Genetic Evidence for an Interaction of the UbiG O-Methyltransferase with UbiX in Escherichia coli Coenzyme Q Biosynthesis

Melissa Gulmezian,1 Haitao Zhang,2 George T. Javor,2 and Catherine F. Clarke1*

Department of Chemistry and Biochemistry and the Molecular Biology Institute, University of California, Los Angeles, California 90095-1569,1 and Department of Biochemistry, Loma Linda University School of Medicine, Loma Linda, California 923542

Received 10 May 2006/Accepted 22 June 2006

IS16 is a thiol-sensitive, Q-deficient mutant strain of Escherichia coli. Here, we show that IS16 harbors a mutation in the ubiG gene encoding a methyltransferase required for two O-methylation steps of Q biosynthesis. Complementation of IS16 with either ubiG or ubiXk12 reverses this phenotype, suggesting that UbiX may interact with UbiG.

Ubiquinone (coenzyme Q or Q) is a prenylated, redox-active lipid that functions as an electron carrier in the respiratory electron transport chain in mitochondria of eukaryotes and the plasma membranes of most prokaryotes (5, 8). In addition to the role of Q in the electron transport chain, the redox poise of the quinone/hydroquinone pool (Q/QH2) acts as a signal for the global two-component ArcB/ArcA (anoxic redox control) system in Escherichia coli (9). Q also functions in the process of disulfide bond formation in E. coli periplasm (2).

Previous work has shown that IS16, a Q-deficient mutant strain of E. coli, is thiol hypersensitive and unable to grow on succinate. Expression of the ubiX gene from E. coli K-12 was found to rescue the IS16 Q-deficient phenotypes (30). E. coli has two distinct genes, ubiD and ubiX, thought to be involved in the decarboxylation of 3-octaprenyl-4-hydroxybenzoate (19, 23). The IS16 mutant strain was found to contain an ubiX gene sequence identical to that of its parental strain THU (an E. coli K-15 strain), encoding a single-amino-acid substitution (S98R) relative to the ubiX sequence from E. coli K-12. It was proposed that strain IS16 harbored a second mutation in ubiD, a gene considered to be isofunctional with ubiX (30). The physical location of ubiD on the E. coli chromosome was established (31). The ubiD gene sequence was determined for both THU and IS16 strains; however, we found no mutation in the ubiD gene of either strain, indicating that the mutation must reside elsewhere.

Here, we show that IS16 harbors a mutation in the ubiG gene encoding a methyltransferase required for two O-methylation steps of Q biosynthesis. We show that Q biosynthesis in IS16 is restored by expression of either the E. coli ubiG or the ubiXk12 gene, providing genetic evidence for an interaction of UbiG and UbiX in Q biosynthesis in E. coli (Table 1 shows a list of strains).

Identification of an ubiG mutation in the IS16 mutant strain. To identify the metabolic block of Q synthesis in strain IS16, a plasmid library was constructed from genomic DNA of E. coli THU. Chromosomal DNA of strain THU was partially digested by Sau3AI, and 3- to 12-kb fragments were inserted into the BamHI sites of pUC18 plasmids (Amersham Pharma- cia Biotech Inc., Piscataway, NJ). IS16 cells were transformed with this library, and transformants were screened for the ability to grow on media containing succinate. The complementing chromosomal insert contained a 6,023-bp hybrid segment of DNA resulting from the ligation of two chromosomal Sau3AI fragments into the vector; one fragment contained a portion of gyrA, complete ubiG, and a portion of xfaL, while the second fragment contained araB, complete araA, and a small stretch of araD. To determine whether IS16 harbors a mutation in the ubiG gene, the sequence was amplified from IS16 and THU genomic DNA. Sequence analysis revealed that the ubiG gene in IS16 contained a unique nucleotide substitution, T395A, which resulted in an amino acid change, L132Q. The L132Q mutation in IS16 lies adjacent to methyltransferase motif II (Fig. 1). The four motifs shown in Fig. 1 are present in a large family of AdoMet-dependent methyltransferases (18).

