OROBOROS INSTRUMENTS
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Protocols

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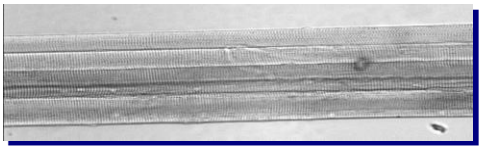
Isolated Mitochondria or Permeabilized Tissues and Cells



Erich Gnaiger

OROBOROS INSTRUMENTS Corp, high-resolution respirometry
Schöpfstr 18, A-6020 Innsbruck, Austria
erich.gnaiger@oroboros.at; www.oroboros.at
Medical University of Innsbruck
D. Swarovski Research Laboratory
A-6020 Innsbruck, Austria

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 have to be considered, too, for optimum experimental design and critical evaluation of results.

1 Permeabilized Tissue or Cells versus Isolated Mitochondria in Respirometry (Ptic vs. Imt): Advantages and Disadvantages

1.1 General Considerations

1. Respiratory flux is frequently related to tissue wet weight or million cells in Ptic, and to mitochondrial protein in Imt. It is important to note that interpretation is very different of changes in respiratory flux per mass of tissue, per million cells, or per mitochondrial protein. For direct comparison of results, a common marker has to be quantified, such as citrate synthase activity [MiPNet08.14], Complex IV activity

[MiPNet08.12], or cytochrome *aa*3 content (Renner et al 2003).

2. Respiratory results on human skeletal muscle using Ptic (Gnaiger 2009) are in excellent agreement 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 various sections of mitochondrial metabolism [MitoPathways: MiPNet11.04, MiPNet11.09, MiPNet12.12, MiPNet12.13, MiPNet12.15].
4. Substrate combinations are required for evaluation of maximum capacity of oxidative phosphorylation, which match physiological intracellular conditions (Gnaiger 2009).
5. Few studies with Ptic are available reporting the dependence of oxygen flux on various substrate concentrations, and direct comparison of Ptic and Imt is scarce or lacking (reviewed by Gnaiger 2009), except for ADP.
6. Optimum uncoupler concentrations for Ptic cannot be deduced from studies with Imt, since the sensitivities are different.

1.2 Advantages of Permeabilized Tissue or Cells (Ptic)

1. Ptic needs less tissue or fewer cells than Imt. Using the OROBOROS Oxygraph-2k, only 1 mg wet weight of cardiac fibres is required per experimental test, in a 2 ml chamber at 37 °C, or 0.3 million cells (fibroblasts, endothelial cells).
2. Optimization of isolation of mitochondria may be significantly more time-consuming compared to the optimization of Ptic preparation. A set of standardized tests can be applied for quality control of Ptic or Imt preparation.
3. All types of mitochondria are experimentally accessible in Ptic, 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. Imt preparation 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 avarage for the tissue sample, and fewer replica are required for averaging over heterogenous subsamples of fibres.
3. The oxygen dependence of respiration in permeabilized muscle fibres is increased by two orders of magnitude, due to oxygen diffusion to the mitochondria in the small unperfused fibre bundle. Imt provide, therefore, the only choice for the study of mitochondrial oxygen kinetics (small isolated cells are a good model, as well). Low oxygen levels have to be strictly avoided in studies with muscle fibres, which is easily done, if one is aware of the problem (Gnaiger 2003).
4. ADP has to be added to Ptic at high concentrations to achieve max. OXPHOS capacity, due to diffusion restriction and since the outer membrane 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 fibre bundles (similar for liver). This is best observed when placing the Petri dish onto a dark background. Appropriate forceps have to be used. Initially, the main difficulties appear to be: (a) 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 weight 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 cells, for the study of routine or endogenous respiration in the cell, cannot be obtained by this approach. Partially permeabilized preparations need additional chemical permeabilization [MiPNet14.14]; saponin or digitonin), by standardized

incubation conditions which leave the outer and inner mitochondrial membranes intact. In merely mechanical permeabilized tissue, full permeabilization must be checked by addition of saponin or digitonin into the respirometer in state 3 with succinate/rotenone. 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 has to be at or better than 1 pmol $O_2 \cdot s^{-1} \cdot ml^{-1}$.
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 (Kuznetsov et al 2002), minimizing storage after permeabilization, and by application of preservation medium [MiPNet14.13] 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/inhibitor titration protcols (Gnaiger et al 2005; Kuznetsov et al 2004).

3 References

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Protocols

MiPNet08.12	NO effect on mitochondrial oxygen kinetics at low oygen.
MiPNet08.14	Citrates synthase – laboratory protocol.
MiPNet11.04	MitoPathways to Complex I.
MiPNet11.09	MitoPathways to Complex II.
MiPNet12.12	MitoPathways to Complexes I+II.
MiPNet12.13	MitoPathways Compilation: Additive effect at the Q-junction.
MiPNet12.15	Mitopathways: Respiratory states and coupling control ratios.
MiPNet14.13	Mitochondrial respiration medium – MiR06
MiPNet14.14	Preparation of permeabilized muscle fibres