MASTER RESEARCH PROJECT

Project title: Functional study of a focal protein in the anaerobic respiratory metabolism of a bacterium capable of power up microbial fuel cells

Host laboratory/Institution: Inorganic Biochemistry & NMR / Instituto de Tecnologia Química e Biológica

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Duration: 1 year

Background:

Bioelectrochemical systems (BESs) have attracted much research attention due to their promising applications in bioremediation, wastewater treatment, electrical power generation, production of valuable compounds and biosensing. The electrode reactions of these systems are catalyzed at high rates by biological entities, either redox enzymes or whole living microorganisms, such as bacteria. One of the most promising approaches is **microbial fuel cells** (MFC), since electricity can be generated from wastewater or other biomass resources. In these devices, microorganisms support their growth by oxidizing organic compounds existing in the wastewater, and transfer metabolic electrons outside the cell to an electrode where electricity can be harvested. For *Shewanella oneidensis* MR-1, one of the best studied organisms that are capable to power up MFC, the electron transfer pathway to the electrode is sustained by several *c*-type cytochromes, which are able to shuttle electrons from the cytoplasm toward the outside of the cell. *S. oneidensis* MR-1 mutants lacking the gene that codes for the **membrane-bound tetraheme cytochrome CymA,** exhibited a decrease of more than 80% of current generation in MFCs, indicating that this protein is essential for extracellular electron transfer. It was proposed that this protein receives electrons from the menaquinone pool and transfers them to periplasmic proteins that can be terminal reductases for soluble electron acceptors, or other multiheme cytochromes that are responsible for delivering electrons across the periplasm to the outer membrane terminal reductases. CymA is a 21 kDa protein that belongs to the NapC/NirT family of quinol dehydrogenases, presenting a N-terminal transmembrane α-helix that anchors the protein to the cytoplasmic membrane, and a globular periplasmic domain that covalently binds four c-type heme groups. Despite the key physiological role of CymA in the anaerobic respiratory metabolism of *S. oneidensis* MR-1, no detailed structural and functional information has been reported to date. One of the main difficulties is the fact that this protein is membrane-bound, and detergents must be used to solubilize it, compromising the stability and folding of the protein. The aim of this work is to produce and study the soluble version of CymA by producing CymA without the transmembrane anchor.

Work plan:

CymA without the membrane anchor will be produced using an over-expression system available in the host laboratory. Briefly, using molecular biology tools, the N-terminal sequence of the *cymA* gene cloned in the pBAD/D-TOPO vector will be removed. The over-expression of the truncated form of CymA will be accomplished by the use of a homologous recombinant expression system, and the purification of the target protein will proceed through standard chromatography protocols. Nuclear magnetic resonance will be one of the tools to obtain structural insights of the redox centers of the target protein, while spectroelectrochemical methods will be used to determine the redox properties of the truncated form of the protein. This characterization will be the first step to obtain molecular insights of the electron transfer process performed by this focal protein.

This information will also be essential to understand the promiscuity of this protein regarding the various periplasmic electron acceptors, elucidating its focal role in the anaerobic respiratory metabolism of *S. oneidensis* MR-1.

Methodologies:

Molecular Biology
Fast protein liquid chromatography (FPLC)
Nuclear resonance spectroscopy
Spectroelectrochemistry

For more information please visit the WebPages:

http://www.itqb.unl.pt/~louro/
http://www.itqb.unl.pt/research/biological-chemistry/inorganic-biochemistry-and-nmr

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