Malate Quinone oxidoreductase (Mqo) from *Staphylococcus aureus*: preliminary data

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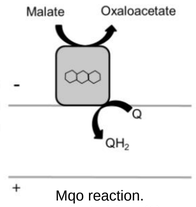
Introduction:

Harnessing of energy is central to life. Organisms obtain most energy by cellular respiration. While multicellular organisms have optimized the mitochondrial respiratory chain, unicellular organisms have developed remarkable diversity, flexibility and robustness their energetic metabolism, which allow them to live or survive under most diverse conditions. In this way these organisms may have alternative and/or multiple enzymes performing the same reaction. One of such examples is malate oxidation, which in mammals performed by the tricarboxylicacid cycle (TCA) enzyme malate:NAD+ oxidoreductase, while in some prokaryotes or unicellular eukaryotes the reaction may be performed by malate:quinone oxidoreductase (Mqo) (1).

Mqo is a one subunit peripheral enzyme which catalyzes the oxidation of malate to oxaloacetate, and reduces quinone to quinol. In this way, the enzyme directly supplies electrons to the respiratory chain. Some organisms may express the two enzymes such a the cases of *Escherichia coli* and *Corynebacterium glutamicum* (2), while others only contain Mdh or Mqo. Curiously, some clinical important pathogens such as *Helicobacter pylori* or *Staphylococcus aureus* only have genes coding for Mqo, being that *S. aure* present two Mqo copies, one of which seems to have more affinity with lactate than with malate (3). Mqo has been escaping the attention of the scientific community, even though it is reported as essential for cell growth and virulence of some bacteria. In fa deficiency in Mqo has been implicated in growth impairment and virulence of some pathogenic bacteria. This observation, together with the fact that Mqos are absent in mammals raised the possibility of Mqo as a potential drug target (4).

Here is shown the preliminary results of a work that aims to explore *S. aureus* Mqos at molecular and cellular levels, contributing to unravel the role of mqo in the bioenergetic metabolism and understating of its molecular structural and functional determinants.



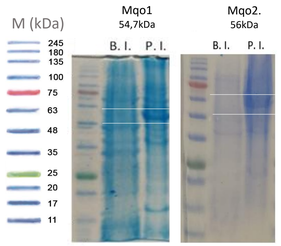
Mqo reaction.
Adapted from(1).

Experimental layout and results:

Mqos expression:

- The genes coding for both Mqo copies from *S. aureus* were inserted into pET-28a(+) plasmid

- *E. coli* C41 cells transformed, separately, with the two constructs were grown in Terrific Broth (TB) medium supplemented with 100 µg/mL of kanamycin, at 37 ºC and 180 rpm. Protein expression was induced with 1 mM of IPTG when an optical density of 0.6 (OD600) was reached. After an overnight growth, cells were harvested by centrifugation at 11305 g, 10 minutes. Samples before and after induction were analysed by SDS-PAGE to evaluate the expression of the protein.



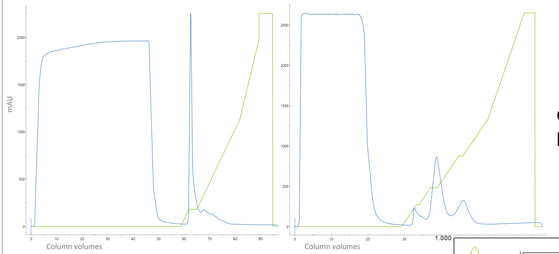
Mqo1 and Mqo2 were successfully expressed in *E. coli*

SDS-PAGE: BI- before induction; PI- post induction

Mqos purification:

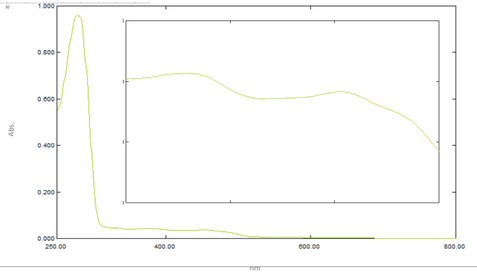
MQOs were extracted from the membrane with 2M of NaCl and purified using a Histrap HP column (5 mL), an affinity column loaded with nickel, which allows a strong interaction with 6xHisTag from the expressed proteins.

The Histrap HP column was equilibrated with potassium phosphate pH 7 buffer with 10 % glycerol and, 500 mM NaCl. The proteins were eluted with an increasing concentration from 0 to 250 mM of histidine



Chromatograms showing the elution of Mqo1 (left pannel) and Mqo2 (right pannel) with ~20 mM of Histidines.

UV/Visible absorption spectroscopy was used to analyse the purified Mqos samples



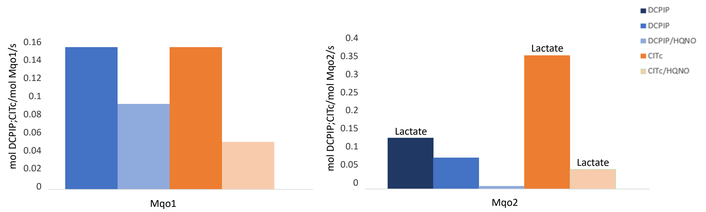
Mqos present a typical flavoprotein UV-visible spectrum with maximum absorbance at 375 and 450nm.

Mqos catalytic activity assays:

The specific enzymatic activities of Mqo1 and Mqo2 were monitored spectrophotometrically at 37ºC either with the enzyme in solution (following DCPIP reduction at xx nm) or reconstituted into liposomes (following cytochrome *c* reduction at 550 nm). Liposomes were composed of a mixture of POPE, POPG and cardiolipin. Malic acid or lactate were used as electron donors.

To confirm the specificity of the activity measured, HQNO, a inhibitor that compete with the quinone for the binding site, was added to the reactional mixture.

Mqo1 activity assays were performed using malate as electron donor. Due to the hypothesis raised of Mqo2 being a lactate:quinone oxidoreductase instead, the experiment was performed with both lactate and malate..



For MqoI, the oxidoreduction activity is the same whether it is in solution or in liposomes. However, in liposomes the inhibition by HQNO is observed to higher than in solution.

In contrast, Mqo2 showed an higher activity when incorporated into liposomes. In solution, Mqo2 has also activity when malate is used as electron donor. Nevertheless, in this conditions lactate seems to be the preferential electron donor.

Future work:

To better understand the role of Mqos, it is necessary to perform its functional, structural and biochemical characterization. Among other things we want to:

- Perform kinetic analysis through Steady-state and Fast Kinetic Assays (Stopped-Flow)
- Obtain the crystallographic structure
- Create and evaluate the effect of Mqo knock-out mutants in cell context and explore the spatial-temporal organization of the enzymes in cell context using GFP proteins.

References:

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3. 17. Fuller J. et al., Identification of a lactate-quinone oxidoreductase in Staphylococcus aureus that is essential for virulence, Frontiers in Cellular and Infection Microbiology, 2011, 1 - 19.
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