

## Low Ubiquinone Content in *Escherichia coli* Causes Thiol Hypersensitivity

H. ZENG, I. SNAVELY, P. ZAMORANO, AND G. T. JAVOR\*

Department of Biochemistry, Loma Linda University School of Medicine,  
Loma Linda, California 92350

Received 15 December 1997/Accepted 9 May 1998

**Thiol hypersensitivity in a mutant of *Escherichia coli* (IS16) was reversed by complementation with a plasmid that carried the *ubiX* gene. The mutant had low ubiquinone content. Complementation elevated the ubiquinone level and eliminated thiol hypersensitivity. Analysis of chromosomal *ubiX* genes indicated that both parent and mutant strains were *ubiX* mutants. The low ubiquinone content of IS16 was possibly caused by a *ubiD ubiX* genotype. A *ubiA* mutant also exhibited thiol hypersensitivity. Neither IS16 nor the *ubiA* mutant strain could produce alkaline phosphatase (in contrast to their parent strains) after 2 h of induction, thus showing Dsb<sup>-</sup> phenotypes. The phenomena of thiol hypersensitivity and low ubiquinone content may be linked by their connections to the periplasmic disulfide bond redox machinery.**

The neutral water-soluble thiols 2-mercaptoethanol, 1-thioglycerol, and dithiothreitol inhibit gram-positive and gram-negative bacteria at millimolar concentrations (19, 21, 24). Although the precise mechanism of growth inhibition is not understood, it is known that several processes are affected. These include the lowering of intracellular concentrations of S-adenosylmethionine (14), inhibition of aerobic respiration (15), and possibly interference with the formation of disulfide bonds of periplasmic and outer membrane proteins (4, 27).

Exposure of aerobically growing *Escherichia coli* to exogenous thiols also results in a pleiotropic reductive stress response, which includes elevation of riboflavin and porphyrin syntheses (16), blockage of septum formation (18), and changes in the expression of hundreds of genes (17, 20).

In an attempt to look for genes which may be regulating this complex response, we searched for thiol-hypersensitive mutants. The search was based on the rationale that should such a gene(s) exist, its (their) inactivation would likely yield a thiol-hypersensitive phenotype.

### MATERIALS AND METHODS

**Reagents.** Amino acids, nucleotides, monosaccharides, coenzyme Q<sub>10</sub>, and dithiothreitol were purchased from Sigma Chemical Co., St. Louis, Mo. Hexane, petroleum ether, and 1-thioglycerol were bought from Aldrich Chemical Co., Milwaukee, Wis. The Wizard genomic purification kit and the Wizard Minipreps and Wizard PCR Preps DNA purification systems were from Promega Corp., Madison, Wis. Cloned *Pfu* DNA polymerase was obtained from Stratagene, La Jolla, Calif.

**Bacterial strains and plasmids.** Strains and plasmids are listed in Table 1.

**Growth media.** All experiments were performed on aerobically growing cells, at 37°C, in Davis minimal salts medium (12). Glucose (0.3% [wt/vol]) was the carbon source. The media were supplemented for strains THU and IS16 and its derivatives with uracil plus thymine at 20 µg/ml each and with histidine at 40 µg/ml. The growth media for strain IS16B1 and all plasmid-bearing strains were supplemented with 100 µg of ampicillin per ml. *dsb* mutant strains were supplemented with 25 µg each of leucine, isoleucine, and valine per ml and 2.5 µg of cystine per ml.

**Growth inhibition studies.** Growth inhibition studies were essentially carried out as described before (15, 21), except that the absorbance was monitored at 540 nm (*A*<sub>540</sub>).

**Alkaline phosphatase assay.** Cultures were grown in Davis minimal salts medium until the *A*<sub>540</sub> was 0.4 to 0.5. The bacteria were centrifuged and washed twice with low-phosphate-containing Davis medium (Davis medium containing only 10<sup>-4</sup> M K<sub>2</sub>HPO<sub>4</sub>) at room temperature. The cells were resuspended in low-phosphate Davis medium containing all growth supplements at their initial volumes and were incubated at 37°C with shaking. At 20- or 30-min intervals, 0.1-ml aliquots were collected into 1.9 ml of ice-cold 1 M Tris-HCl buffer (pH 8.0). The enzyme assays were done as described by Brickman and Beckwith (6) except that *A*<sub>540</sub> was used instead of *A*<sub>600</sub>.