The structure of UbiG is not currently available; hence, it is difficult to ascertain the functional role of this amino acid substitution. However, based on the known crystal structure of the rat catechol O-methyltransferase (COMT), the post-motif II region is known to comprise the active site of the enzyme, with specific residues that contact AdoMet and the catechol

<table>
<thead>
<tr>
<th>Strain or plasmid</th>
<th>Description</th>
<th>Source and/or reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli strains</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HW272</td>
<td>Parental strain of GD1</td>
<td>28</td>
</tr>
<tr>
<td>GD1</td>
<td>ubiG::Kan</td>
<td>13</td>
</tr>
<tr>
<td>THU</td>
<td>Derivative of strain 15, thy-43 his-67 pyr-37</td>
<td>7</td>
</tr>
<tr>
<td>IS16</td>
<td>Mutagenized derivative of THU (ubiG T395A)</td>
<td>29; this study</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Plasmids</th>
<th>Source and/or reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>pUbDGTHU</td>
<td>Harbors ubiGTHU (Glu8)</td>
</tr>
<tr>
<td>pAHG</td>
<td>Harbors ubiGk12 (Val8)</td>
</tr>
<tr>
<td>pHZ1</td>
<td>Harbors ubiXk12 (Arg98)</td>
</tr>
<tr>
<td>pPZ2</td>
<td>Harbors ubiXk12 (Ser98)</td>
</tr>
</tbody>
</table>
substrate (29). The alignment between *E. coli* UbiG, *Saccharomyces cerevisiae* Coq3p, human Coq3, and rat COMT amino acid sequences over methyltransferase motifs I, post-I, II, and III is shown in Fig. 1. The UbiG L132Q mutation occurs at the position corresponding to K144 in COMT, which is shown to be involved in substrate binding. It is reasonable to assume that the L132Q mutation may impact the substrate binding specificity of the UbiG polypeptide. Use of PHYRE, a protein fold recognition server (http://www.sbg.bio.ic.ac.uk/phyre/), identified mycolic acid cyclopropane synthases from *Mycobacterium tuberculosis* as protein structures closely related to the UbiG polypeptide sequence. Cyclopropane synthases catalyze the transfer of the methyl group from AdoMet to a double bond of the acyl substrate. Residues 137 to 144 of mycolic acid cyclopropane synthases are involved in cofactor binding (15), are located in the post-motif II region, and also implicate the UbiG L132Q substitution in UbiG as potentially affecting cofactor or substrate binding.

*IS16 can be complemented by either ubiG or ubiX*. The *ubiG* gene from *E. coli* THU was amplified by PCR and inserted into pHZ1, pHZ2, pHX1, and pHX2. The resulting plasmid, pHUbiGTHU, restored growth on succinate and partially restored production of Q8 in the IS16 mutant (Fig. 2). Rescue of Q8-deficient phenotypes can be achieved with levels of Q that are significantly lower than the amount normally present in wild-type cells (3, 6, 17). Lower steady-state levels for the UbiGTHU polypeptide than for...
UbiGK-12 were observed when each construct was expressed in the GD1 mutant strain (data not shown). Both IS16 and ubiG null mutant strain GD1 were rescued by ubiGK-12. Based on this, it seems likely that the differential rescue may be due to efficiency of expression: ubiGK-12 in pAHG is expressed from the yeast CYC1 promoter, while ubiGTHU is expressed from its native promoter sequence. A plasmid harboring ubiX from E. coli THU, pHZ1, failed to rescue either IS16 or GD1, while pPZ2, a plasmid harboring ubiX from E. coli K-12, rescued IS16, as observed previously (30). pPZ2 failed to rescue GD1 (data not shown). For the determination of E. coli Q8 content, cultures were grown in Davis minimal media at 37°C overnight and collected by centrifugation. E. coli cells (0.1 to 0.2 g wet weight) were extracted and quinones separated by reverse-phase high-pressure liquid chromatography (HPLC) and quantified with an electrochemical detector as described previously (16). Q10 (Sigma-Aldrich, St. Louis, MO) was added as an internal standard (final concentration of 20 pmol/mg). The areas of the peaks corresponding to Q8 and Q10 samples and standards were determined with Gilson Unipoint version 5.1 software.

