EXPERIMENT : 7

LIPID PEROXIDATION

Oxidative Stress and Free Radicals

The term “oxidative stress” began to be used frequently in the 1970s, but its conceptual origins can be traced back to the 1950s to researchers pondering the toxic effects of ionizing radiation, free radicals, and the similar toxic effects of molecular oxygen (Gerschman et al., 1954), and the potential contribution of such processes to the phenomenon of aging (Harman, 1956). The acceptance of free radical biology was remarkably slow, probably due to the largely theoretical and hypothetical nature of its beginnings, the evanescent nature of free radicals, and the lack of experimental tools to study them. The recognition in 1968 that biological systems could produce substantial quantities of the superoxide free radical, $O_{2}^{\bullet -}$, through normal metabolic pathways (McCord and Fridovich, 1968) and that enzymes, the superoxide dismutases (SOD), had evolved with the apparent sole purpose of protecting aerobic organisms from the presumed toxicity of this free radical (McCord et al., 1969 and McCord et al., 1971) spurred much interest. These enzymatic tools to both produce (via xanthine oxidase) and eliminate superoxide (via SOD) facilitated additional research in a number of areas of physiology and pathology.

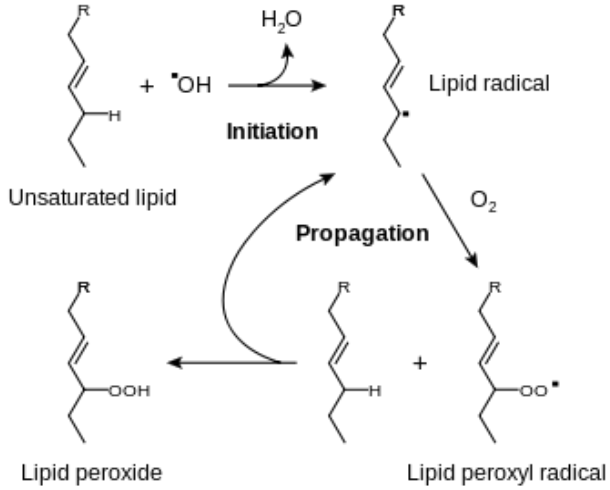
For several decades, free radical biology has been “superoxide-centric”, owing largely, perhaps, to the fact that superoxide is quantitatively the predominant free radical produced by biological systems. An example of a biologically-important free radical process that does not necessarily involve superoxide is lipid peroxidation, propagated by the characteristic “free radical chain reaction”. Oxidative stress, however, is a broader term than free radical biology, as few oxidants are actually free radicals. The superoxide radical, in fact, is a fairly good reducing agent in addition to being a mild oxidizing agent. In the dismutation reaction one superoxide radical acts as an oxidant, the other acts as a reductant. As the term “oxidative stress” came into broad usage in the 1970s it frequently described imbalances in redox couples such as reduced to oxidized glutathione (GSH/GSSG) or NADPH/$NADP^{+}$ ratios. Such metabolic disturbances need not involve the overproduction of reactive free radicals at all. Thus, the terms “oxidative stress” and “free radical damage” are not synonymous and may not always be interchangeable. Similarly, the terms “free radicals” and “reactive oxygen species” (ROS) are also not synonymous, as many reactive oxygen species (singlet oxygen, hydrogen peroxide, peroxynitrite) are not free radicals.

Table 1. Main oxygen reactive species and their performances.

Species	Chemical Structure	Description	Occurence	Action
Superoxide radical	$O_2^{\cdot}$	Most potent radical in the induction of cellular damage.	Almost all aerobic cells	Majority of reactions as a reducing agent
Hydroxyl radical	$OH^{\cdot}$	O_2 acid conjugate, highly reactive	Formed through water radiolysis	DNA, proteins, carbohydrates and lipids
Hydroperoxyl radical	$HO_2^{\cdot}$	Protonated from the O_2	From hydrogen peroxide	Biological membranes
Hydrogen peroxide	H_2O_2	It is not a free radical because didn’t submit electrons paired in the last layer	Reactions for the production of $OH^{\cdot}$	Proteins and lipids
Singlet oxygen	$^1O_2$	Excited form of molecular oxygen. It is not a free radical because did not submit electrons paired in the last layer	Generated by phagocytes, luminous induction and catalyzed by peroxidases	DNA changes

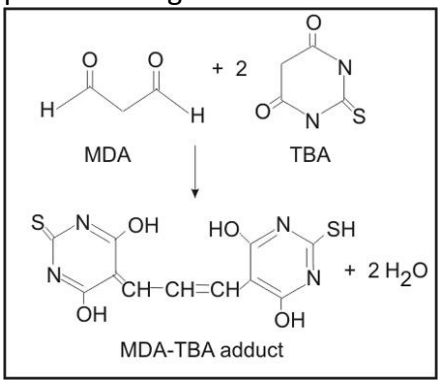
Lipid Peroxidation

Lipid peroxidation refers to the oxidative degradation of lipids. It is the process in which free radicals "steal" electrons from the lipids in cell membranes, resulting in cell damage. This process proceeds by a free radical chain reaction mechanism. It most often affects polyunsaturated fatty acids, because they contain multiple double bonds in between which lie methylene bridges ($-CH_2-$) that possess especially reactive hydrogens. As with any radical reaction, the reaction consists of three major steps: initiation, propagation, and termination.



Measurement of Malondialdehyde

Malondialdehyde is one of the most commonly reported biomarkers of lipid peroxidation in clinical studies. The reaction of thiobarbituric acid (TBA) with Malondialdehyde to yield a pink chromogen attributable to an MDA-TBA adduct is a common assay.



Experimental Design

Preparation of Solutions

<u>0.8% TBA:</u> Dissolve 0.8 g TBA in100 mL distilled water and *heat* until *dissolved*

<u>20% Acetic Acid:</u> Mix 20 mL acetic acid with 80 mL distilled water. Adjust pH to 3.5 by pipetting NaOH solution.

<u>8.1% Sodium Dodecyl Sulphate:</u> Add 0.81 g sodium dodecyl sulfate into 10 mL distilled water.

<u>Tissue Homogenization</u> (this procedure is given as an additional information)

To prepare 10% tissue sample homogenanate, 100 mg sample was measured and homogenized with 0.9 mL 1.15% KCl in the glass tube in the ice bucket. After 10 minutes centrifuge in 1000g, the supernatant is collected.

Preparation of Study Sample

Add 0.1 mL sample or standard (0.1 mL %1.15 KCl in blank tube) into the tube which contains 0.75 mL %0.8 TBA, 0.75 mL 20% Acetic acid and 8.1% sodium dodecyl sulphate.

Place the tubes into 100° C water bath for 30 minutes. Read the absorbances at 532 nm using a spectrophotemeter.

Preparation of Standard Curve

Malondialdehyde is used as the standard. Dilute 1mM stock MDA solution and prepare 2.5,5,10 nmol standard MDA solutions.

Create a standard curve by plotting the absorbance for each standard concentration on the ordinate. To determine the sample values the slope of the curve is determined. Because of the samples have been diluted, the standard curve must be multiplied by the dilution factor.