**Measurement of coenzyme Q<sub>8</sub>.** Coenzyme Q<sub>8</sub> was routinely extracted by the method of Krivankova and Dadak (23). For its use as a high-performance liquid chromatography (HPLC) marker and for the purpose of quantitation, an extracted sample was further purified by thin-layer chromatography. On a Whatman linear-K silica gel thin-layer plate, with a 70:30 chloroform-light petroleum ether solvent mixture, coenzyme Q<sub>8</sub> had an *R<sub>f</sub>* value of 0.71. This substance was eluted from the plate with methanol, and its concentration was determined by its Δ*A* (oxidized-reduced)<sub>275</sub>, with 12.7 as the millimolar extinction coefficient (10).

Quantitation of coenzyme Q<sub>8</sub> was performed by the HPLC method of Anderson (3). A Spherisorb octyldecyl silane 2 column (manufactured by Phenomenex Co., Torrance, Calif.) was used. The mobile phase was 10% (vol/vol) *n*-hexane in methanol, at a flow rate of 0.6 ml/min. Ubiquinone was detected at 275 nm, with a V<sup>4</sup> variable UV-visible detector (manufactured by ISCO, Inc., Lincoln, Neb.) under the control of Dynamax software from Rainin Instrument, Inc., Woburn, Mass.

**PCR.** Genomic DNA was isolated with the Promega Wizard Genomic DNA kit. Cloned *Pfu* DNA polymerase purchased from Stratagene Cloning Systems was used. The *ubiX* genes of strains THU and IS16 were isolated by PCR, with the following primers: forward, 5'-ttcgaagcagtgcaacgtcagagcg-3', and reverse, 5'-gaattcaaacagggcaacagcggag-3'. (The 5' end of the forward primer was given a *Hind*III cutting site, and that of the reverse primer was given an *Eco*RI cutting site). The primers were synthesized on an Applied Biosystems 394 DNA synthesizer. The products of PCR were purified with the Promega Wizard PCR Preps DNA purification kit. They were sequenced on an Applied Biosystems Model 373 sequencer at the Center for Molecular Biology and Gene Therapy of Loma Linda University.

The PCR fragments were ligated into pBR322 plasmids by a standard protocol (30).

### RESULTS

**Isolation of a thiol-hypersensitive derivative of *E. coli* THU.** A culture of strain THU was mutagenized by nitrosoguanidine (1), and the cells were grown on minimal glucose plates containing 25 mM 1-thioglycerol. The smallest colonies were replica plated onto minimal medium- and minimal medium-plus-25 mM-1-thioglycerol-containing dishes. For controls, nonmutagenized THU colonies were replica plated as well. Colonies which were normal size on minimal plates but small on thioglycerol plates were isolated for further study. The isolate exhibiting the greatest hypersensitivity to 1-thioglycerol, named IS16, was used for further studies.

\* Corresponding author. Mailing address: Department of Biochemistry, Loma Linda University School of Medicine, Loma Linda, CA 92350. Phone: (909) 796-7311, ext. 48663. Fax: (909) 824-4887. E-mail: gjavor@cmail.llu.edu.

TABLE 1. *E. coli* strains and plasmids used in this study

Strain or plasmid	Relevant description or genotype	Source
<i>E. coli</i> strains		
THU	<i>thy-43 his-67 pyr-37</i> ; colicin resistant	S. S. Cohen (7)
IS16	Mutagenized (1) derivative of THU	This study
IS16B1	IS16 with pPZ2	This study
AN387	F <sup>-</sup> ; wild-type <i>E. coli</i>	Catherine Clarke
AN385	F <sup>-</sup> ; <i>ubiA420</i>	Catherine Clarke
JCB 570	MC1000 [ <i>araD139</i> Δ( <i>araABC-leu</i> )7679 <i>galU galK</i> Δ( <i>lac</i> )X74 <i>rpsL thi</i> ] <i>phoR</i> Tet <i>zih-12::Tn10</i>	James C. A. Bardwell
JCB 571	JCB 570 <i>dsbA::kan1</i>	James C. A. Bardwell
JCB 758	JCB 570 <i>dsbA::kan1 dsbB::kan5</i>	James C. A. Bardwell
JCB 789	JCB 570 <i>dsbB::kan5</i>	James C. A. Bardwell
Plasmids		
pPZ2	pBR322 with a 1,282-bp <i>E. coli</i> chromosomal insert into the <i>Bam</i> HI site	This study
pHZ1	pBR322 with a 699-bp <i>E. coli</i> THU chromosomal insert into the <i>Eco</i> RI- <i>Hind</i> III site	This study
pHZ2	pBR322 with a 699-bp <i>E. coli</i> IS16 chromosomal insert into the <i>Eco</i> RI- <i>Hind</i> III site	This study

Strain IS16 had no additional growth requirements. Its mean growth rate (0.53 doubling/h) was lower than that of THU (0.87 doubling/h) in minimal glucose medium. It had a longer adaptation lag period when shifting down from glucose to lactate or malate and could not utilize succinate for growth.

