NORTHERN ILLINOIS UNIVERSITY

The *ubiX* encoded decarboxylase is not required for the *ubiG* O-methyltransferase activity

A Thesis Submitted to the
University Honors Program
In Partial Fulfillment of the
Requirements of the Baccalaureate Degree

With Upper Division Honors

Department Of

Biology

By

Carolyn Russie, Usman Beg, Debarati Ghose

Faculty Adviser: Prof. R. Meganathan

DeKalb, Illinois

May 9, 2014
University Honors Program

Capstone Approval Page

Capstone Title:
The *ubiX* encoded decarboxylase is not required for the *ubiG* O-methyltransferase activity

Student Name:
Carolyn Russia, Usman Beg, Debarati Ghose
Faculty Adviser: Prof. R. Meganathan

Faculty Supervisor: Prof. R. Meganathan

Faculty Approval Signature

Department of Biology

Date of Approval: 12/3/2014
HONORS THESIS ABSTRACT
THESIS SUBMISSION FORM

AUTHOR:
Carolyn Russie, Usman Beg, Debarati Ghose
Faculty Adviser: Prof. R. Meganathan

THESIS TITLE:
The ubiX encoded decarboxylase is not required for the ubiG O-
methyltransferase activity

ADVISOR: Prof. R. Meganathan

ADVISOR'S DEPARTMENT: Biology

DISCIPLINE: Microbiology YEAR: 2014

PAGE LENGTH: 12

BIBLIOGRAPHY:


**ILLUSTRATED:** N/A  
**PUBLISHED (YES OR NO):** No  
**LIST PUBLICATION: N/A**  
**COPIES AVAILABLE (HARD COPY, MICROFILM, DISKETTE):** N/A  

**ABSTRACT (100-200 WORDS):**  
An essential component of the respiratory chain of aerobic prokaryotes and eukaryotes is Coenzyme Q (Ubiquinone; Q), which acts as an electron carrier thereby serving as a link between dehydrogenases and the subsequent electron acceptors in the respiratory chain. In *Escherichia coli*, during Q biosynthesis, the decarboxylation step is catalyzed by two isofunctional enzymes, UbiD and UbiX. UbiD carries out the decarboxylation of 80% of the substrate, 3-octaprenyl-4-hydroxybenzoate whereas UbiX is responsible for the remaining 20%. It has been reported that the loss of *ubiX* decreases UbiG O-methyltransferase activity by 3.6 fold and the growth in Luria-Bertani (LB) broth as well as in succinate minimal medium is severely affected. Contrary to this report, we
discovered that the ΔubiX mutant grows to wild-type levels in both media and synthesizes close to wild-type levels of Q. We constructed an in-frame deletion mutant of ubiD using the Datsenko and Wanner method. The ΔubiD mutant however, showed severely impaired growth in LB broth and succinate minimal medium. Complementation of the ΔubiD mutant by the ubiD gene restores the growth back to wild type levels. ubiD, ubiX and ubiI (visC) were cloned separately into a multi-copy plasmid and used to complement the ΔubiX mutant. High Performance Liquid Chromatography (HPLC) analysis of the levels of Q produced by these strains showed that when ubiD complemented the ΔubiX mutant, it makes significant levels of Q which in turn shows that the loss of ubiX has no effect on UbiG O-methyltransferase activity.
The *ubiX* encoded decarboxylase is not required for the *ubiG* O-methyltransferase activity

