MSc Project

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TITLE: Structural and functional characterization of acetate membrane transporters

BACKGROUND

Glucose is one of the principal fuels when it comes to cancer cells. More recently it has become clear that other fuel sources play an important role in cancer. A variety of cancer cells are able to capture (from outside the cell) and metabolize acetate, a metabolic adaptation that facilitate their growth. Acetate can be a major contributor to acetyl-CoA and is transported into cells by members of the monocarboxylate transporter family. Acetyl-CoA is essential for the synthesis of nucleotides, amino acids and cell membrane components (e.g fatty acids and cholesterol). The ability to recoup acetate and use it as fuel may promote tumor cell growth or survivability in face of nutritionally challenging or hypoxic microenvironments as in cancer cells (tumors need nutrients and oxygen from the blood to grow and spred). This may also explain how cancer cells may become resistant to cancer treatments using angiogenesis inhibitors (block formation on new blood vessels), and more studies are needed in this emerging topic.

We are interested in studying acetate uptake transporters (AceTr) found in bacteria and archaea, namely Succinate-Acetate Transporter SatP-YaaH and acetate permease ActP with 6 and 14 predicted transmembrane helices, respectively. A number of these proteins are homologues to those found in man, so they can provide a convenient model for elucidating by proxy the molecular mechanisms of mammalian transporters and valuable for rational drug design.

OBJECTIVES

In this project we aim for the structural and functional characterization of the acetate transporters. We have already optimized the growth conditions of several SatP-YaaH and ActP homologues to produce sufficient amounts of proteins to proceed with biochemical and functional studies. We have already obtained crystals for one target, which is under optimization trails. The goal is to unravel the mechanism of acetate translocation and identify the amino acid residues responsible for monocarboxylate specificity. Several mutants have been engineering to evaluate their effect in acetate uptake and help locating the substrate binding site. It is envisaged that the shapes and structures of the substrates determine their recognition or exclusion by the two transport systems.

PROJECT DESCRIPTION

The tasks to be performed within this project comprise an experimental component and also involve some computational work. Tasks include gene cloning and expression in *Escherichia coli*, purification of several selected membrane transporters from various sources; functional and biochemical experimental assays, crystallization of the purified protein and subsequent structure determination. The elucidation of the molecular structure of these target proteins will provide insights into the mode of action and specificity of these transport systems.

The work will be done in the Laboratory of Membrane Protein Crystallography at ITQB under supervision of Dr Margarida Archer Frazão, in close collaboration with Prof. Margarida Casal, Univ. Minho. In the scope of the project, trips to European synchrotron radiation sources to collect X-ray diffraction data can take place and short visits to University of Minho and Membrane protein Laboratory (Oxfordshire, UK) may be arranged.

Task 1- Gene cloning and expression tests in Escherichia coli and purification of selected transporter targets, screening for the best detergents for membrane extraction and protein stabilization

Task 2- Stability and characterization of the purified proteins (e.g. circular dichroism, homogeneity tests), protein-ligand interactions (isothermal titration calorimetry, surface plasmon resonance,) and functional characterization (activity assays in proteolipossomes, mutagenesis)

Task 3- Crystallization experiments of the purified proteins (apo-, ligand-bound and mutants).

Task 4- X-ray diffraction data measurements, in-house or at synchrotron sources, structure determination and structure-function analysis

TIMELINE (use fill tool for the cells)

	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										