Inhibitory activities by exogenous 1-thioglycerol and dithiothreitol for strains THU and IS16 were compared. Percents inhibition of the mean growth rates were determined in a series of growth experiments, as described earlier (14), and the results are shown in Fig. 1. The experiments were performed on cells growing aerobically in minimal salts medium, with glucose as the carbon energy source. It can be seen that strain IS16 was substantially more inhibited than its parent strain THU by both thiols. This hypersensitivity was seen only under aerobic conditions. Thiols are less inhibiting to *E. coli* under anaerobic conditions (15), and both strains were inhibited to a similar extent (results not shown).

Exogenous methionine provides significant protection against thiols (14) by increasing the *S*-adenosylmethionine pool of the cells. This protection remained for strain IS16, so that in the

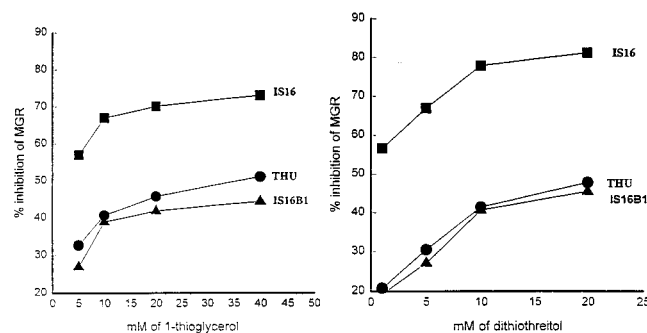


FIG. 1. Inhibition by 1-thioglycerol and dithiothreitol of growth of strains THU, IS16, and IS16B1. The mean growth rates (MGR) of cultures in the presence of the indicated concentrations of thiols were compared with the MGR of untreated cultures.

**purF:**  
 D L N D D A K A V Q R R Q  
 GAT CTG.....564 nucleotides ..... AAT GAT GAC GCC AAA GCA GTG CAA CGT CAG  
 N E V E N L E M H N E G STOP  
 AAC GAA GTG GAA AAT CTC GAA ATG CAT AAC GAA CGA TGA tgcctctgaggtgccc  
**ubiX:**  
 M K R  
 gtctgcacctgactgcaactcccgcaaaactctgcaagctgctgcaagctgacaggcaactatt ATG AAA CGA  
 L I V G I S G A S G A I Y G V R L  
 CTC ATT GTA GGC ATC AGC GGT GCC AGC GGC GCG ATT TAT GGC GTG CGC TTA  
 L Q V L R D V T D I E T H L V M S  
 TTA CAG GTT CTG CGC GAT GTC ACA GAT ATC GAA ACG CAT CTG GTG ATG AGC  
 Q A A R Q T L S L E T D F S L R E  
 CAG GCA GCG CGC CAG ACC TTA TCC CTC GAA ACG GAT TTT TCT CTG CGC GAA  
 V Q A L A D V T H D A R D I A A S  
 GTG CAG GCA TTA GCC GAT GTC ACG CAC GAT GCG CGC GAT ATT GCC GCC AGC  
 I S S G S F Q T L G M V I L P C S  
 ATC TCT TCC GGT TCT TTC CAG ACG CTG GGG ATG GTG ATT TTA CCC TGT TCA  
 I K T L S G I V H S Y T D G L L T  
 ATC AAA ACC CTT TCC GGC ATT GTC CAT AGC TAT ACT GAT GGC TTA CTG ACC  
 R A A D V V L K E R P L V L C V  
 CGT GCG GCA GAT GTG GTG CTG AAA GAG CGT GCG CGC TTG GTG CTC TGC GTG  
 R E T P L H L G H L R L M T Q A A  
 CGT GAA ACA CCA TTG CAC TTA GGC CAT CTG CGT TTA ACT GAT ACT GCG GCA  
 E I G A V I M P P V P A F Y H R P  
 GAA ATT GGT GCG GTG ATT ATG CCT CCC GTT CCG GCG TTT TAT CAT CGC CGG  
 Q S L L D D V I N Q T V N R V L D Q  
 CAA TCC CTT GAT GAT GTG ATA AAT CAG ACG GTT AAT CGT GTT CTT GAC CAG  
 F A I T L P E D L F A R W Q G G A STOP  
 TTT GCG ATA ACC CTT CCT GAA GAT CTC TTT GCC CGC TGG CAG GGC GCA TAA  
 ctccctgttgcctgtttcaggccaatttgca.....

