SCIENCESPRINGDAY

FACULDADE DE CIÊNCIAS E TECNOLOGIA
UNIVERSIDADE NOVA DE LISBOA

Chemistry Department

Bacterial Aldehyde Oxidases: Structure & function

Macromolecular Crystallography Group

xtal Crystallography Group @ REQUIMTE

http://xtal.dq.fct.unl.pt/

requimte
rede de química e tecnologia

Márcia Correia

Postdoctoral Research Fellow (since 2010)

2006-2010 – PhD in Science and Animal Technology (Molecular biology)

1999-2004 – Graduated in Biochemistry

Published 9 article in I.J.

Supervisor: Prof. Maria João Romão

Objectives

Molybdoenzymes are involved in a large number of enzymatic reactions in the nitrogen, carbon and sulfur cycles. Aldehyde oxidases are present in different species, and can be found in many tissues. In humans, AO is abundantly expressed in the liver, with a major role in drug clearance, similar to CYP450. AO is able to perform different oxidative and reductive transformations, namely heterocyclic oxidations. In recent years, exhaustive work has been done regarding its biochemical characterization with identification of numerous inhibitors and substrates of the enzyme, which has given rise to pharmacological interest on the protein. Xanthine oxidases are also present in several organisms and, in humans, XO is mainly involved in the metabolism of purines. Gout and xanthinuria are the most common pathologies in which this enzyme is involved.

Methodology

3D structure characterization of a new, heterotrimeric periplasmic aldehyde oxidase (PaoABC) from *Escherichia coli* is going to be achieved by X-ray crystallography. PaoABC, is the first example of an *E.coli* protein containing a molybdopterin cytosine dinucleotide (MCD) and it is the only heterotrimer (aßγ) of the XO family known in the literature. This 135 kDa enzyme contains a large subunit (PaoC - 78.1 kDa), a medium subunit (PaoB - 33.9 kDa), and a small subunit (PaoA - 21.0 kDa). Crystal were prepared using a protein concentration of 20 mg/ml and a solution of salts and poly(ethylene glycol) as precipitant. Vapor diffusion-hanging drops were setup at 20 °C and crystals appeared in two or three days (Figure 1). Using synchrotron radiation, a complete data sets was collected at PXIII (X06DA) beamline of the SLS (Paul Scherrer Institut, Switzerland).

Expected Results

The PaoABC crystals diffracted up to 1.8 Å resolution and belong to C2 space group with cell constants a=109.42, b=78.08, c=151.77 Å; ß=99.77º. The Structure was solved by molecular replacement (Figure 2). In spite of the high similarities to members of the XO family, the presence of approximately 30 additional amino acids with four conserved cysteine residues and a clear peak in the anomalous difference maps proved the existence of this unexpected [4Fe-4S] cluster (Figure 3). PaoABC, together with 4-hydroxybenzoyl-CoA reductase (4-HBCR) from Thauera aromatica (PDB ID: 1RM6), are the only know proteins to carry such a cluster. Furthermore, PaoABC is the first example of a molybdopterin cytosine dinucleotide-containing enzyme in E.coli and also the first example of a monomeric enzyme from the XO family.

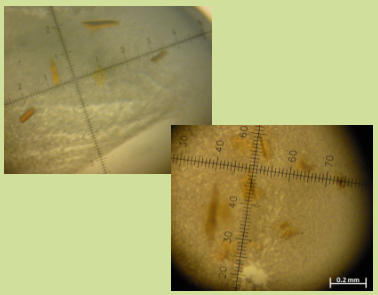

Figure 1 -. PaoABC crystals

Figure 2 - Structure of PaoABC from *E.coli.* Subunits A, B and C are represented by orange, green and blue respectively.

NEW [4Fe4S] CLUSTER!

Figure 3 - [4Fe-4S] cluster from PaoABC.

Funding: Portuguese Foundation for Science and Technology
BPD SFRH/ 64917/2009 and Projects PEst-C/EQB/LA0006/2011 and PTDC/BIA-PRO/118377/2010

FCT Fundação para a Ciência e a Tecnologia