively, and continued to grow gradually to 0.6 within the 18 h incubation. In contrast, the *trxB::kan* mutant strain could not grow over 0.35 even after the

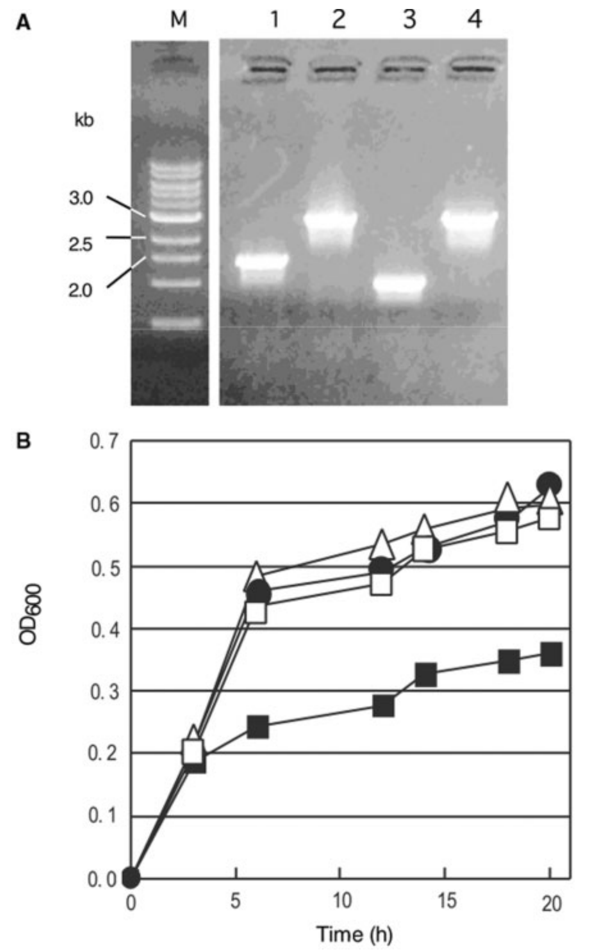

Fig. 1. **Disruption of *gor* and *trxB* genes in *E. coli* MC4100 by P1 transduction.** (A) The *gor* gene was amplified from genome DNAs of MC4100 (Lane 1) and of *gor522::Tn10* strain (Lane 2). The *trxB* gene was amplified from MC4100 (Lane 3) and from *trxB::kan* (Lane 4). (B) The effect of the gene knock-out on the anaerobic growth of the wild-type strain MC4100 (open triangle), *gor522::Tn10* (filled circle), *trxB::kan* (filled square) and *trxB::kan* / pTrc-*trxB* (open square) strains. The strains were grown in the Stadtman medium at 30°C under anaerobic conditions.

mutant reached a stationary phase by 4 h culture (Fig. 1B).

The production of FDH_H activity was assayed by the formate-dependent benzyl viologen (BV)-reducing activity of the whole *E. coli* cells (Table 2). The bacterial cells expressing the FDH_H developed deep blue color when overlaid with benzyl viologen and formate (Fig. 2). Wild-type strain MC4100 and the *gor* mutant produced FDH_H activity, while *trxB* mutation resulted in the loss of the enzyme activity. Because selenoprotein and seleno-tRNAs are normally not produced under the aerobic cultures of *E. coli*, the lack of FDH_H activity in the cells of *trxB* mutant might be attributed to the repression of the bacterial SPS due to the more-oxidizing redox states. Therefore, we carried out the similar experiments with the WL400 strain, in which the bacterial *Sps* was complemented by a recombinant human lung *Sps* gene. *Escherichia coli* WL400 and its *gor*-mutant produced

FDH_H activity but no activity was detected in cells of WL400 *trxB* mutant that harbours a recombinant human lung *Sps2Cys*. Because the recombinant *Sps2Cys* gene was expressed under the pTrc promoter, it is unlikely that the loss of FDH_H activity was due to the suppression of bacterial SPS in the cells of *trxB* mutants.