FIG. 2. Nucleotide sequence of *dedF* (*ubiX*) and its flanking regions. The *Bgl*II cutting sites are double underlined. Plasmid pPZ2's chromosomal insert sequence begins at the 5' end with G and ends at the 3' end with A. The abbreviated 3' end of *purF* shows only 69 nucleotides. The single-underlined sequences bracket the portions of the chromosome that were isolated by PCR from IS16 and THU and were inserted into pBR322 to form pHZ1 and pHZ2. Codon 98 in *dedF* (AGC), printed in boldface, is the site of the mutation in IS16 (AGA).

presence of 50 μg of methionine per ml thiol hypersensitivity could not be shown (results not shown).

**Complementation of thiol hypersensitivity.** Strain IS16 was transformed with a pBR322-based *E. coli* chromosomal library of *Bgl*II-cut fragments, and a transformant which lost its thiol hypersensitivity was isolated. This strain, IS16B1, contained a pBR322 plasmid with a 1,267-bp chromosomal fragment inserted in the *Bam*HI site. This plasmid was named pPZ2.

The chromosomal insert in pPZ2 was sequenced, and with the help of the Blast program of the National Biomedical Library, its location on the *E. coli* chromosome was determined. It was at the 50-min segment of the *E. coli* chromosome and contained the 3' end of the *purF* gene and the *dedF* gene (minus 8 codons from its 3' end) (29) (Fig. 2). The nucleotide sequence of *dedF* shown in Fig. 2 is somewhat at variance with that in an earlier publication (29) but is in complete agreement with the latest sequence data (5).

The *dedF* gene's sequence is identical to that of the *ubiX* gene of *Salmonella typhimurium*, which codes for the enzyme polyprenyl *p*-hydroxybenzoate carboxylase (13, 25). Located at around 86 min of the *E. coli* chromosome is the *ubiD* gene, which codes for 3-octaprenyl-4-hydroxybenzoate decarboxylase, an enzyme functionally analogous to the product of *Salmonella's ubiX* gene. It appears, therefore, that *E. coli* possesses two distinct genes, whose products catalyze the conversion of 3-octaprenyl-4-hydroxybenzoate to 2-octaprenyl phenol in the ubiquinone biosynthetic pathway (25). Studies with *ubiD* mutants suggest that in wild-type cells 80% of the enzyme activity is due to the *ubiD* gene product and 20% is due to the *ubiX* product (25). Although the *ubiD* mutation has been mapped to the 86-min segment of the *E. coli* chromosome (9), and the nucleotide sequence of that region is known (5, 11), the actual location of the gene is yet to be found (26).

**Measurement of ubiquinone content of strain IS16.** Since the complementing plasmid contained only one functional gene, *dedF* (*ubiX*), a ubiquinone-biosynthetic gene, the ubiquinone contents of strains THU, IS16, and IS16B1 were mea-

