Characterization of the RNF complex (NADH:ferredoxin oxidoreductase) in *Escherichia coli* and *Vibrio cholerae*

by

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ABSTRACT

Cholera is a severe disease and a major health problem worldwide. *Vibrio cholerae* is the causative agent of cholera, and it has caused seven pandemics since 1817 (Reidl 2002). The bacterium's ability to survive and persist in a variety of extreme environments including estuarine waters and in the stomach of humans has facilitated the occurrence of cholera epidemics. The molecular processes involved in the virulence of *V. cholerae* are continuously being studied because of the importance and severity of this disease. The objective of this project is to elucidate the function of RNF (*Rhodobacter* nitrogen fixation) and its possible role in adaptation and pathogenicity of the cycle of infection of *V. cholerae*.

The cell can use bioenergetics and sodium motive force (smf) to transport solutes and nutrients, synthesize ATP, and most importantly, cause flagellar rotation. The virulence of *V. cholerae* may be linked to flagellar rotation, so elucidating the function and role of sodium pumps within the cell is essential (Kojima 1999). A known sodium pump in *V. cholerae* is Na^+-NQR, and through sequence comparisons, the RNF complex was discovered in *Rhodobacter capsulatus* (Jouanneau 1998). The gene cluster is composed of six genes *rnf*A, *rnf*B, *rnf*C, *rnf*D, *rnf*G, and *rnf*E which encode the components of a membrane complex that are required for nitrogen fixation and electron transport to nitrogenase. An interesting aspect is that the *rnf* genes exhibit close homology to genes from other bacteria including *Haemophilus influenzae*, *Escherichia coli*, *Vibrio alginolyticus*, and *Vibrio cholerae*, all which are non-nitrogen fixing species. This discovery proposes the questions of why the RNF complex is located in bacteria that do not use nitrogen fixation, if it is a sodium pump like its homologue Na^+-NQR enzyme, and what role this complex plays within the cell.

The first method for studying the RNF protein complex is to purify it from the membrane. To accomplish the purification of the complex, a recombinant form of the *rnf* operon was over-expressed in *V. cholerae* and purified using affinity chromatography. Once the purified protein has been obtained, the enzymatic activity and ion translocation across the membrane can be determined. It is important to determine if the reaction that RNF catalyzes goes from NADH to ferredoxin or if it proceeds from ferredoxin to

NADH, and whether it acts as a sodium or proton pump for the cell. Also, site directed mutagenesis studies on the purified protein would aid in determining which residues are essential in electron transfer.

In order to characterize the function of RNF in *V. cholerae*, it is necessary to construct a deletion strain without the genomic copy of RNF. This deletion strain will allow the physiological role of the enzyme to be determined by growing RNF in different conditions to mimic the life cycle of the cell. The effects of sodium and oxygen concentrations, pH, and temperature will be studied.

Another technique for analyzing the role of RNF is to identify and characterize the redox cofactors in *V. cholerae*. RNF has been proven to be a flavoprotein and in order to characterize the different flavin cofactors of the RnfD and RnfG subunits, it is essential to isolate and express them individually. The RnfG subunit has been purified and will be analyzed by mass spectroscopy to conclusively determine if the flavin cofactor is either FAD or FMN. Site-directed mutagenesis studies have been performed to change the threonine binding site of the flavin from the distinctive S(T)GAT motif to a leucine. Through UV-visible and mass spectroscopy, it will be demonstrated that the threonine is the binding ligand for the FMN flavin. These studies will further our understanding of RNF and the importance this protein may play in the adaptation and virulence of *V. cholerae*.