**FIG. 3.** O methylation of early Q intermediates is defective in IS16 and is restored by expression of E. coli UbiXK-12. Permeabilized E. coli cells were prepared from HW272 (wild-type, parental strain of GD1), GD1 (ubiG disruption mutant), THU (parental strain of IS16), IS16 (UbiG-L132Q), and IS16:pPZ2 (IS16 harboring UbiXK-12 on a plasmid) and incubated with farnesylated analogs of 1 mM 3,4-dihydroxy-5-farnesylbenzoic acid (open bars). 250 μM 5-farnesyl-2-hydroxyphenol (closed bars) for 10 min. S-Adenosyl-[methyl-3H]-methionine (6.9 μM, 81.5 Ci/mmol; PerkinElmer Life Sciences) was added to the reaction mixture. Following incubation at 37°C for 45 min, lipids were extracted and separated by reverse-phase HPLC (BetaBasic C18 column, 5 μM, 4.6 by 250 mm; Thermo Electron Corporation). Radioactivity present in fractions 6 and 7 (open bars) or fractions 10 to 12 (closed bars) is expressed in pmol of CH3 groups/hr/mg wet weight and eluted with the 3H-labeled product standard. Error bars represent standard deviations obtained from two O-methyltransferase assays of the same sample, and data shown represent two independent experiments.

**FIG. 4.** The final O-methylation reaction is defective in the IS16 mutant and is restored by expression of either E. coli ubiG or ubiX. The purified E. coli UbiG polypeptide has been shown to function as a soluble enzyme and catalyze the O methylation of three different farnesylated analogs of intermediates in Q biosynthesis (24). To examine the effect of the L132Q mutation on UbiG O-methyltransferase activity in the IS16 mutant, we employed a cell permeabilization assay because this method has been shown to preserve O-methyltransferase activity in analyses of 

VOL. 188, 2006 NOTES 6437

E. coli strains harbored plasmids expressing the designated genes as follows: pAHG, UbiGK-12; pUbiGTHU, UbiGTHU; pHZ1, UbiXTHU; and pPZ2, UbiXK-12.
another E. coli ubiG mutant (14). In vitro assays of O-methyltransferase activity employed the farnesylated analogs of the E. coli intermediate (5-farnesyl-2-hydroxyphenol), demethyl-Q, (2-farnesyl-5-hydroxy-6-methoxy-3-methyl-1,4 benzoquinone), or the yeast intermediate (3,4-dihydroxy-5-farnesylbenzoic acid) and S-adenosyl-[methyl-3H]L-methionine as previously described (22, 24). O-methyltransferase activity assays were linearly dependent on time of incubation, substrate concentration, and amount of permeabilized cells. O-methyltransferase activity with 3,4-dihydroxy-5-farnesylbenzoic acid (Fig. 3, open bars) or 5-farnesyl-2-hydroxyphenol (Fig. 3, closed bars) was readily detected in the HW272 and THU E. coli parental strains, while activity was either not detected or present at significantly lower levels in the GD1 and IS16 ubiG mutant strains. Complementation of IS16 with either ubiX or ubiG from K-12 or ubiG from THU rescued O-methyltransferase activity with the demethyl-Q substrate (Fig. 4). However, O-methyltransferase activity in the ubiG disruption mutant GD1 was restored only by ubiG from K-12.

Steady-state levels of UbiG and cytochrome o oxidase were measured in E. coli (26) and suggests that synthesis of the isoprenoid tail occurs in complex with ring modifications. Based on the genetic evidence presented here, UbiX, a protein thought to be involved in a decarboxylation step in Q biosynthesis, may be required to stabilize the catalytic activity of UbiG L132Q by direct interaction or by channeling substrates (Fig. 6). These results provide support for a polypeptide complex involved in E. coli Q biosynthesis, first described by Knoell (20, 21). Knoell demonstrated that a complex of membrane-associated polypeptides in E. coli converts 2-octaprenylphenol to Qs in vitro.

