Oxygen Toxicity

Oxygen Toxicity: Radicals, Damage & Defense

The Radical Nature of O_2

Superoxide Anion

Highly reactive free radical with limited lipid solubility; cannot diffuse far from formation site.

Hydrogen Peroxide

Weak oxidizing agent that generates hydroxyl radicals. Lipid soluble, diffuses through membranes to mitochondria.

Hydroxyl Radical

Most powerful ROS. Initiates chain reactions forming lipid peroxides and organic radicals.

Major Sources of ROS

01

Coenzyme Q

Electron transporter in mitochondria. Not protein-bound, diffuses through inner mitochondrial membrane. Major site for toxic oxygen free radical generation when accidentally transferring electrons to O_2.

02

Oxidases, Oxygenases & Peroxidases

Found in mitochondria, peroxisomes, lysosomes. Manage oxygen and ROS. May accidentally release free radical intermediates during single-electron transfers to O_2.

03

Ionizing Radiation

Alpha particles, beta particles, gamma rays, X-rays split water into hydrogen and hydroxyl radicals, causing skin damage, mutations, cancer, and cell death.

ROS Damage to Cellular Components

Membrane Attack

Hydroxyl radicals extract hydrogen from lipids, forming lipid peroxyl radicals and peroxides. Lipid degradation produces malondialdehyde (blood/urine indicator of free radical damage).

Proteins & Peptides

Radicals cause protein fragmentation and amino acid cross-linking. Attack on cysteine residues forms aggregates preventing degradation. Increases susceptibility to enzymatic digestion.

DNA

Hydroxyl radicals cause base alterations and strand breaks. ROS extract hydrogen from C-4 of deoxyribose sugar, leading to single strand breaks.

- **The Radical Nature of O_2**

  – Most of Radical are bounded

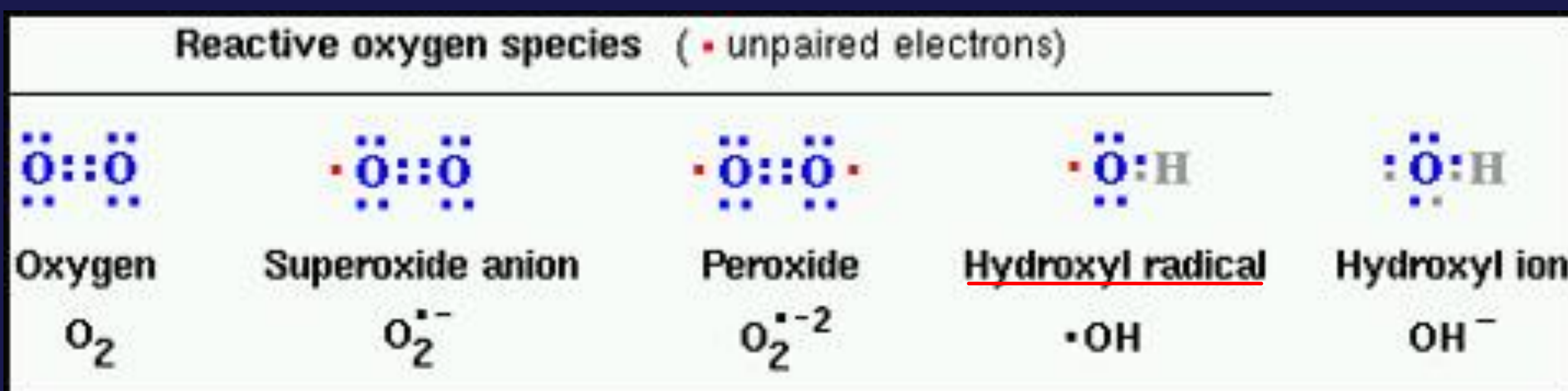
- A radical is a molecule that has a single unpaired electron in an orbit.
- A free radical is a radical capable of independent existence.

  → They are steal electron from Neghbouring molecule.

