O2k-Procedures: Isolated mitochondria and permeabilized cells

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Isolated mitochondria or permeabilized tissues and cells

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Summary: Whereas isolated mitochondria remain one of the gold-standards in studies of bioenergetics and mitochondrial physiology, permeabilized tissues and cells have become an alternative with several advantages. But some disadvantages must be considered, too, for optimum experimental design and critical evaluation of results.

Contents

1. Permeabilized tissue or cells versus isolated mitochondria in respirometry (pti vs. imt): advantages and disadvantages

1.1. General considerations

1. Respiratory flux is frequently related to mitochondrial protein mass in isolated mitochondria imt or tissue wet mass in permeabilized tissue pti (including permeabilized muscle fibers pfe), whereas O_2 flow is commonly normalized per cell in living cells ce and permeabilized cells pce (Gnaiger et al 2020). It is important to note that interpretation is different of changes in respiratory flux per mitochondrial protein (a mitochondria-specific marker), per mass of tissue (tissue-specific but not mitochondria-specific flux), or per million cells (an extensive rate that varies with cell size and is thus not comparable from platelets to fibroblasts and cardiomyocytes). For comparison of results, a common mitochondrial marker has to be quantified, such as citrate synthase activity

(MiPNet17.04), Complex IV activity, or cytochrome aa_3 content (Renner et al 2003).
2. Respiratory results on human skeletal muscle using permeabilized fibers pfe (Gnaiger 2009) agree with data on imt (Rasmussen et al 2001).
3. Substrates specific for feeding electrons into different complexes of the respiratory system, for various segments of the tricarboxylic acid cycle, and for transporters are used for functional evaluation of specific mitochondrial pathways (Gnaiger 2020).
4. Substrate combinations are required for evaluation of maximum capacities of electron transfer and oxidative phosphorylation, to reconstitute physiological TCA cycle function (Gnaiger 2009; 2020).
5. Few studies with pfe report the dependence of oxygen flux on various substrate concentrations, and direct comparison of pfe or pce and imt are scarce, except for ADP and O_2 (reviewed by Gnaiger 2009).
6. Optimum uncoupler concentrations for pce and pti cannot be deduced from studies with imt since the sensitivities are different.

1.2. Advantages of permeabilized tissue or cells (pti, pce)

1. pti and pce need less tissue or fewer cells than imt. In high-resolution respirometry (O2k, Oroboros Instruments, Innsbruck, Austria), only 1 mg wet mass of cardiac fibers is required per experimental test, in a 2 mL chamber at 37 °C, or 0.3 million cells (fibroblasts, endothelial cells).
2. Optimization of imt preparations may be significantly more time-consuming compared to the optimization of pti pr pce preparation. A set of standardized tests can be applied for quality control of pti, pce, or imt preparation.
3. All types of mitochondria are experimentally accessible in pti and pce, whereas imt preparation allows for the separation of different mitochondrial populations (advantage), or it has been argued (but probably never shown experimentally) that imt may involve the selective loss of damaged mitochondria.

1.3. Advantages of isolated mitochondria (imt)

1. Preparation of imt is required for separation and study of different mitochondrial subpopulations (Palmer et al 1977; Riva et al 2005).
2. The homogeneous suspension of imt yields a representative average for the tissue sample, and fewer replicas are required for averaging over heterogenous subsamples of fibers.
3. The oxygen dependence of respiration in permeabilized muscle fibers is increased by two orders of magnitude, due to O_2 diffusion to the mitochondria in the small nonperfused fiber bundle. Therefore, imt are chosen for the study of mitochondrial oxygen kinetics. Small, suspended cells are a good model, as well (Sandurra, Gnaiger 2010). Low oxygen levels must be strictly avoided in studies with muscle fibers, which is easily done, if one is aware of the problem (Gnaiger 2003; Pesta, Gnaiger 2012). O_2 concentration are increased in the O2k-chamber using electrolytically produced O_2 gas (Oxia, Oroboros Instruments; MiPNet26.14) and H_2O_2 titrations into MiR06 containing catalase (MiPNet14.13).
4. ADP must be added to pfi at high concentrations to achieve maximum OXPHOS capacity, due to diffusion restriction and since the mitochondrial outer membrane mtOM may exert a barrier function different from imt (Saks et al 2000; Gnaiger 2001).

2. Permeabilized tissue preparation and respirometry

1. Evaluation of mechanical tissue separation: The degree of mechanical tissue separation may be evaluated by observing a change to a pale colouring of the separated fiber bundles (similar for liver tissue). This is best observed when placing the Petri dish onto a dark background. Appropriate sharp forceps must be used. Initially, the main difficulties appear to be the application of too much tissue, which makes difficult the full attention to the mechanical separation of small amounts of tissue. A practical limit for routine experiments may be 10-20 mg wet mass of tissue, subsequently separated into 2-5 mg samples for experiments.
2. Mechanical and chemical permeabilization of the cell membrane: The mechanical tissue preparation leads to partial (skeletal muscle) or full permeabilization of the cell membrane (heart muscle; liver tissue). A preparation of fully intact living cells, for the study of ROUTINE respiration, cannot be obtained by this approach. Partially permeabilized preparations need additional chemical permeabilization (saponin or digitonin), by standardized incubation conditions which leave the mitochondrial outer and inner membranes, mtOM and mtIM, intact. In merely mechanical permeabilized tissue, full permeabilization must be checked by addition of saponin or digitonin into the respirometer in the OXPHOS state (Pesta, Gnaiger 2012). Under these conditions, no stimulation of respiration is expected in fully permeabilzed cells, whereas partial permeabilization is indicated by a stimulatory effect of added detergent (Gnaiger et al 1998).
3. Measurement of oxygen consumption of 1-2 mg of tissue per experimental run requires high-resolution respirometry. The instrumental limit of detection of the O2k is 1 pmol $O_2 \cdot s^{-1} \cdot mL^{-1}$ up to air saturation (O_2 concentrations of c. 200 µM; Gnaiger 2001), but instrumental O_2 background corrections become more variable up to 600 µM and lower the limit of detection to 4 pmol $O_2 \cdot s^{-1} \cdot mL^{-1}$ (MiPNet14.06).
4. Stability of tissue biopsies before the experiment is usually sufficient following standard procedures. Stability is prolonged by storing the biopsy in an intracellular preservation medium, since some cells are permeabilized during tissue sampling, minimizing storage after permeabilization, and by application of preservation medium (Gnaiger et al 2000) after permeabilization and prolonged storage (Skladal et al 1994).
5. Stability of the tissue preparation in the respirometer depends on the application of a high-quality mitochondrial respiration medium (Gnaiger et al 2000). High stability allows for application of complex and extended substrate-uncoupler-inhibitor titration (SUIT) protocols (Gnaiger 2020).

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