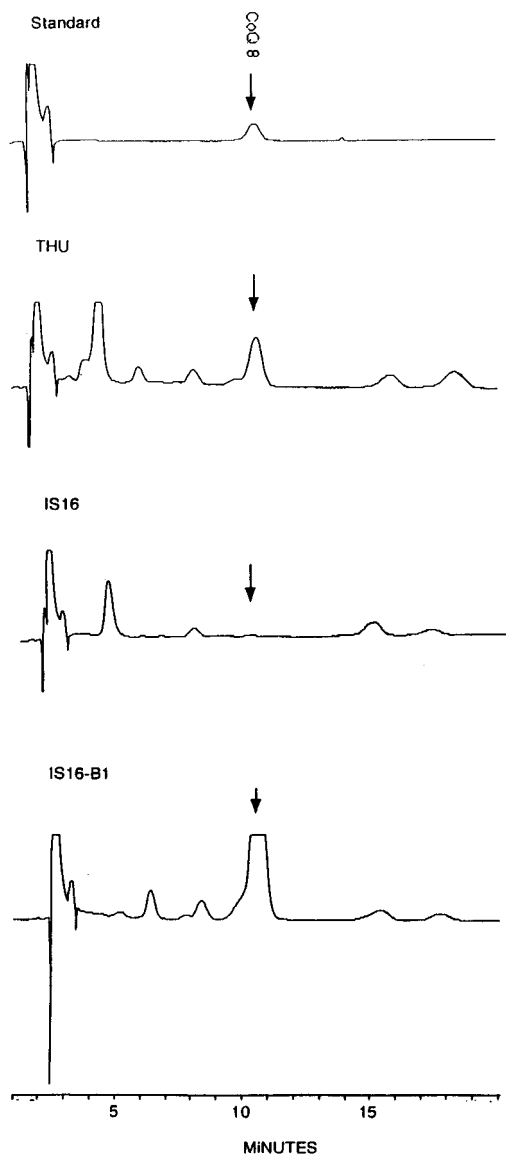


FIG. 3. Analysis of lipid extracts for ubiquinone by HPLC. Shown are the tracings of the absorption of the effluents at 275 nm. The arrows represent the peaks of ubiquinone  $Q_8$ . From the areas under the peaks, the ubiquinone contents were calculated. The results are 0.21, 0.03, and 0.31 nmol/mg (dry weight) for strains THU, IS16, and IS16B1, respectively.

sured. The results are shown in Fig. 3. Strain IS16 contained 85% less ubiquinone than did THU, and strain IS16B1, carrying multiple copies of *ubiX*, had 1.5 times as much ubiquinone.

**Search for the locus of mutation on *ubiX* in strain IS16.** Chromosomal *ubiX* genes of strain IS16 and strain THU were isolated by PCR, as described in Materials and Methods. Cloned *Pfu* polymerase was used, because of its considerably greater fidelity than *Taq* polymerase. The isolated fragments were sequenced from both directions. The *ubiX* sequence of IS16 differed from the published sequence only at codon 98 (shown in boldface in Fig. 2), where a serine residue (AGC) changed to arginine (AGA). Surprisingly, the *ubiX* sequence of the parent strain THU was identical to that of strain IS16.

To test the biological activity of the mutated *ubiX* gene, it was cloned into pBR322, and the new plasmid (pHZ1), when transformed into strain IS16, could not restore the ubiquinone

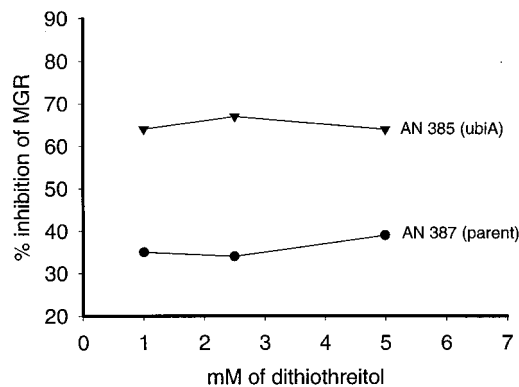


FIG. 4. Inhibition by dithiothreitol of growth of strains AN387 and AN385 (*ubiA*). MGR, mean growth rate.

levels of the parent strain. The thiol sensitivity of this strain was intermediate between that of IS16 and that of THU.

The correlation between low ubiquinone content and thiol sensitivity was extended to another *E. coli* strain, AN385, which carries a *ubiA* mutation. The ubiquinone content of this strain was 0.06 nmol/mg (dry weight), 26% of that of its parent strain AN387, which had 0.23 nmol/mg (dry weight). Their sensitivities to dithiothreitol were compared, and the results are shown in Fig. 4. It can be seen that the *ubiA* mutant strain was also hypersensitive to this thiol.

All previously reported thiol-hypersensitive *E. coli* strains belong to the *dsb* class of mutants (4, 27). These mutants are deficient in forming disulfide bonds in periplasmic proteins. The operational definition of hypersensitivity for these strains is their lack of ability to grow on Luria-Bertani agar plates containing 7 or 10 mM dithiothreitol (27). It was of interest to compare the thiol hypersensitivity of some *dsb* mutant strains with that of IS16, with liquid cultures and minimal growth medium. The results in Fig. 5 confirm the thiol hypersensitivities of the *dsb* mutant strains. A comparison with the results of Fig. 1 indicates that these cells were less sensitive to thiols than was strain IS16.

Strains IS16, IS16B1, and AN385 were tested for any evidence of the *Dsb*<sup>-</sup> phenotype. The test was the induction of the periplasmic enzyme, alkaline phosphatase, which needs two disulfide bonds for activity (2). The results are shown in

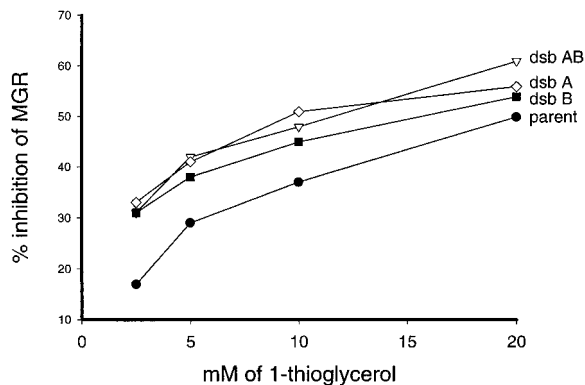


FIG. 5. Inhibition of *dsb* mutant strains by 1-thioglycerol. Closed circles, parent JCB 570; open diamonds, *dsbA* mutant JCB 571; closed squares, *dsbB* mutant JCB 789; open inverted triangles, *dsbAB* mutant JCB 758. The cultures were grown in minimal medium as described in Materials and Methods. MGR, mean growth rate.