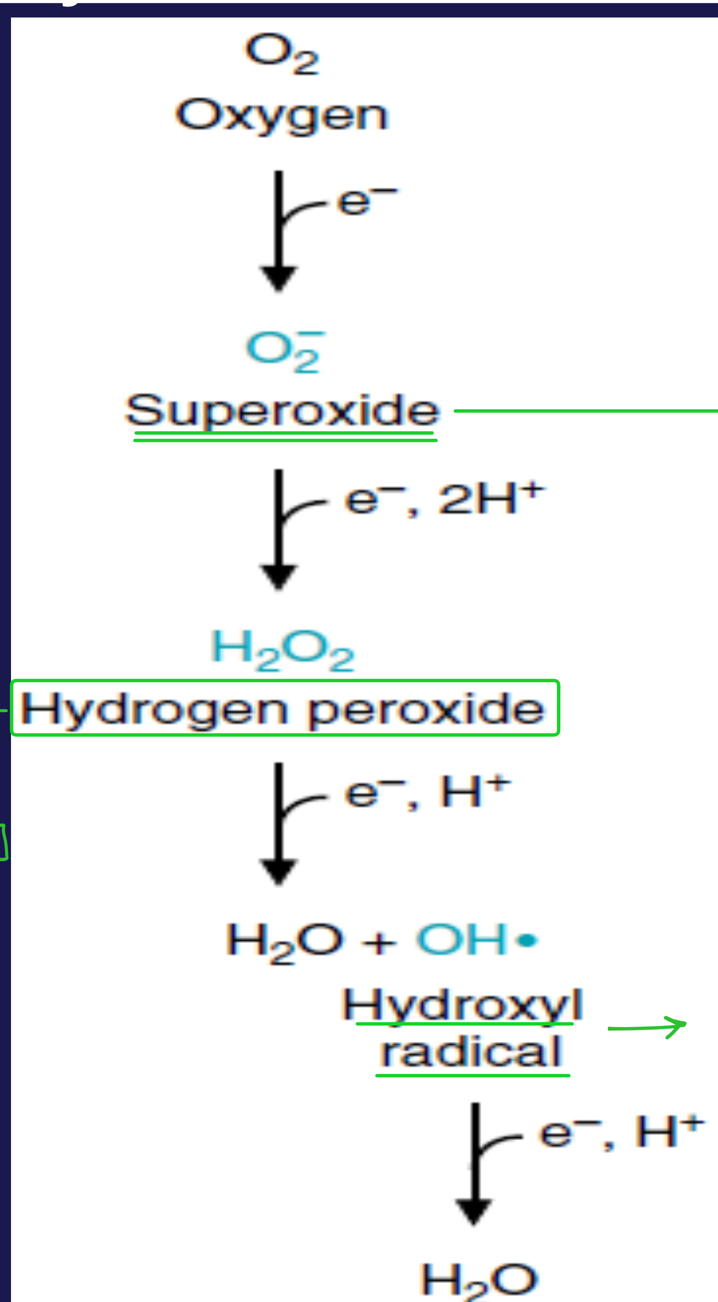
- Radicals are highly reactive and unstable that create a **chain reaction**
- The oxygen atom has two single electrons in different orbitals
- Oxygen single electrons cannot react rapidly with the paired electrons found in the covalent bonds of organic molecules. O_2 reacts slowly through the acceptance of single electrons in reactions that require a catalyst (such as a metal-containing enzyme).

Name: Oxygen
Symbol: O
Atomic Number: 8
Number of Protons/Electrons: 8
Number of Neutrons: 8
Oxygen: electron configuration $1s^2\ 2s^2\ 2p^4$

	Atomic number	1*s*	2*s*	$2p_x$	$2p_y$	$2p_z$	3*s*
Oxygen	8	↑↓	↑↓	↑↓	↑	↑	



Formation of ROS by one-electron reduction steps for O_2



Superoxide → Lipid insoluble (Water soluble)

Hydrogen peroxide:
- Not a Radical
- Lipid soluble
- Creat most dangerous oxy-Radical [OH]

Hydroxyl radical → Highly dangerous!

Characteristics of Reactive Oxygen Species

- Free radicals initiate chain reactions by extracting an electron from a neighbouring molecule to complete their own orbit.

- **The superoxide anion** a highly reactive free radical, but has limited lipid solubility and cannot diffuse far.
- **Hydrogen peroxide**, although not actually a radical, is a weak oxidizing agent that is classified as a ROS because it can generate the hydroxyl radical (OH•).
- Because hydrogen peroxide is lipid soluble, it can diffuse through membranes and generate OH• at localized Fe^{2+} or Cu^{+}-containing sites, such as the mitochondria.

- **The hydroxyl radical** is probably the most powerful of the ROS. It initiates chain reactions that form lipid peroxides and organic radicals.

- **Radicals are useful in:**
- Hydrogen peroxide is the precursor of hypochlorous acid (HOCl), a powerful oxidizing agent that is produced by white blood cells to destroy invading organisms.
- Nitric oxide is extremely important in relaxation and dilation of blood vessels.