Carolyn Russie, Usman Beg, Debarati Ghose

Faculty Adviser: Prof. R. Meganathan

Department of Biological Sciences, Northern Illinois University

ABSTRACT

An essential component of the respiratory chain of aerobic prokaryotes and eukaryotes is Coenzyme Q (Ubiquinone; Q), which acts as an electron carrier thereby serving as a link between dehydrogenases and the subsequent electron acceptors in the respiratory chain. In *Escherichia coli*, during Q biosynthesis, the decarboxylation step is catalyzed by two isofunctional enzymes, UbiD and UbiX. UbiD carries out the decarboxylation of 80% of the substrate, 3-octaprenyl-4-hydroxybenzoate whereas UbiX is responsible for the remaining 20%. It has been reported that the loss of *ubiX* decreases UbiG O-methyltransferase activity by 3.6 fold and the growth in Luria-Bertani (LB) broth as well as in succinate minimal medium is severely affected. Contrary to this report, we discovered that the Δ*ubiX* mutant grows to wild-type levels in both media and synthesizes close to wild-type levels of Q. We constructed an in-frame deletion mutant of *ubiD* using the Datsenko and Wanner method. The Δ*ubiD* mutant however, showed severely impaired growth in LB broth and succinate minimal medium. Complementation of the Δ*ubiD* mutant by the *ubiD* gene restores the growth back to wild type levels. *ubiD*, *ubiX* and *ubiI* (*visC*) were cloned separately into a multi-copy plasmid and used to complement the Δ*ubiX* mutant. High Performance Liquid Chromatography (HPLC) analysis of the levels of Q produced by these strains showed that when *ubiD* complemented the Δ*ubiX* mutant, it makes significant levels of Q which in turn shows that the loss of *ubiX* has no effect on UbiG O-methyltransferase activity.
INTRODUCTION:

One of the essential components of the respiratory chain of aerobic prokaryotes and eukaryotes (mitochondrial respiratory chain) is Coenzyme Q, (Ubiquinone ;Q). Q plays a major role in electron transport chain by carrying electrons and hence serving as a link between dehydrogenases and electron acceptors.

*E. coli* synthesize the precursor of Q, p-hydroxybenzoate (PHB) directly from the shikimate pathway intermediate chorismate by the enzyme encoded by the *ubiC* gene with the elimination of pyruvate. The PHB is prenylated by UbiA resulting in the formation of 3-octaprenyl-4-hydroxyebnzoate. The latter then undergoes decarboxylation to form 2-octaprenylphenol. In *E. coli* and *Salmonella enterica*, this decarboxylation is carried out by two isofunctional enzymes, UbiD and UbiX, resulting in the formation of 2-octaprenylphenol (Gulmezian et. al., 2007). The 2-octaprenylphenol undergoes three hydroxylations alternating with three methylations. The two O-methylation steps are carried out by UbiG. It is generally accepted that in *E. coli*, 80% of the decarboxylation of 3-octaprenyl-4-hydroxybenzoate is carried out by UbiD and the residual 20% is carried out by UbiX (Meganathan, et. al., 2010).

According to Gulmezian et. al. the loss of *ubiX* gene leads to a reduction in growth on Luria-Bertani(LB) medium as well as succinate minimal medium. It has been reported that, Δ*ubiX* mutant produces very low levels of Q and has reduced UbiG O-methyltransferase activity.
Figure 1: Ubiquinone Biosynthetic Pathway (adapted from Nowicka and Kruk, 2010)

MATERIALS AND METHODS:

♢ Construction of *ubiD* in-frame deletion mutant

We successfully created an in-frame deletion mutant of *ΔubiD::Kan* based on the Datsenko and Wanner method (Datsenko & Wanner, 2000). The *ubiD* gene was replaced by a kanamycin cassette thereby making the mutant resistant to kanamycin, which aids in the selection of the mutant. The voltage used for electroporation was 2.5kV. After electroporation, the culture tubes containing the cells were allowed to grow in a 28C shaker incubator for 4hrs. to allow enough time for recovery. The transformants were selected on LB plates containing kanamycin and glucose. The *ΔubiX::Kan* deletion mutant was obtained from Coli Genetic Stock Center, Yale University, Connecticut.
Growth curve of *Δubi*D and *Δubi*X mutants in glucose minimal medium and succinate minimal medium
The mutants were individually grown in LB broth, pelleted, and starved in minimal salts for 3-4 hours, followed by inoculation into either glucose minimal medium or succinate minimal medium. Growth was monitored every hour to determine the rate and maximal yield of growth.

Complementation of the *Δubi*X mutant with *ubi*D, *ubi*X and *vis*C (*ubi*) individually cloned into a multi-copy plasmid
Genomic DNA was isolated from wild-type *E. coli*. The *ubi*D, *ubi*X and *vis*C inserts were prepared using gene-specific cloning primers, followed by purification of the PCR product. The inserts were cloned into the multi-copy plasmid pUC18. pUC 18 and the purified inserts were then digested with the restriction enzymes BamHI and Hind III, followed by agarose gel electrophoresis and gel purification. This was followed by dephosphorylation of the purified digested plasmid. The digested inserts were individually ligated into the vector plasmid using T4 DNA ligase. The constructed plasmids were then transformed into the *Δubi*X mutant by chemical transformation and verified by PCR using gene specific verification primers.

