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Scientific area: Biochemistry
TITLE: Unraveling a key O_2/NO detoxification mechanism of an enterohemorrahagic *E. coli* strain

BACKGROUND

Nosocomial infections are a major health problem worldwide, maintaining a pool of affected people of over 1.4 million; in Portugal for example, an average of 12 deaths per day is due to hospital acquired infections. These are caused by many pathogens, from which bacteria are the most common ones, namely gram-positive bacteria as Clostridium sp., or gram-negative bacteria such as *Escherichia coli*.

The ability of human immune system to deal with the infections caused by some of these organisms relies on several defence mechanisms, including the generation of nitric oxide (NO). In this sense, evolution has provided defence mechanisms that enable these organisms to survive to these molecules.

One of these mechanisms is based on flavodiiron proteins, FDPs. Widespread among all Kingdoms, FDPs may have a dual role regarding O_2 and NO detoxification, contributing to the microbial survival to the stress imposed by host immune system. All FDPs have a central core composed by a metallo-β-lactamase-like diiron containing domain, followed by a flavodoxin one. Extensive bioinformatic studies have recently shown that FDPs may have up to three additional domains, having extra redox centers. The FDP from *Escherichia coli*, has one extra domain, predicted as a small-rubredoxin-like domain.

OBJECTIVES

The main objectives of this Project are to analyse site directed mutants of a still intriguing putative NO reductase from an enterohemorrahagic *Escherichia coli* strain. The mutants were constructed on the basis of the already available three-dimensional structure, in order to underpin key events of the enzyme´s catalytic cycle.

In particular, the analysis of these mutants may contribute to disentangle the substrate specificity of these enzymes, some of which are promiscuous regarding O_2 and NO.

PROJECT DESCRIPTION

The project will be divided in four tasks, towards achieving the main goals of this project.

Task 1 – Enzyme production and purification: The clones of all enzyme targets are already available at the host laboratory, and preliminary tests revealed the overexpression of the targets. Therefore, this task will involve their production, by overexpression in *E.coli*, and purification, by standard chromatographic processes.

Task 2- General biochemical characterization: Determination of molecular masses and quaternary structure, metal and flavin contents, using several analytical tools (SDS PAGE, gel filtration, ICP, reverse phase HPLC).

Task 3 – Kinetic, thermodynamic and spectroscopic analysis: This task will start by an overall analysis of the thermodynamic (redox) and spectroscopic studies (UV-Visible, Electron Paramagnetic Resonance), aiming at determining the effects on the redox/catalytic centers due to the mutations, as well as analysing the newly studied enzyme from the pathogenic *E.coli* strain. Subsequently, a thorough kinetic study, by steady state (using specific NO and O_2 electrodes) and single turnover measurements, by fast kinetics (stopped flow) will be undertaken, to assess the effect of the mutation on the catalytic rates, stability of the enzymes during turnover and establish the function of the novel FDP.

Task 4 – Complementation assays: To further analyse the wt and mutant enzymes, complementation assays will be performed in an *E.coli* strain deleted in the FDP encoding gene, also available at the host Laboratory

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										