Major Sources of ROS include

- **1. Coenzyme Q** **(Q refers to the quinone chemical group)**

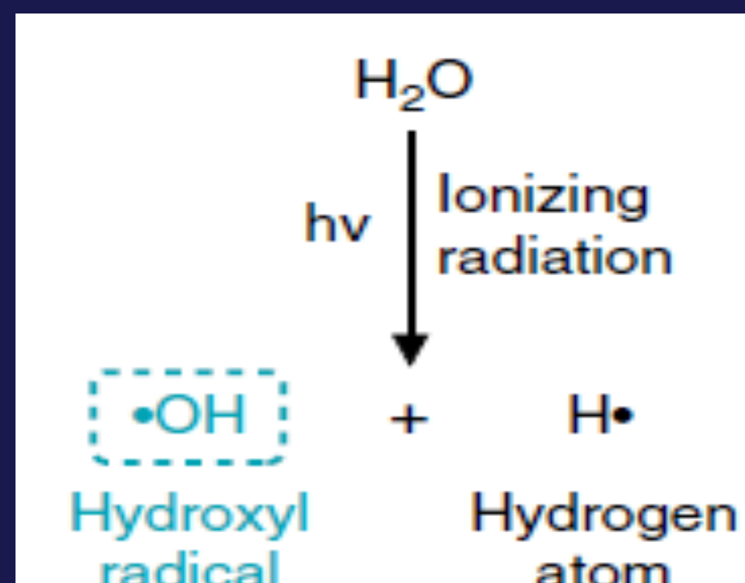
- CoQ is an electron transporter in mitochondria.
- CoQ the only component of the electron transport chain that is not protein bound.
- CoQ is hydrophobic thus it can diffuse through the lipids of the inner mitochondrial membrane.
- When CoQ accepts a single electron it may accidentally transfer an electron to dissolved O_2, thereby forming superoxide. → By mistake عن طريق الخطأ
- The transfer of single electrons makes it the major site for generation of toxic oxygen free radicals in the body.

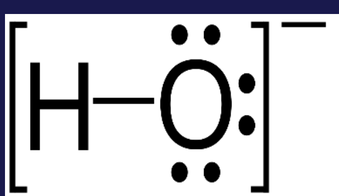
- **2. Oxidases, Oxygenases, and Peroxidases**
- Fond in mitochondria, peroxisomes and lysosomes.
- There function is to manage oxygen and ROS.
- Most of the oxidases (transfer electrons from the substrate to O_2), peroxidases, and oxygenases (incorporate one or both of the atoms of oxygen into the organic substrate) in the cell bind O_2 and transfer single electrons to it via a metal.
- Free radical intermediates of these reactions may be accidentally released before the reduction is complete.
- Because these enzymes catalyze reactions in which single electrons are transferred to O_2 and an organic substrate, the possibility of accidentally generating and releasing free radical intermediates is high.

- **3. Ionizing Radiation**

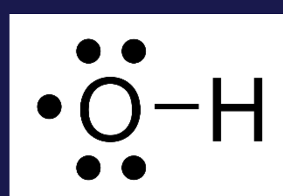
High energy → water → OH, H+

- Alpha particles, beta particles, gamma rays, and X rays are forms of ionizing radiation.
- Ionizing radiation has a high energy level that it can split water into hydrogen and hydroxyl radicals, thus leading to radicals formations and radiation damage to skin, mutations, cancer, and cell death.





