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Título: Oxaloacetate decarboxylase complex from Vibrio cholerae

Life relies on the constant exchange of different forms of energy, i.e., on energy transduction. All living organisms need energy to fuel life processes. External energy sources, light or chemical compounds, are converted to biologically usable forms of energy, such as adenosine triphosphate (ATP) or electrochemical gradients (Δ𝜇̃). The energy transduction is performed by membrane proteins that couple their exergonic catalytic reactions to the translocation of charges (electrons or ions, H+ or Na+) across the membrane. The energy stored in the form of the Δ𝜇̃, is used to power all kinds of essential cellular processes such as ATP synthesis, active transport across the membrane, flagellum rotation or thermal dissipation, among others.

The majority of the organism seem to rely on a proton motive force (Δ𝜇̃𝐻+); but some microorganisms depend on sodium gradient to survive (Δ𝜇̃𝑁𝑎+). Many human and animal pathogens rely on the use of Na+ as a coupling ion instead of or in addition to the H+. This capability to use a sodium motive force might have an important role in energy metabolism and pathogenicity of some pathogens, such as Vibrio cholerae, a Gram-negative pathogen responsible for 3 to 5 million cases of cholera annually and 100,000 to 120,000 of deaths. The discovery of korormicin, an effective antibiotic targeted against the Na+-translocating NADH:ubiquinone oxidoreductase, suggests a potential use of Na+-translocating complexes, such as OAD, as drug targets candidates.

Oxaloacetate decarboxylase (OAD) was the first enzyme of the Na+-transport decarboxylases family demonstrated to act as a Na+ primary pump. OAD couples the Gibbs energy change of the decarboxylation reaction to the transport of Na+ across the membrane, thus contributing to the establishment of Δ𝜇̃𝑁𝑎+. In V. cholerae were identified two gene clusters encoding OAD, termed oad-1 and oad-2. oad-2 is inserted into the citrate fermentation operon and the oad-1 is not associated with genes for a specific fermentation pathway.

The main goal of this project is to explore the structure and function of the transmembrane complexes OAD-1 and OAD-2 present in V. cholerae. In this project, the MSc student will learn microbiology, molecular biology, biochemistry and biophysics methodologies. Specifically, the student will perform, among other techniques, cell growths, protein expression, purification and biochemical and biophysical characterizations.

Task 1 – Preparation of the studying systems

A) Molecular Biology methodologies will be used to insert fluorescent tags and to create knock-out mutants that will allow the investigation of the function, localization and expression conditions of the OAD-1 and OAD-2 complexes in vivo (task 2).

B) This task aims at obtaining the target protein for subsequent studies (task 3).

The enzymes will be cloned in a vector to be expressed with a His-tag both in E. coli and in V. cholera or Vibrio natriegens. Expression conditions will be optimized.

After cell growth, cells will be harvested by centrifugation and disrupted; membranes will be obtained by ultracentrifugation and will be solubilized with detergent. The protein will be isolated by chromatographic procedures in ÄKTAprime systems. All the purifications steps will be monitored by activity assays, UV-visible spectroscopy and electrophoretic procedures.

Task 2- In vivo studies

Cell growths using different carbon sources and oxygen conditions will be done to evaluate the conditions in which the proteins are expressed. Fluorescent microscopy and Fluorescence spectroscopy will be used to detect the localization and the expression of the proteins in the cell, respectively.

Task3- In vitro studies

Task3 will biochemically and spectroscopically characterize the OAD-1 and OAD-2 enzymes. Biochemical characterization will be performed using standard methods, such as electrophoreses, chromatography and activity assays. For the quinone reductase, the oligomerization state of the protein, its reduction potential and kinetic parameters such as Km and Vmax are expected to be determined.

Work to be developed at FCUL