Similarly, a growing body of evidence suggests that a complex of Coq polypeptides is involved in Q biosynthesis in S. cerevisiae. Gel filtration chromatography shows that Coq3p, Coq4p, Coq6p, and Coq7p coelute as a high-molecular-weight complex (22, 28). Coq3p, Coq4p, and Coq7p comigrate as high-molecular-mass complexes as assessed by two-dimensional blue native analysis (22, 28). O-Methyltransferase activity is decreased in coq null mutants relative to that in atp2 and cor1 respiratory deficient mutants (13). Deletions in any of the COQ genes affect the steady-state levels of Coq3p, Coq4p, and Coq6p (11). Unlike yeast coq3-coq9 mutants that accumulate

boring ubiXK-12) cells were assessed by Western blot analysis as previously described (12). Levels of UbiG polypeptide in IS16 were similar to those in the parental strain THU (Fig. 5).

Complementation studies revealed that multiple copies of ubiXK-12 restore growth on succinate, Qs levels (30), and O-methyltransferase activity in IS16 (Fig. 2, 3, and 4). This is a surprising finding because the O-methyltransferase activity of the purified UbiG polypeptide does not require other Ubi polypeptides (24). It is possible that overexpression of UbiX may stimulate another O-methyltransferase with overlapping substrate specificity to UbiG. We consider this unlikely; the rescue is unique to the L132Q UbiG point mutant. Interactions between Ubi polypeptides were not identified by use of tagged constructs to identify multisubunit complexes (7). However, an interaction was detected between UbiX and FldA. FldA plays an essential role in the synthesis of isoprenoid precursors in E. coli (26) and suggests that synthesis of the isoprenoid tail occurs in complex with ring modifications. Based on the genetic evidence presented here, UbiX, a protein thought to be involved in a decarboxylation step in Q biosynthesis, may be required to stabilize the catalytic activity of UbiG L132Q by direct interaction or by channeling substrates (Fig. 6). These results provide support for a polypeptide complex involved in E. coli Q biosynthesis, first described by Knoell (20, 21). Knoell demonstrated that a complex of membrane-associated polypeptides in E. coli converts 2-octaprenylphenol to Qs in vitro.

Similarly, a growing body of evidence suggests that a complex of Coq polypeptides is involved in Q biosynthesis in S. cerevisiae. Gel filtration chromatography shows that Coq3p, Coq4p, Coq6p, and Coq7p coelute as a high-molecular-weight complex (22, 28). Coq3p, Coq4p, and Coq7p comigrate as high-molecular-mass complexes as assessed by two-dimensional blue native analysis (22, 28). O-Methyltransferase activity is decreased in coq null mutants relative to that in atp2 and cor1 respiratory deficient mutants (13). Deletions in any of the COQ genes affect the steady-state levels of Coq3p, Coq4p, and Coq6p (11). Unlike yeast coq3-coq9 mutants that accumulate
an early predominant intermediate, 3-hexaprenyl-4-hydroxybenzoic acid (HHB) (1, 4, 12, 25). *E. coli* *ubi* mutants tend to accumulate immediate precursor substrates at the blocked steps (10). However, no *ubiG* mutant has been reported to accumulate 3,4-dihydroxy-5-octaprenyl-benzoic acid (14, 27), possibly due to the instability of the catechol moiety. We are currently investigating the lipid quinone intermediate formed in the IS16 strain to further characterize the polypeptide Q-biosynthetic complex in the THU genetic background strain of *E. coli*.

We thank J. N. Shepherd for the farnesylated Q intermediate analogs and W. W. Poon for the generation of the UbiG antibody. We thank N. Lee for the purified *E. coli* protein His6-UbiG and R. H. Kaback for the generous gift of the *E. coli* cytochrome oxidase antibody. We also thank members of the Clarke laboratory for helpful suggestions in this study.

This work was supported in part by National Institutes of Health grant GM45952.

REFERENCES