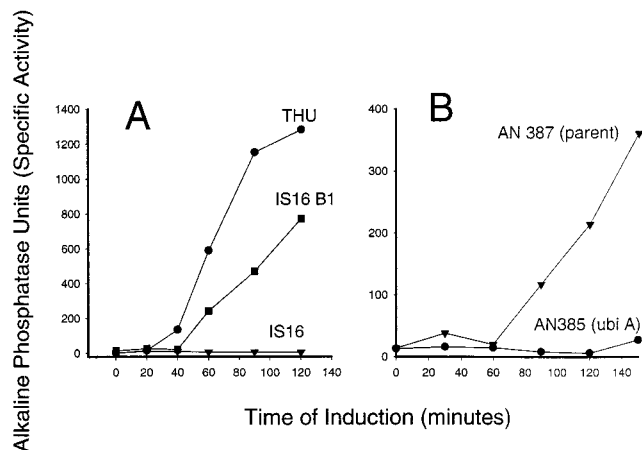


FIG. 6. Induction of alkaline phosphatase in strains THU, IS16, IS16B1, AN385, and AN387. Cultures were grown in repressing, high-phosphate-containing medium until  $A_{540}$  was 0.4 to 0.5. Following washing and resuspension in low-phosphate, inducing medium, the enzyme levels were determined at the indicated times. (A) Strains THU (closed circles), IS16 (closed inverted triangles), and IS16B1 (closed squares). (B) Strains AN385 (closed circles) and AN387 (closed inverted triangles).

Fig. 6. Strains IS16 and AN385 could not produce functional enzyme within 120 min of induction. In contrast, the parent strains and strain IS16B1 began making alkaline phosphatase within an hour. It was concluded that the low-ubiquinone-containing strains exhibited  $Dsb^-$  phenotypes.

## DISCUSSION

The low ubiquinone level of strain IS16 was puzzling, since *E. coli* has two genes, *ubiD* and *ubiX*, for the decarboxylation of octaprenyl *p*-hydroxybenzoate. It required multiple copies of the wild-type *ubiX* gene to increase this strain's ubiquinone content. The *ubiX* gene of IS16 had a single deviation from the wild-type sequence, and increasing the number of copies of this variant gene was insufficient to elevate its ubiquinone content to near that of the wild type. It appears that the mutation at codon 98 of the *ubiX* gene practically inactivated the enzyme. Therefore, serine residue 98 is likely essential for the catalytic role of polyprenyl *p*-hydroxybenzoate lyase.

Since strain THU, the parent strain of IS16, also carried this mutation, both strains must have been *ubiX* negative. A reasonable guess is that the mutation in IS16, which resulted in very low ubiquinone levels, had to occur in the *ubiD* gene. This will have to be confirmed when the *ubiD* gene is identified.

Ubiquinone deficiency in *E. coli* is known to give rise to a pleiotropic phenotype of increased resistance to some antibiotics and heat inactivation; the inability to grow on succinate as the sole carbon source; and increased sensitivity to hydrogen peroxide, methyl methanesulfonate, and gamma radiation (8). The results reported here add another phenotypic characteristic to low ubiquinone content, thiol hypersensitivity.

Correlation between low ubiquinone content and thiol hypersensitivity was shown by (i) the loss of thiol hypersensitivity when the ubiquinone level was raised via complementation, (ii) the lessening of thiol hypersensitivity when ubiquinone levels were slightly elevated with multiple copies of the mutated *ubiX* gene, and (iii) the demonstration of thiol hypersensitivity in a *ubiA* mutant strain.

Previously published screens for thiol hypersensitivity, with dithiothreitol as the reducing agent, turned up *dsbA dsbB*, as

well as *trxA* thioredoxin and *trxB* (thioredoxin reductase), mutants (27). The screen reported here employed 1-thioglycerol, a weaker reducing agent than dithiothreitol. This could be the explanation for finding a thiol-hypersensitive mutant which differed from the previously reported varieties.