Incorporation of ^{75}*Se-labelled Selenite into* E. coli *FDH_H*—Labelling experiments using a radioactive ^{75}Se-selenite was carried out to clarify whether the *trxB* was essential for the synthesis of selenoprotein or the mutation has only caused inactivation of the oxygen-sensitive FHL system. *Escherichia coli* cells were grown overnight in the low-salt medium containing ^{75}Se-labelled selenite, and proteins in the cell extract were separated on SDS/12% PAGE gels for the subsequent visualization by PhosphorImager analysis (Fig. 3).

Extract from the wild-type MC4100 and MC4100*gor::Tn10(tet^R)* (lanes 1 and 3) clearly showed the specific incorporation of ^{75}Se in FDH_H and into the smaller molecular mass region, which presumably represents the ^{75}Se-labelled tRNA (*25*). *Escherichia coli* WL400 strain, which is incapable of producing monoselenophosphate, showed no specific incorporation of ^{75}Se into FDH_H and tRNA (lane 2). There observed non-specific labelling of ^{75}Se in the broad mass range of 14–50 kDa, whose chemical identity is not elucidated in the present study. *Escherichia coli Sps* gene can be partly complemented by recombinant pTrc-*Sps2Cys* gene as demonstrated in WL400/pTrc-*Sps2Cys* (lane 5) and WL400*gor::Tn10(tet^R)*/pTrc-*Sps2Cys* (lane 6), but the radioactivity was not incorporated into the tRNA region, suggesting that the radioactivity was less efficiently incorporated in tRNA when the bacterial SPS was replaced by human lung *Sps2Cys*.

The *trxB::kan* strains failed to incorporate ^{75}Se-labelled selenite into FDH_H as observed for MC4100*trxB::kan* (lane 4) and WL400*trxB::kan/Sps2Cys* (lane 7). It is interesting to note that cell extracts from these *trxB::kan* strains represented labelling proteins in the mass region around 20 kDa (lanes 4 and 7). Our preliminary experiment indicated that the radioactive compounds in that mass region were resistant to RNase treatment, but chemical property of the ^{75}Se-labelled molecules was not investigated any further in the present study. Based on a previous report that *E. coli* thioredoxin can be readily oxidized by selenite *in vitro* (*16*), we currently speculate that the lack of thioredoxin reductase might have caused accumulation of thioredoxin

Table 2. **FDH_H activity of *E. coli* MC4100, WL400 strains and the *gor522::Tn10(tet^R)*, *trxB::kan* mutants.**

Strain/Plasmid	Genotype	FDH_H activity (U/mg protein)
MC4100	wild-type	0.113
MC4100 Δ*gor*	*gor522::Tn10(tet^R)*	0.094
MC4100 Δ*trxB*	*trxB::kan*	0.008
MC4100 Δ*trxB*/pTrc*TrxB*	*trxB::kan*	0.036[a]
WL400	*selD::crm*	not detected
WL400/*Sps2Cys*		0.097
WL400 *Δgor/Sps2Cys*	WL400 *gor522::Tn10(tet^R)*	0.084
WL400 *ΔtrxB/Sps2Cys*	WL400 *trxB::kan*	0.006

Cells were grown anaerobically, and FDH_H activity in cell extract was measured as described in Materials and Methods section.
[a]MC4100*ΔtrxB* was complemented with a recombinant *trxB* gene expressed from pTrcHis2-TOPO vector (Invitrogen).

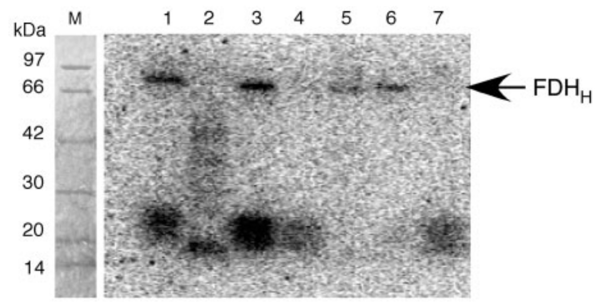

Fig. 3. **PhosphorImager analysis of SDS/12% PAGE gel containing ^{75}Se-labelled cell extract.** Cell-free extract from *E. coli* MC4100 (lane 1), WL400 (lane 2), MC4100 *gor522::Tn10* (lane 3), MC4100*trxB::kan* (lane 4), WL400/pTrc-*Sps2Cys* (lane 5), WL400*gor522::Tn10*/pTrc-*Sps2Cys* (lane 6) and WL400 Δ*trxB*/pTrc-*Sps2Cys* (lane 7).

