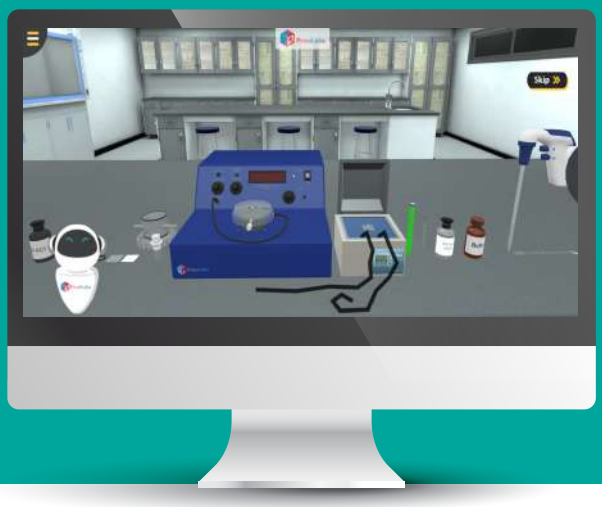
Biology | Biochemistry - Bioenergetics

Polarographic Oxygen Respirometry



General Aim

To study Electron Transport Chain (ETC) in yeast mitochondria using an oxygen electrode.

Method

Polarographic oxygen respirometry.

Learning Objectives (ILOs)

- Set up a polarographic oxygen respirometry.
- Run a simple biological specimen's response to both the inhibition and the uncoupling of the electron transport chain.
- Collect and analyze the raw data.
- Plot the data and draw informed conclusions.

Theoretical Background/Context

- Electron transport chain (ETC) is the process by which eukaryotic cells can produce ATP. ETC constitutes five complexes located at the inner membrane of the mitochondria. ATP production from ADP phosphorylation by ATP synthase (complex V) is the final step in ETC.
- ETC basically involves the transfer of high energy electrons from reducing equivalents (NADH,H+, FADH2) through respiratory carriers called complexes. Eventually electrons are reduced to molecular oxygen. Subsequent addition of protons (H+ ions) – also coming from NADH,H+ and FADH2 - to the molecular oxygen results in the formation of molecular water as a by-product.
- During the transfer of electrons, the Q-cycle ensures protons from inside the mitochondrial matrix are transferred to the intermembrane space of the mitochondria. This creates a low pH proton gradient. Therefore, protons seek discharge back to the mitochondrial matrix through Complex V (ATP synthase). Protons passage through Complex V activates the ATP synthase subunit which produces ATP. ETC is feedback inhibited by increased ATP.
- Reducing equivalents originally result from another cycle, also located in the mitochondria, called the TriCarboxylic Acid cycle (TCA). TCA can be considered the final common path for the metabolism of Carbohydrates, Lipids and Proteins inside the body. So, this is how your body can produce energy from food.
- Uncouplers of the ETC allow protons in the intermitochondrial space to pass back into the mitochondrial matrix but through channels other than the ATP synthase. This results in the dissociation of oxidation from phosphorylation (ATP production). Thus energy is liberated in the form of heat instead. The ETC in this case loses self control and oxygen consumption is dramatically increased. This is a hallmark of pathological and toxicological mitochondrial diseases, ranging from rare mitochondriopathies to cardiovascular and neurological diseases.
- Inhibitors of ETC can act on the ETC complexes stopping them, for example, Oligomycin can inhibit complex V.
- Azide is an oxygen absorbing substance so it brings OCR to 0%.

Principle of Work

- In this simulation, users will learn how to use an oxygen sensor electrode while measuring Oxygen Consumption Rate (OCR) of yeast mitochondria. Basal OCR is to be measured; moreover, various substances that can modulate the OCR will be sequentially added to the experimental setup in order to measure their effect on the OCR.

The Clarke oxygen electrode

The oxygen electrode measures changes in the concentration of dissolved oxygen. If a suitable potential difference (voltage) is applied between a platinum cathode (-ve) and a Ag/AgCl anode (+ve), then oxygen can diffuse through a thin (0.0005mm) PTFE membrane and is reduced (gain of electrons) at the platinum cathode:

O2 + 2e- + 2H+ → H202
H202 + + 2e- + 2H+ → 2H2O
Ag then reacts at the (+ve) anode:
4Ag + 4Cl- → 4AgCl + 4e-

Thus there is a flow of four electrons per molecule of oxygen reduced. This electrical current is directly proportional to the concentration of oxygen in the solution.

The anode and cathode are situated below the base in a thermostatically controlled environment provided by the water jacket. A rotating magnetic stirrer bar ensures oxygen is evenly distributed in the buffer during consumption. After pre-warming the buffer to 25˚C (as oxygen saturation varies with temperature/pressure), the solution becomes saturated with atmospheric oxygen. The chamber is then sealed to prevent the ingress of further oxygen during the experimental run and the oxygen HIGH value can be recorded. Addition of the biological sample commences the reaction and analysis is recorded and plotted.