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The role of UbiX in *Escherichia coli* coenzyme Q biosynthesis

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Abstract

The reversible redox chemistry of coenzyme Q serves a crucial function in respiratory electron transport. Biosynthesis of Q in *Escherichia coli* depends on the *ubi* genes. However, very little is known about UbiX, an enzyme thought to be involved in the decarboxylation step in Q biosynthesis in *E. coli* and *Salmonella enterica*. Here we characterize an *E. coli ubiX* gene deletion strain, LL1, to further elucidate *E. coli ubiX* function in Q biosynthesis. LLI produces very low levels of Q, grows slowly on succinate as the sole carbon source, accumulates 4-hydroxy-3-octaprenyl-benzoate, and has reduced UbiG *O*-methyltransferase activity. Expression of either *E. coli ubiX* or the *Saccharomyces cerevisiae* ortholog *PAD1,* rescues the deficient phenotypes of LL1, identifying *PAD1* as an ortholog of *ubiX*. Our results suggest that both UbiX and UbiD are required for the decarboxylation of 4-hydroxy-3-octaprenyl-benzoate in *E. coli* coenzyme Q biosynthesis, especially during logarithmic growth.

Keywords: Ubiquinone; Coenzyme Q biosynthesis; Decarboxylase; *Escherichia coli*; *ubiX*; *ubiD*; *Saccharomyces cerevisiae*; PAD1; YDR538W; YDR539W

In *Escherichia coli* and *Salmonella enterica*, UbiD and UbiX are generally considered to operate as isofunctional decarboxylases that convert 4-hydroxy-3-octaprenylbenzoic acid (HP_8B)[1] to 2-octaprenylphenol, an early step in the biosynthesis of coenzyme Q (Fig. 1) [1]. The *ubiX* gene was first identified in a *S. enterica* mutant TA1851 [2]. Subsequent work by Bar-Tana et al., lent support to the involvement of UbiX in Q biosynthesis [3]. The TA1851 mutant, harboring a deletion including the *ubiX* gene, was deficient in the decarboxylation of HP_8B [4], a reaction already known to be catalyzed by the product of the *E. coli ubiD* gene. Interestingly, the accumulation of 2-octaprenylphenol (and partial rescue of Q_8 biosynthesis) was observed when TA1851 was cultured in the presence of hexanoate or benzoate [4]. The authors speculated that UbiD (or another decarboxylase) might be responsible for the induced decarboxylation activity in the TA1851 mutant.

E. coli ubiD$^-$ mutants have defects in Q_8 biosynthesis, accumulate HP_8B, and lack decarboxylase activity *in vitro* [5,6]. However, *E. coli ubiD*$^-$ mutants retained the ability to produce about 20–25% of the normal levels of Q [6]. Leppik et al. also observed that the amount of HP_8B declined under conditions of prolonged incubation, and the amount of Q_8 increased. Based on these observations, Leppik et al. concluded that a second enzyme must exist in *E. coli* that functions *in vivo* to decarboxylate HP_8B [6]. When the homologous *ubiX* sequence was later identified in *E. coli* [7], it was presumed that the residual 20% of Q_8 present in the *E. coli ubiD*$^-$ mutant was due to UbiX [8].

Our interest in the putative UbiX/UbiD protein pair began with the observation that increasing the amount of UbiX in a strain of *E. coli*, hypersensitive to growth inhibition by thioglycerol, restored the cell's ability to cope with

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[1] *Abbreviations used:* HP_8B, 4-hydroxy-3-octaprenylbenzoic acid; LB, Luria–Bertani; TMS-DS, trimethylsilane-diazomethane; APCI, atmospheric pressure chemical ionization; Q, coenzyme Q.

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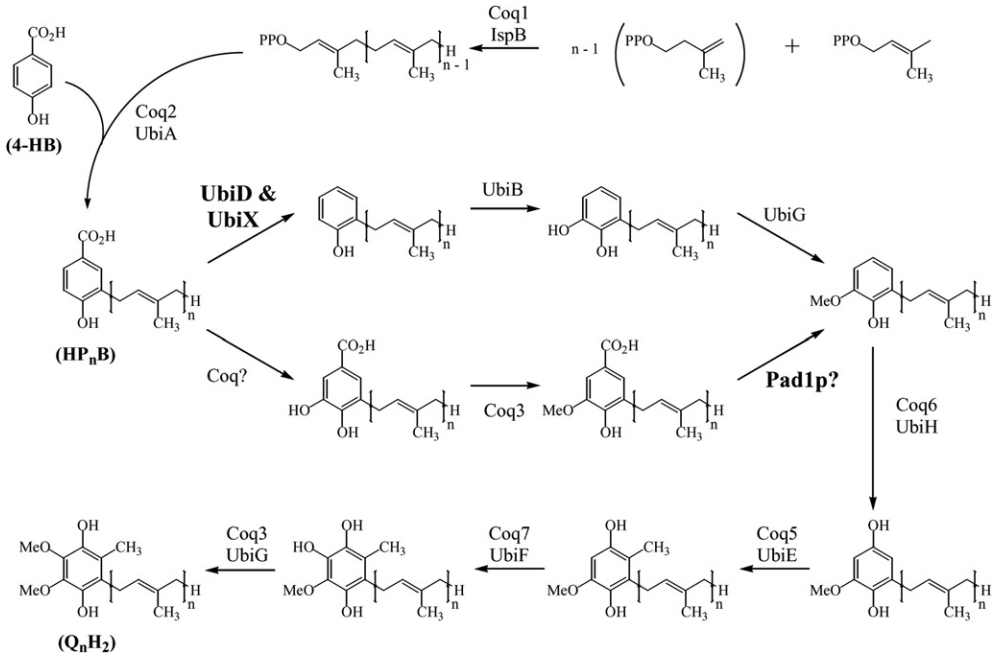