Growth curve of the complemented strains in glucose minimal medium and succinate minimal medium
*Δubi*X mutant complemented individually with *ubi*D, *ubi*X and *vis*C were grown in LB broth, pelleted and starved in minimal salts for 3-4 hours. The cells were then inoculated into the glucose minimal medium and succinate minimal medium. Growth was monitored every hour.
Extraction of Coenzyme Q and analysis by High Performance Liquid Chromatography

The cells were grown in glycerol minimal medium and harvested at late logarithmic phase. The cells were centrifuged at 6000xg at 4°C, washed with phosphate buffer (pH 7.0) and pellets were frozen at -20°C. The isolation of quinones from wet pellets was done by Soxhlet extraction (Young, 1973). Acetone was used as the lipid extraction solvent. Weighed cell pellets were used in cellulose thimbles (Whatmann 25mmx80mm) and continuously extracted with 170ml acetone in a round bottomed flask for 20-25 cycles. Quinones were dried under reduced pressure at 37°C in a rotary vacuum evaporator. Light exposure was minimized at all stages. The dried quinone was resuspended in 1ml acetone and 30μl was injected using an autosampler Waters (model) reverse phase high performance liquid chromatography (RHPLC) equipped with Machery-Nagel Nucleodur C18 Gravity 3μm column. Samples were eluted with methanol:isopropyl ether (3:1) at 0.8ml/min. (Suvarna Et. al., 1998)
RESULTS

1. Figure 2: Growth curves of the deletion mutants ΔubiD::Kan, ΔubiX::Kan and ΔvisC::Kan in glucose minimal medium

![Glucose Minimal Medium Graph]

2. Figure 3: Growth curves of the deletion mutants ΔubiD::Kan, ΔubiX::Kan and ΔvisC::Kan in succinate minimal medium

![Succinate Minimal Medium Graph]
3. Figure 4: Growth comparison of ΔubiX::Kan mutant complemented with ubiD, ubiX and visC (ubiL) individually in a multi-copy plasmid in glucose minimal medium and succinate minimal medium

Glucose Minimal Medium

Sucinate Minimal Medium
4. Figure 5: Growth curves of \( \Delta ubiD::\text{Kan} \), \( \Delta ubiX::\text{Kan} \) and \( \Delta visC::\text{Kan} \) complemented with \( ubiD \), \( ubiX \) and \( visC \) genes respectively in glucose minimal medium and succinate minimal medium.

**Glucose Minimal Medium**

![Glucose Minimal Medium Graph](image)

**Succinate Minimal Medium**

![Succinate Minimal Medium Graph](image)
5. Figure 6: HPLC analysis of Coenzyme Q produced by the mutants ΔubiD::Kan, ΔubiX::Kan and the complemented strains
visC+pUC18 in ΔubiX

ΔubiD::Kan

ΔubiX::Kan
DISCUSSION

The growth curves suggest that the mutant ΔubiX::Kan grows to wild-type levels at the same rate as the wild type, whereas the ΔubiD::Kan mutant grows at a much slower rate than the wild-type. The loss of the visC(ubil) gene too has no effect on the growth of E. coli aerobically. The growth of the ΔubiD::Kan mutant is restored to wild type levels when it is complemented with the ubiD gene, thus showing that the loss of ubiD affects growth in succinate minimal medium whereas the loss of ubiX does not. Also when levels of Coenzyme Q produced are analyzed using HPLC, it is seen that the mutant ΔubiX produces significantly high levels of Q, when either complemented with the gene ubiD or ubiX in a multi-copy plasmid. This in turn, shows that the loss of ubiX gene does not affect the activity of UbiG O-methyltransferase, otherwise the mutant ΔubiX would not have synthesized wild-type levels of Q when complemented with the ubiD gene.

This shows that the loss of the ubiX encoded decarboxylase does not affect the ubiG O-methyltransferase activity downstream of the Coenzyme Q biosynthetic pathway in E. coli.
References


5. Meganathan, R., (1996), Escherichia coli and Salmonella, Cellular and Molecular Biology, American Society for Microbiology, Washington, DC.


