Dissertation Project – 2nd Cycle

Supervisor(s): Américo G. Duarte; Inês Cardoso Pereira
Supervisor(s) email address: americoduarte@itqb.unl.pt, ipereira@itqb.unl.pt
Lab/Institution: Bacterial Energy Metabolism

TITLE: Study of the QmoABC membrane complex involved in energy conservation in gut anaerobes

BACKGROUND

Sulfate reducing bacteria (SRB) are common members of the human gut microbiota [1,2]. These anaerobes have been implicated in inflammatory bowel diseases (e.g., ulcerative colitis and Crohn's disease), due to the toxic and corrosive effects of sulfide produced from sulfate reduction, which lead to cell inflammation [1,3,4]. Other illnesses of the human gut tract have also been linked to SRB activity, such as cholocystitis, abdominal abscesses, spondylitis or even colorectal cancer (where sulfide is considered to be an initiator of the disease by activating a number of biochemical pathways) [5]. In the human gut hydrogen and several organic acids are used as electron sources for respiration with sulfate as the terminal electron acceptor, relying on respiratory chain membrane complexes that conserve energy through redox loops in order to build a proton motive force for ATP synthesis.

The QmoABC is a quinone-interacting membrane complex highly conserved in SRB that was proven to be essential for sulfate reduction [6]. In our lab, we demonstrated that Qmo interacts with adenosine 5'-phosphosulfate reductase (AprAB) [7], and links the quinol pool oxidation to the sulfate reduction [8]. The QmoABC complex is composed by two cytoplasmic subunits (QmoAB) and one membrane subunit (QmoC). Its biochemical characterization revealed the presence of multiple redox cofactors: two *b*-type hemes, two FAD and several FeS clusters [9]. The QmoC subunit belongs to the family of the membrane cytochromes *b* where its hydrophobic C-terminal domain includes six transmembrane helices binding two heme *b* groups. These type of proteins are often involved in quinone redox chemistry, and present in several respiratory chains but their mechanism is dependent on which side of the membrane (P-side or N-side) the quinone interacts.

The objective of this project will be to study the molecular mechanism of QmoABC in terms of energy conservation. This work will provide a better understanding of the respiratory mechanism for sulfide production in the gut, and may provide a possible target for therapeutic intervention.

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OBJECTIVES

The main objective of this project is to understand the functional mechanism of the QmoABC complex. To accomplish this goal, a homologous overexpression system will be established, which will allow the large scale production of the complex for biochemical and kinetic experiments.

Therefore, the project is divided in four major tasks:

1) Cloning the *qmoABC* operon in an independent plasmid for expression in *Desulfovibrio vulgaris* Δ*qmoABC*;
2) Production and purification of the QmoABC complex;
3) Reconstitution of QmoABC into liposomes;
4) Fast-kinetic experiments with proteoliposomes to determine H^+/e^- stoichiometry.

PROJECT DESCRIPTION

Task 1 – Cloning the *qmoABC* operon in an independent plasmid for expression in *Desulfovibrio vulgaris* Δ*qmoABC*

The *qmo* operon will be cloned into an independent vector for homologous expression in *D. vulgaris*. Since it is a large sequence, sequence-and ligation-independent cloning (SLIC) will be used to produce the final plasmid. In this procedure, affinity tags will be introduced to facilitate the complex purification (task 2).

Task 2 – Production and purification of the QmoABC complex

The plasmid containing the complete *qmoABC* operon will be transformed into *D.* vulgaris Δ*qmoABC* strain, which is already available at the lab. Expression optimization will be carried in different growth conditions and the complex will be purified with an established protocol or by optimizing an affinity chromatography step.

TIMELINE

	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6	Month 7	Month 8	Month 9	Month 10
Task 1										
Task 2										
Task 3										
Task 4										
Thesis										