

7.343. The Radical Consequence of Respiration: Reactive Oxygen Species (ROS) in Aging and Disease

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Class Scope

The goal of this course is to give you an overview of the complex biological functions of oxygen radicals with emphasis on how they affect the rate of aging and the onset/progression of a number of serious diseases.

By the end of this course, you should have a clear idea of the current thinking regarding ROS in normal and diseased states, and the pros and cons of the experimental approaches utilized to study their effects.

Class Specifics and Requirements

- This class is graded pass/fail.
- A background in basic chemistry (redox, organic), basic molecular biology and biochemistry concepts/techniques will be helpful to you.
- **Attendance and active participation are crucial!**
- Reading assignments: Two papers from primary literature/week (these are listed in the syllabus and will also be posted on the website).
- To facilitate discussion, please email me two questions based on your reading by 8pm of the night before class.
- There will be two short writing assignments and one related presentation required.

Assignment Details

Assignment#1

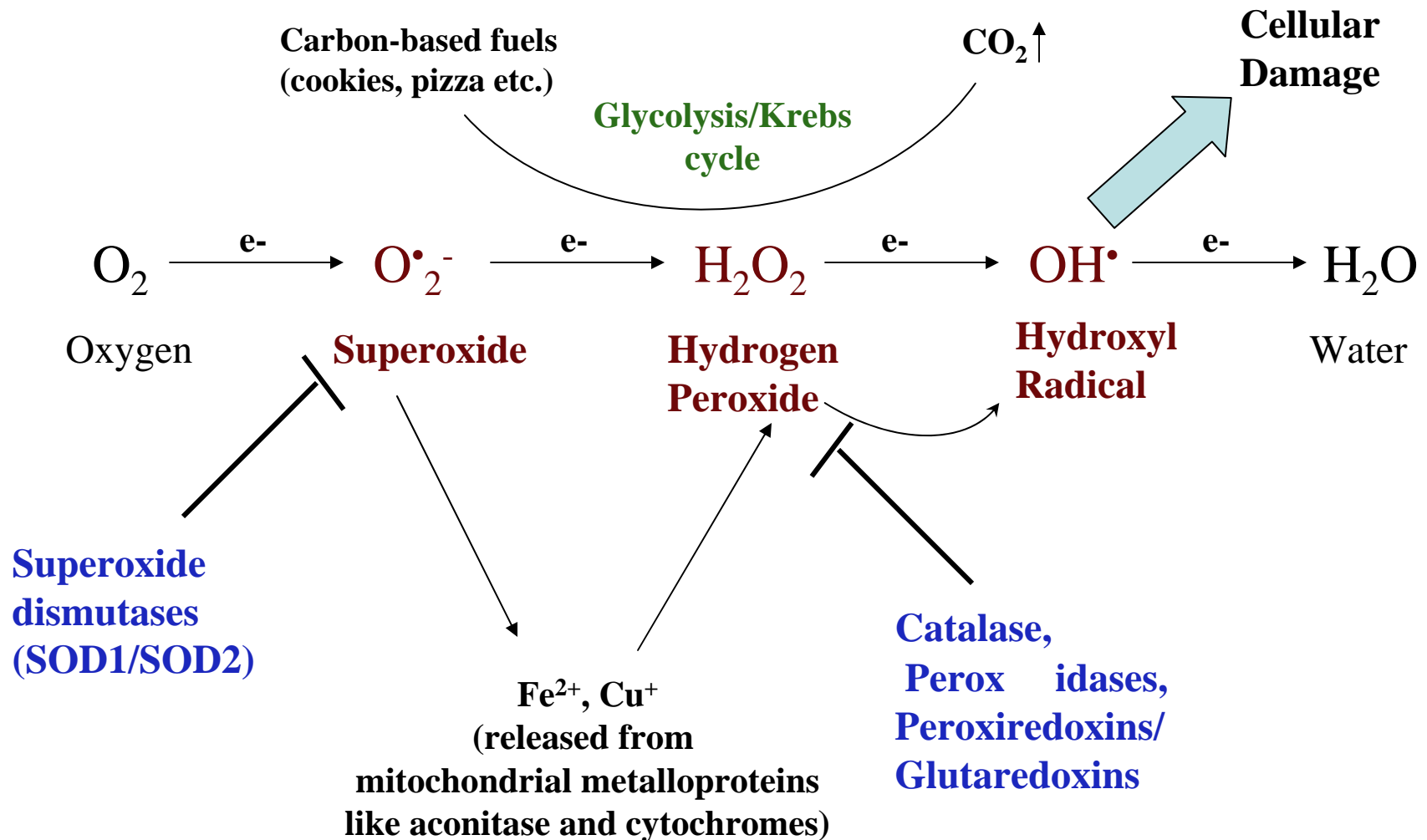
You will be given the text of a paper with its title and abstract omitted. Your assignment will involve writing a title and a no-more-than 250 word abstract for the paper based on its results and conclusions

Assignment#2

You will be given a selection of topics - each topic comprises a pair of papers which address a similar issue but come to different conclusions. You will need to write a 2-3 double-spaced paper and prepare a related 10-minute presentation describing additional experiments to reconcile the results or validate one paper over the other. The introductory paragraph of your paper will be due on Nov 15th.

What are **ROS** and where do they come from?

The Mitochondrial Respiratory Chain



Reactive Oxygen Species & Us

Mitogenic Signaling
(cell growth and
proliferation)

**Immune cell
activation**
(Pathogen destruction)

Oxygen sensing
(Activation of hypoxia-
inducible genes, metabolic
adjustment)

Cytokine signals
(Programmed cell death)

DNA Damage, Mutations
Genetic Instability
(Aging, Cancer, Stem cell loss)

Fat Oxidation or Lipid Peroxidation
(Damage to blood vessels,
nerve sheaths, heart tissue,
brain tissue)

Protein Carbonylation/Aggregation
(Loss of protective antioxidants,
amyloid formation)

Aberrant signaling through ROS
(Insulin sensitivity, tumorigenic
transformation)

Mitochondrial Efficiency: Coupling Oxidation and Phosphorylation

To generate ATP, energy is needed from the proton motive force (PMF). Therefore:

- The inner mitochondrial membrane must remain intact
- The concentrations of H^+ must be higher in the intermembrane space relative to the inner lumen.

Measurement of ROS levels in live cells

1. Electron paramagnetic resonance (EPR) imaging
2. Cell-permeant redox-sensitive dyes (fluorophores)
 - Dichlorofluorescein-diacetate (DCF-DA)
It is deacetylated by intracellular esterases, allowing the fluorescein moiety to be oxidized by ROS into the green fluorophore, dichlorofluorescein. It leaks from the cell, so not very stable.
 - Hydroethidine (measures superoxide)
It is dehydrogenated in the presence of superoxide to give ethidium bromide
 - Amplex Red (measures hydrogen peroxide)
In the presence of horseradish peroxidase, it reacts with 1:1 stoichiometry with H₂O₂ to form a red fluorescent compound called resorufin.

Next week's assignment

Chen Q, Vazquez EJ, Moghadda s S, Hoppel CL, Lesnefsky EJ. Production of reactive oxygen species by mitochondria: central role of complex III. J Biol Chem. 2003 Sep 19;278 (38):36027-31 .

Campian JL, Qian M, Gao X, Eaton JW. Oxygen tolerance and coupling of mitochondrial electron transport. J Biol Chem. 2004 Nov 5;279(45):46580-7