Fig. 1. Coenzyme Q biosynthetic pathway of *Escherichia coli* and *Saccharomyces cerevisiae*. Polyprenyl diphosphate synthases, Coq1p (*S. cerevisiae*) and IspB (*E. coli*), catalyze the formation of the isoprenoid tail. The tail lengths among different organisms vary and are specified by the specific polyprenyl diphosphate synthase enzyme present. For example, *S. cerevisiae* synthesize Q_6, bearing six isoprene units, and *E. coli* produce Q_8. In *S. cerevisiae*, the isoprene tail is attached to 4-hydroxybenzoic acid (4-HB) by Coq2p to form 3-hexaprenyl-4-hydroxybenzoate (HP_6B); in *E. coli,* UbiA catalyzes this reaction to form 4-hydroxy-3-octaprenyl-benzoate (HP_8B). At this point the pathways are proposed to diverge between prokaryotes and eukaryotes. *E. coli* genes products are identified as Ubi (and include IspB) and *S. cerevisiae* gene products are identified as Coq.

this exogenous reducing agent. The actual cause of thiol hypersensitivity was a deficiency of Q [9], a condition that hinders the periplasmic oxidizing system in removing electrons via the electron transport system [10]. The cause for Q-deficiency was traced to a mutation in the *ubiG* gene (L132Q) [11] encoding an *O*-methyltransferase required for Q biosynthesis [12]. It was demonstrated that the thiol hypersensitive mutant had normal UbiG protein levels but low *O*-methyltransferase enzyme activity. Expression of wild-type *ubiX* restored *O*-methyltransferase activity and Q_8 synthesis, implicating the interaction of the UbiX and UbiG polypeptides [11]. These observations suggest that UbiX function in Q biosynthesis plays a more central role than previously appreciated.

A homolog of *E. coli ubiX* is also present in *Saccharomyces cerevisiae,* the *PAD1* gene (50% amino acid sequence identity). A *S. cerevisiae pad1* mutant is defective in decarboxylating phenyl acrylic acids and displays sensitivity to cinnamic acid in plate media [13]. In this previous study, it was assumed that Pad1p functioned as a decarboxylase. However, *E. coli* overexpressing yeast Pad1 lacked decarboxylase activity with a variety of phenyl acrylic acids [14]. Based on its 50% sequence identity with *E. coli* UbiX, the authors postulated that Pad1 might function in Q biosynthesis. Other investigators suspect partner proteins may be required for decarboxylation activity [15]. Thus the functional roles of UbiX and Pad1 in *E. coli* and yeast Q biosynthesis are not known.

In the work reported here, we generated an *E. coli ubiX* null mutant to characterize its functional roles in Q biosynthesis and in supporting the UbiG *O*-methyltransferase. We characterize the growth properties of the *ubiX* deletion mutant, determine its Q_8 content, and identify the accumulating Q-intermediate. We determine whether the absence of UbiX affects *O*-methylation in *E. coli* Q biosynthesis and also test whether expression of yeast Pad1 can rescue the Q-deficiency of the *E. coli ubiX* null mutant. The results identify yeast Pad1 as an ortholog of *E. coli* UbiX, and indicate that both UbiD and UbiX are required for the decarboxylation of HP_8B. We present a model implicating 2-octaprenylphenol as a lipid component that is required for later enzymatic steps in Q biosynthesis, including the *O*-methylation steps catalyzed by UbiG.

Materials and methods

Bacterial strains, plasmids, media, and growth conditions

Strains and plasmids are listed in Table 1. Bacteria were grown in either Luria–Bertani (LB) or minimal salts [16] media at 30 °C or at 37 °C with shaking. Glucose 0.3% (w/v) was used as carbon/energy source with minimal salts media. Antibiotics used both in liquid media and in plates were: ampicillin (100 μg/ml), chloramphenicol (20 μg/ml) and kanamycin (50 μg/ml). Growth was measured by optical density at 600 nm with a Kontron DB-3500 UV/VIS spectrophotometer (Research Instruments International). Growth of the LL1/pYDR538 W strain was conducted at 30 °C in order to optimize expression of the yeast Pad1 polypeptide. Thiol sensitivity was measured as described previously [9].