A recent report suggests that there is a direct link between the respiratory chain and the DsbA-DsbB disulfide bond-forming system (22). Electrons removed from the periplasmic cysteine residues during disulfide bond formation pass first to the DsbA protein, then to the cytoplasmic membrane-associated DsbB protein, and finally to the respiratory chain. In support of their thesis, the authors showed that a *ubiA menA* double mutant, when deprived of *para*-hydroxybenzoate, slowed its growth (presumably because of reduction of ubiquinone content) and accumulated first reduced forms of DsbA and DsbB proteins and then the DsbA-DsbB complex.

This finding could explain how continually low ubiquinone levels such as those seen in strains IS16 and AN385 would reduce the ability of the respiratory chain to accept electrons from the DsbA-DsbB complex. Accumulation of reduced DsbA and DsbB proteins and the DsbA-DsbB complex in turn would result in a  $Dsb^-$  phenotype.

The thiol hypersensitivity of *dsb* mutants could be the result of accumulation of reduced periplasmic and outer membrane proteins. Dysfunctional (reduced) DsbA proteins would prevent the appropriate folding and integration of outer membrane proteins (28). This scenario gains further credence from an earlier observation that exposure to 1-thioglycerol causes a rapid shift-up in the synthesis of outer membrane proteins OmpA and OmpF (17).

Thiol hypersensitivity of low-ubiquinone-containing cells would be the final outcome of the inability of the respiratory chain to absorb electrons from reduced Dsb proteins in the presence of excess exogenous thiols. Here millimolar concentrations of 1-thioglycerol or dithiothreitol outcompete the cysteine residues of periplasmic proteins for oxidation by DsbA. Growth inhibition would be for the same reasons as in the case of *dsb* mutants.

It was of interest that multiple copies of the *ubiX* gene in strain IS16B1 resulted in a 1.5-fold increase in ubiquinone content over that of the parent strain of THU. This implied that, in strain THU, the decarboxylation of octaprenyl *p*-hydroxybenzoate was rate limiting. However, since strain THU itself is a *ubiX* mutant, this observation may be applicable only to this strain. Further work will be required to determine whether this reaction is one of the flux-determining steps in ubiquinone biosynthesis in *E. coli*.

## ACKNOWLEDGMENTS

We thank James Bardwell and Catherine Clarke for sending us bacterial strains and for helpful discussions. We also thank R. Meganathan for advising us and sharing unpublished results from his laboratory.

## REFERENCES

- Adelberg, E. A., M. Mandel, and G. C. C. Chen. 1965. Optimal conditions for mutagenesis by N-methyl-N'-nitro-N-nitrosoguanidine in *Escherichia coli* K12. *Biochem. Biophys. Res. Commun.* **18**:788-795.
- Akiyama, Y., S. Kamitani, N. Kusukawa, and K. Ito. 1992. *In vitro* catalysis of oxidative folding of disulfide-bonded proteins by *Escherichia coli dsbA (ppfA)* gene product. *J. Biol. Chem.* **267**:22440-22445.
- Andersson, S. 1992. Determination of coenzyme Q by non-aqueous reversed-phase liquid chromatography. *J. Chromatogr.* **606**:272-276.
- Bardwell, J. C. A., K. McGovern, and J. Beckwith. 1991. Identification of a protein required for disulfide bond formation in vivo. *Cell* **67**:581-589.
- Blattner, R. R., G. Plunkett III, C. A. Bloch, N. T. Perna, V. Burland, M. Riley, J. Collado-Vides, J. D. Glasner, C. K. Rode, G. F. Mayhew, J. Gregor,

