Characterization of the Na\(^+\)-transporting NADH:ubiquinone oxidoreductase (NQR) in Vibrio cholerae

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Abstract

We previously reported that inhibition of the Na\(^+\) translocating NADH:ubiquinone oxidoreductase (NQR), either by chemical inhibition or mutation, increased toxT transcription in Vibrio cholerae. In this study, we revealed that the nqr mutant strain showed similar phenotypes as the Escherichia coli NADH dehydrogenase I (nuo) mutant strain (e.g., growth defect after the mid log growth phase and higher acetic acid production). The increased growth and toxT expression in the nqr mutant relative to the wild type strain appears to be growth phase dependent. However, after longer growth, nqr showed lower amounts of cholera toxin production compared to the parent strain. Interestingly, the nqr mutant strain showed a similar level of toxT expression in the presence of L-lactate, which is known to stimulate respiration. Through Biolog\textsuperscript{®} Phenotype Microarray (PM) analysis, we found that the V. cholerae nqr mutant strain had defects in a broad spectrum of metabolism functions, including amino acid, carboxylic acid, phosphorus, and sulfur utilization, indicating an important role of NQR in V. cholerae metabolism. In addition, nqr mutation increased osmotic sensitivity in V. cholerae. Some of the defects identified by PM analysis, including NaCl sensitivity, were restored by the addition of L-lactate.

Background

The Na\(^+\) translocating NADH:ubiquinone oxidoreductase (NQR) is an unique respiratory enzyme catalyzing the electron transfer from NADH to quinone, coupled with the translocation of sodium ions across the membrane. A genomic analysis of Vibrio cholerae revealed that it does not possess an ortholog of Complex I of the electron transport chain, NADH:ubiquinone oxidoreductase (NADH:QO), which often acts as the main respiratory NADH dehydrogenase in bacteria. This suggests NQR plays an important role in energy metabolism in V. cholerae. We previously reported that the inactivation of NQR either by chemical inhibition or mutation increased virulence gene expression in V. cholerae. However, the details of the mechanisms of the link between NQR and virulence gene expression are still unclear.

Conclusions

- Lack of functional NQR affects wide ranges of V. cholerae metabolism, suggesting an important role for NQR in V. cholerae metabolism.
- The effects of NQR on virulence gene expression are growth phase dependent.
- We hypothesize that the changes in certain metabolic functions are linked to the observed changes in virulence gene expression following loss of NQR in V. cholerae.

References


Table 1. Biolog\textsuperscript{®} Phenotype Microarray analysis of the V. cholerae nqr mutant.

<table>
<thead>
<tr>
<th>Mode of action</th>
<th>Substrates</th>
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<tbody>
<tr>
<td>C-Source</td>
<td>L-Aspartic acid, L-Proline, L-Histidine, L-Glutamine, L-Glutamic acid, Gly-Glu, Acetic acid, D,L-Malic acid, Fumaric acid, Malic acid Mono-Methylsuccinate, Succinic acid</td>
</tr>
<tr>
<td>S-Source</td>
<td>Glutathione</td>
</tr>
<tr>
<td>Osmotic sensitivity</td>
<td>1% NaCl, 5% Ethylene Glycol, 2% Sodium Sulfate, 2% Urea</td>
</tr>
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Phenotype microarray analysis of the V. cholerae nqr mutant indicated defects in broad ranges of amino acid and carboxylic acid metabolism and osmotic sensitivity. Substrates in red were confirmed by growth-based assays.

Fig. 1. NQR in the aerobic respiration system in V. cholerae.

The electron transport system of V. cholerae possesses a redox driven sodium pump (NQR) instead of the NADH:ubiquinone oxidoreductase (NQR) found in E. coli and other bacteria. LDH: lactate dehydrogenase, Q: quinone.

Fig. 2. Effects of NQR mutation on growth (A), acetate secretion (B), virulence gene expression (C), and cholera toxin production (D).

(A) The nqr mutant growth was similar to the wild type strain at early log growth phase but showed a growth defect after mid-log growth phase. (B) The nqr mutant showed higher acetate excretion compared to the wild type. (C) The nqr mutant showed higher virulence gene expression levels only at early log growth phase. (D) The nqr mutant produced lower cholera toxin levels compared to the wild type when measured after 16 hr growth in LB at 30ºC.

Fig. 3. Effects of L-lactate on toxT transcription.

The nqr mutant strain did not show increased toxT transcription in the presence of L-lactate.

Fig. 4. Affect of L-Lactate on V. cholerae growth in high NaCl media.

In the presence of L-lactate, the nqr mutant strain grew similarly to wild type levels, even in the presence of high NaCl (500 mM). The results indicate that the combination of NQR and the Na\(^+\)/H\(^-\) antiporter, NhaA, is essential for NaCl resistance, especially at pH 8.5.