MASTER RESEARCH PROJECT

Project title: Detailed characterization of UndA: a redox protein from a Uranium reducing bacterium

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

Supervisor: Catarina M. Paquete
Co-supervisor: Ricardo O. Louro

Duration: 1 year

Background:

Dissimilatory metal reducing bacteria can use a variety of exotic terminal electron acceptors for respiration, including soluble metallic compounds or even metallic minerals. This metabolic capability has a tremendous potential for the development of bioremediation strategies of environments contaminated with heavy metals and radionuclides. The reduction of insoluble terminal electron acceptors, such as metallic ores, requires that electrons are delivered to the cell surface. This form of metabolism is called extracellular respiration, and in numerous bacteria it was demonstrated that it is linked to the action of multiheme cytochromes that exist in the outer surface of the cells. UndA is the outer-membrane cytochrome from *Shewanella* sp. strain HRCR-6 that was isolated from near-shore sediments from the Columbia River along the Hanford Reach in an area contaminated by radioactive metal residues. This protein belongs to the outer-membrane family of proteins that may contain proteins with 10 or 11 hemes. UndA is the only of the undecaheme cytochromes for which a structure is known.

We have previously cloned and over-expressed UndA, and prepared plasmids coding for mutations in the vicinity of each heme. This work, together with the knowledge of the structure provides the first opportunity to characterize in detail the electron transfer properties of a protein with this degree of complexity, setting the stage to improve the bioremediation capabilities of organisms capable of performing extracellular electron transfer.

Work plan:

The over-expression of native and mutated forms of UndA will be accomplished by the use of a homologous recombinant expression system already available in the host laboratory, while the purification process will proceed through standard chromatography protocols. The correct fold of the mutated proteins will be confirmed by biochemical and spectroscopic methods including NMR spectroscopy. Once pure well folded proteins are available, spectroelectrochemical methods will be

used to determine the redox properties of this protein and its mutants. The results will be analysed to extract the midpoint reduction potentials of the individual hemes, providing for the first time a detailed characterization of a protein of this level of complexity.

Methodologies:

Fast protein liquid chromatography (FPLC)
Nuclear magnetic resonance spectroscopy
Spectroelectrochemistry
Modeling of redox properties

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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