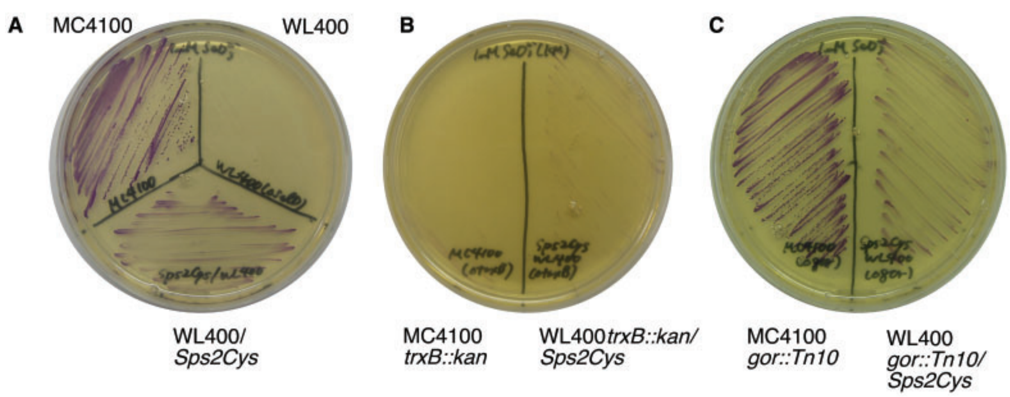

Fig. 2. **Benzyl viologen-reducing activity of *trxB/gor* mutant strains.** (A) *Escherichia coli* strains MC4100, WL400, WL400/*Sps2Cys* were grown anaerobically on the Stadtman medium, and assayed for FDH_H activity. (B) The *trxB* mutant strains MC4100Δ*trxB* and WL400Δ*trxB/Sps2Cys* could grow on the Stadtman medium containing 50 μg/ml kanamycin, but did not show FDH_H activity by BV assay. (C) The *gor* mutants grown in the presence of 50 μg/ml tetracycline showed FDH_H activity.

molecules that were dimerized (Trx-S-^{75}Se-S- Trx) or conjugated with other thioredoxin family proteins through seleno-trisulfide linkage (-S-^{75}Se-S-). It has been reported that such chemical species can survive the reductive conditions employed for SDS-PAGE (*26*). Further investigation is required to identify the product of ^{75}Se-labelled compounds formed in the *trxB*-mutant cells.

Transcription of FDH$_H$ Gene in Wild-type, gor-, *and* trxB- *Strains*—It has been suggested that the *trxB* mutation might alter the intracellular redox state, and thus make the cytoplasm rather oxidizing state even under anaerobic conditions (*27*). Because FHL complex including FDH$_H$ is produced only under anaerobic conditions, the *trxB* mutation might have resulted in transcriptional regulation due to the elevated redox potential in the cells. Real-time PCR and northern blot analysis were carried out for quantitative detection of the transcript for FDH$_H$. The relative amounts of FDH$_H$ transcript for wild-type, *gor*, and *trxB* strains were 1, 1.60 and 1.86, respectively. The result obviously ruled out the possibility that *trxB* mutation suppressed the transcription of FDH$_H$ through the redox equilibrium in the cells. The result also demonstrated that the FDH$_H$ gene was transcribed when the *E. coli* strains were grown in the low-salt medium without formate and D-glucose. Northern blot analysis also confirmed the production of mRNA for FDH$_H$ in *trxB* mutant as well as in *gor* mutant and wild-type strains (data not shown).