- N. W. Davis, H. A. Kirkpatrick, M. A. Goeden, D. J. Rose, B. Mau, and Y. Shao. 1997. The complete genome sequence of *Escherichia coli* K-12. *Science* **277**:1453–1474.
6. Brickman, E., and J. Beckwith. 1975. Analysis of the regulation of *Escherichia coli* alkaline phosphatase synthesis using deletion and  $\phi$ 80 transducing phages. *J. Mol. Biol.* **96**:307–316.
  7. Cohen, S. S., M. Sekiguchi, J. L. Stern, and H. D. Barner. 1963. The synthesis of messenger RNA without protein synthesis in normal and phage-infected thymineless strains of *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* **49**:699–703.
  8. Collis, C. M., and G. W. Grigg. 1989. An *Escherichia coli* mutant resistant to phleomycin, bleomycin, and heat inactivation is defective in ubiquinone synthesis. *J. Bacteriol.* **171**:4792–4798.
  9. Cox, G. B., I. G. Young, L. M. McCann, and F. Gibson. 1969. Biosynthesis of ubiquinone in *Escherichia coli* K-12; location of genes affecting the metabolism of 3-octaprenyl-4-hydroxybenzoic acid and 2-octaprenyl phenol. *J. Bacteriol.* **99**:450–458.
  10. Crane, F. L., and R. Barr. 1971. Determination of ubiquinones. *Methods Enzymol.* **18C**:137–165.
  11. Daniels, D. L., G. Plunkett III, V. Burland, and F. R. Blattner. 1992. Analysis of the *Escherichia coli* genome: DNA sequence of the region from 84.5 to 86.5 minutes. *Science* **257**:771–778.
  12. Davis, B. D., and E. S. Mingioli. 1950. Mutants of *Escherichia coli* requiring methionine or vitamin B<sub>12</sub>. *J. Bacteriol.* **60**:17–28.
  13. Howlett, B. J., and J. Bar-Tana. 1980. Polyprenyl *p*-hydroxybenzoate carboxylase in flagellation of *Salmonella typhimurium*. *J. Bacteriol.* **143**:644–651.
  14. Javor, G. T. 1983. Depression of adenosylmethionine content of *Escherichia coli* by thioglycerol. *Antimicrob. Agents Chemother.* **24**:860–867.
  15. Javor, G. T. 1983. Inhibition of respiration of *Escherichia coli* by thioglycerol. *Antimicrob. Agents Chemother.* **24**:868–870.
  16. Javor, G. T. 1985. Thiol-stimulated secretion of riboflavin and porphyrins by *Escherichia coli*. *FEMS Microbiol. Lett.* **27**:243–245.
  17. Javor, G. T. 1989. Thiol-sensitive genes of *Escherichia coli*. *J. Bacteriol.* **171**:5607–5613.
  18. Javor, G. T. 1990. Morphological changes in thioglycerol treated *Escherichia coli*. *Curr. Microbiol.* **20**:57–62.
  19. Javor, G. T. 1995. Growth inhibition of *Escherichia coli* by dithiothreitol, abstr. A-43, p. 151. *In* Abstracts of the 95th General Meeting of the American Society for Microbiology 1995. American Society for Microbiology, Washington, D.C.
  20. Javor, G. T., C. D. Stringer, and J. I. Ryu. 1988. Thiol-sensitive promoters of *Escherichia coli*. *J. Bacteriol.* **170**:3291–3293.
  21. Jensen, K., and G. T. Javor. 1981. Inhibition of *Escherichia coli* by thioglycerol. *J. Bacteriol.* **19**:556–561.
  22. Kobayashi, T., S. Kishigami, M. Sone, H. Inkuchi, T. Mogi, and K. Ito. 1997. Respiratory chain is required to maintain oxidized states of the DsbA-DsbB disulfide bond formation system in aerobically growing *Escherichia coli* cells. *Proc. Natl. Acad. Sci. USA* **94**:11857–11862.
  23. Krivankova, L., and V. Dadak. 1980. Semimicro extraction of ubiquinone and menaquinone from bacteria. *Methods Enzymol.* **67**:111–114.
  24. Limbosch-Rolin, S. 1963. Les effets du  $\beta$ -mercaptoethanol et du dithioglycol sur la croissance de *Escherichia coli* et de *Saccharomyces cerevisiae*. *Exp. Cell Res.* **24**:61–72.
  25. Meganathan, R. 1996. Biosynthesis of the isoprenoid quinones menaquinone (vitamin K<sub>2</sub>) and ubiquinone (coenzyme Q), p. 642–656. *In* F. C. Neidhardt, R. Curtiss III, J. L. Ingraham, E. C. C. Lin, K. B. Low, B. Magasanik, W. S. Reznikoff, M. Riley, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella*: cellular and molecular biology, 2nd ed., vol. 1. ASM Press, Washington, D.C.
  26. Meganathan, R. 1997. Personal communication.
  27. Missiakas, D., C. Georgopoulos, and S. Raina. 1993. Identification and characterization of the *Escherichia coli* gene *dsbB*, whose product is involved in the formation of disulfide bonds *in vivo*. *Proc. Natl. Acad. Sci. USA* **90**:7084–7088.
  28. Missiakas, D., and S. Raina. 1997. Protein folding in the bacterial periplasm. *J. Bacteriol.* **179**:2465–2471.
  29. Nonet, M. L., C. C. Marvel, and D. R. Tolan. 1987. The *hisT-purF* region of the *Escherichia coli* K-12 chromosome. *J. Biol. Chem.* **262**:12209–12217.
  30. Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.