Exploring the technique using Mg Green to analyse mitochondrial ATP production.

Luiza H. D. Cardoso

Mitochondrial respiration and coupling control ratios have been broadly used as parameters to assess mitochondrial function and efficiency. Nevertheless, these methods allow to analyse the O_2 consumption coupled to oxidative phosphorylation (OXPHOS), but not the ATP production itself.

Magnesium Green (MgG), is a Mg^{2+}-sensible fluorescent probe that has been used to measure the ADP/ATP exchange by the adenine nucleotide translocase (ANT), exploiting the fact that ADP and ATP have different affinities to Mg^{2+} [1]. This method can be used to assess mitochondrial ATP production concomitantly with high-resolution respirometry using the Oroboros O2k FluoRespirometer [2]. We aim to further develop the use of these two methods combined to analyse P»/O_2 ratios in different mitochondrial preparations.

The first experiments conducted aimed to compare the mitochondrial respiration in two different media, one specifically developed for the ANT assay ("ANT buffer") [1,2], and MiR05, developed for oxygraphic applications. The MiR05 had its composition modified with 1 mM $MgCl_2$ instead of 3 mM, a change that was necessary to use the MgG technique. The experiments were performed with our gold standard, the cryopreserved HEK293 cells, permeabilized in the O2k chamber, or with mouse brain isolated mitochondria (imt), using the SUIT-002 protocol, in which several ET-pathway states are analysed in OXPHOS. With HEK293 cells, in MiR05 medium with 1 mM $MgCl_2$, OXPHOS respiration was higher than in ANT buffer in the presence of FAO-linked substrates (octanoylcarnitine and low concentration of malate) and *circa* 4 times higher with a combination for FAO- and NADH-linked substrates (octanoylcarnitine, malate, pyruvate and glutamate). With mouse brain imt such differences were not detected. Furthermore, the decrease in the amount of $MgCl_2$ in MiR05, from 3 mM to 1 mM, did not lead to changes in respiration with the samples studied. MiR05 (with modified $MgCl_2$ concentration) was the medium chosen for the next experiments. This medium has been broadly used for respirometry allowing the comparison with previously published data in respiration.

Further experiments were performed to analyse whether MgG could affect mitochondrial respiration. This was investigated with coupling control protocols with different substrates/inhibitors (pyruvate and malate or succinate and rotenone) using mouse cardiac isolated mitochondria. The fluorescent dye did not impact the respiration in any of the conditions studied.

In conclusion, MgG does not interfere with mitochondrial respiration. These results indicate that concomitant measurement with O_2 consumption in the O2k for the determination of the P»/O_2 ratios is possible and these measurements can be done using a coupling control protocol. The next steps will include the comparison of this technique with other published techniques for analysing P»/O_2 ratios [3]. These topics, among other developments to the technique will be discussed during the seminar.

[1] - Chinopoulos C. *et al.* (2009). A novel kinetic assay of mitochondrial ATP-ADP exchange rate mediated by the ANT. Biophys J. 96 (6): 2490-504.

[2] - Chinopoulos C. et al., (2014). Measurement of ADP-ATP exchange in relation to mitochondrial transmembrane potential and oxygen consumption. Methods Enzymol. 542: 333-48.

[3] - Gnaiger E. *et al.*, 2000. High phosphorylation efficiency and depression of uncoupled respiration in mitochondria under hypoxia. Proc Natl Acad Sci U S A. 97 (20): 11080-5.