SCIENCESPRINGDAY

FCT FACULDADE DE CIÊNCIAS E TECNOLOGIA UNIVERSIDADE NOVA DE LISBOA

Chemistry Department

Insights into the structure and reactivity of the catalytic site of nitrous oxide reductase

BioIn and Microbial Stress and Bioremediation Groups

Supervisor: Isabel Moura, Co-Supervisors: Sofia R. Pauleta and Oliver Einsle

Cíntia Carreira

(PhD Student)

2010-Bachelor degree in Biochemistry (FCT/UNL).
2012-Master degree in Biotechnology (FCT/UNL).
2012-Research scholarship: "Proteins with heterometallic centers involved in stress response."
2013-PhD student in the Biochemistry doctoral program.

Objectives

Nitrous oxide (N_2O) is a potent greenhouse gas and its emission to the atmosphere has been enhanced in the last century through the intensification of agriculture. In nature, N_2O can only be converted to N_2 by the enzyme nitrous oxide reductase (N_2OR), in a metabolic pathway known as denitrification.
Nitrous oxide reductase (N_2OR) is a functional homodimer containing two copper centers, CuA, the electron transfer center and CuZ, the catalytic center (Fig.1). The complex redox chemistry and structural features of this unique catalytic center still needs to be unravel. The aim is to purify *Marinobacter hydrocarbonoclasticus* N_2OR under anaerobic conditions, crystallize it in a glove box with CuZ center in the different redox forms and in the presence of substrate, as well as, spectroscopically identify and characterize intermediates in its catalytic cycle.

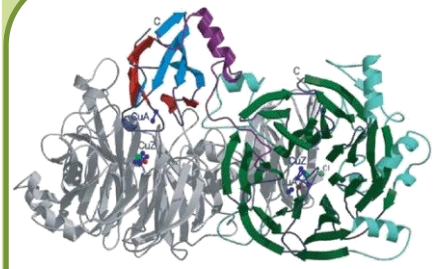

Figure 1: Structure of Nitrous Oxide Reductase and functional homodimer. Brown, K. *et al.*, *J. Biologial Chemistry*, 2000.

Methodology

- N_2OR from *Marinobacter hydrocarbonoclasticus* and its redox partner, cythocrome c_{552}, will be purified using different chromatographic steps, from anaerobically grown cells.
- In order to clarify the catalytic mechanism of reduction of N_2O, the spectroscopic data will be correlated with kinetic characterization of the activated form of N_2OR (Fig.2).
- X-ray studies will be conducted to unravel the structure of the catalytic center of *Marinobacter* N_2OR in the different redox states.
- Electrochemical tools will be used to determine redox potencial of CuA and CuZ centers. Different redox forms of N_2OR will be immobilized in the electrode surface, either directly or using modified electrodes, in order to obtain a good and reversible signal.

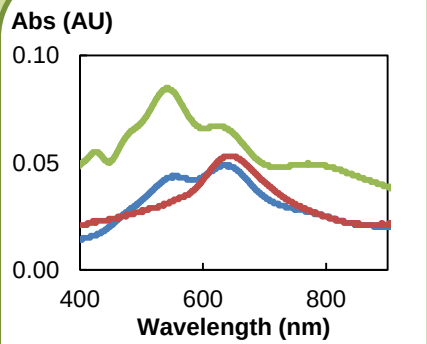

Figure 2: UV-visible absorption spectra of different redox forms of *Marinobacter hydrocarbonoclasticus* N_2OR. – Enzyme as isolated, – enzyme with CuZ center in the [$1Cu^{2+}$-$3Cu^{+}$] state and the CuA center reduced, – enzyme with CuZ center in the [$2Cu^{2+}$-$2Cu^{+}$] state and the CuA center oxidized.

Expected Results

- Isolation of N_2OR in different redox forms that are characterized by presenting CuZ center in different redox states, ([$2Cu^{2+}$-$2Cu^{+}$] or [$1Cu^{2+}$-$3Cu^{+}$] or [$4Cu^{+}$]), and CuA center in the oxidized ([$Cu^{1.5+}$-$Cu^{1.5+}$]) or reduced state ([Cu^{+}-Cu^{+}]).
- Structure characterization of the different N_2OR redox forms. Clarification of the structural changes in the coordination of the copper centers and the substrate binding site in each redox form.
- Unravel the activation mechanism of N_2OR and identify intermediates in the catalytic cycle.

Funding:

FCT Fundação para a Ciência e a Tecnologia

PhD scholarship financed by Fundação para a Ciência e Tecnologia (FCT), SFRH/BD/87898/2012.