DISCUSSION

FHL complex is highly oxygen sensitive and their genes encoded in *hyc* operon are transcribed only under anaerobic conditions and in the absence of other electron acceptors such as nitrate. In this study, we attempted to identify novel regulatory factors and biochemical requirements for the anaerobic expression of the selenoprotein, FDH$_H$, which catalyses the first step in the FHL system. Elucidation of novel biochemical factors that regulate the bacterial hydrogen fermentation can lead to a key innovation for developing an economic fuel cell that depends on hydrogen, which is currently a very expensive energy source (*28*). Under the low-salt conditions, we identified that FDH$_H$ was expressed constitutively in the absence of formate, which is an effector molecule for the transcriptional factor FhlA to be activated (*29–32*). The mechanism behind the formate-independent de-repression of FHL system under the low-salt medium has yet to be elucidated, and it would provide an interesting subject for the investigation by DNA microarray, which allows us to examine a global gene expression under anaerobic culture conditions.

The constitutive expression of FHL system in a novel culture conditions allowed us to characterize the reductive assimilation of selenite, which has long remained as an enigma in the selenium metabolism. In the present study, we disrupted either gene of glutathione reductase (*gor*) or thioredoxin reductase (*trxB*), which provides two of the major flows of reducing equivalents in *E. coli* MC4100, and examined their effects on production of FDH$_H$ activity and the selenite assimilation under the anaerobic conditions. Despite the widely accepted hypothesis that selenite is reduced by glutathione reductase-dependent system (*10*), our results indicated that the *gor* mutation did not affect the anaerobic growth of *E. coli*, nor did it disturb the FDH$_H$ production in the MC4100 strain and in the WL400 strain that is complemented by human lung *Sps2Cys* gene. A previous study has reported that essentiality of glutathione reductase may not to be very high for the normal growth of *E. coli*, and it might even be unnecessary to keep glutathione reduced in the cells unless the cells are exposed to some oxidative stress (*33*). Because the amount of intracellular GSH under the anaerobic growth has not been determined in the present study, we cannot rule out the possibility that reduced form of glutathione was abundantly synthesized in the *gor* mutant strains, and GSH could be involved in selenite assimilation. In fact, an *in vitro* study has proposed the involvement of sulfur-transferases in the reductive selenite assimilation in the presence of reduced form of glutathione; rhodanese (EC 2.8.1.1) or glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12) reacted with selenite in the presence of excess GSH at neutral pHs to generate a stable selenium–protein complex (*34*, *35*). The selenium-bound protein could effectively replace the high concentrations of selenide normally used in the SPS assay, suggesting that the reaction of SeO_3^{2-} with GSH could produce a stable selenium donor if only such a protein existed and served as a selenium-carrier protein for the bacterial SPS.

Our results on *trxB* mutation clearly showed that *trxB* was required for the formation of selenocysteine-containing FDH$_H$ polypeptide in *E. coli*, but there still remains possibility that *trxB* might be involved in a process other than the reductive assimilation of selenite. The catalysis of FDH$_H$ essentially depends not only on the SeCys residue but it also requires iron–sulfur complex and molybdopterin guanine dinucleotide, which are assembled in the active site (*36*). Therefore, the formation of catalytically active FDH$_H$ requires not only the co-translational insertion of SeCys, but it also needs post-translational implementation of these co-factors that might need reducing equivalents. If these prosthetic groups were not properly implemented in the FDH$_H$ protein, the selenoprotein as a premature enzyme might have been eliminated by proteolysis. Although the biogenesis of iron–sulfur cluster [Fe_4–S_4] would essentially involve binding and transport of sulfur and iron, the process appears more likely to utilize reducing equivalents supplied by ferredoxin system (*37*). The biogenesis and installation of molybdopterin guanine dinucleotide into FDH$_H$ might remain as a possible candidate for the *trxB*-involvement, but it has already been shown that L-cysteine serves as the direct sulfur source in molybdopterin synthesis in *E. coli* (*38*). Further studies are now in progress to establish the *trxB*-dependent selenite assimilation by a reconstituted biochemical reaction that involves thioredoxin, thioredoxin reductase and the bacterial SPS to demonstrate the *in vitro* formation of monoselenophosphate on ^{31}P-NMR.

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