Hydroxide ion



Hydroxyl radical

ROS Reactions that damages Cellular Components

A. Membrane Attack:

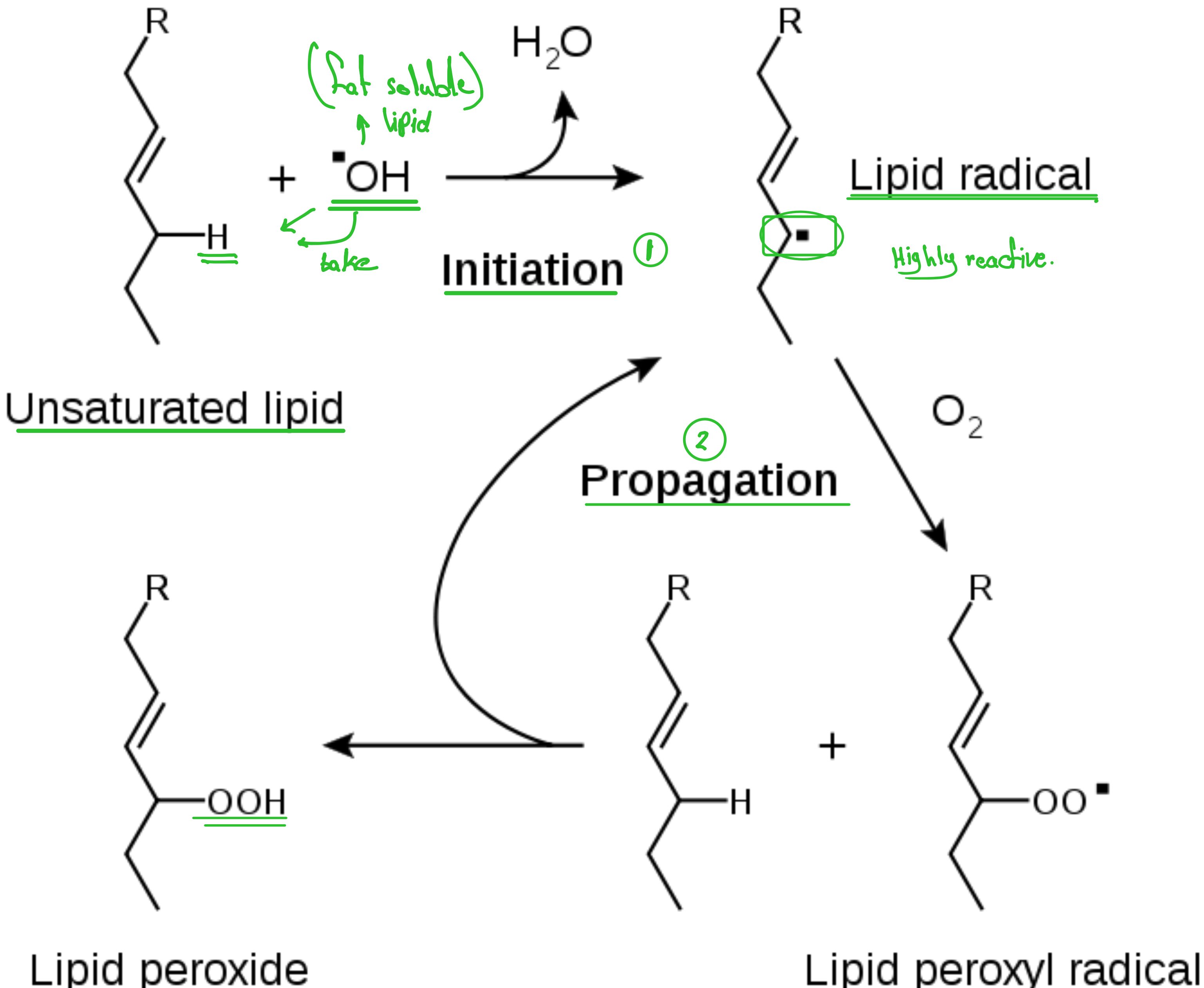
Chain reactions that form lipid free radicals and lipid peroxides in membranes make a major contribution to ROS-induced injury:

1- An initiator such as a hydroxyl radical begins the chain reaction by extracting a hydrogen atom from lipids.

2- The chain reaction is propagated when O_2 adds to form lipid peroxyl radicals (ROO·) and lipid peroxides (ROOH).

3- Eventually lipid degradation occurs, forming such products as malondialdehyde.

- Malondialdehyde appears in the blood and urine and is used as an indicator of free radical damage.



Forming at the end Malondialdehyde

B. Proteins and Peptides

- Radicals may cause protein fragmentation and amino acids cross-link [bond between A.A] with other amino acids. (sulfhydral)
- Free radical attack on protein cysteine residues can result in cross-linking and formation of aggregates that prevents their degradation. Change folding so will be No Active, No enzyme will degrade [most dangerous]
- Oxidative damage increases the susceptibility of other proteins to enzymatic digestion

C. DNA

- The hydroxyl radical can cause base alterations in the DNA it also can attack the deoxyribose backbone and cause strand breaks.
- The principle cause of single strand breaks is through ROS extract a hydrogen atom from C-4 of deoxyribose sugar leading to strand break.

- **Nitric Oxide and Reactive Nitrogen-Oxygen Species (RNOS)**

ممتاز ←No low ↓
No High ↑
bind O_2

- Nitric oxide (NO) is an oxygen-containing free radical which is both essential to life and toxic.
- NO is a gas that diffuses through the cytosol and lipid membranes and into cells.
- NO is produced by the lining of the blood vessels known as endothelium.
- NO has a single free electron.
- At low concentrations, it functions physiologically as a neurotransmitter that causes vasodilation.
- At high concentrations, it combines with O_2 or with superoxide to form additional reactive and toxic species containing both nitrogen and oxygen (RNOS).
- RNOS are involved in neurodegenerative diseases, such as Parkinson’s disease, and in chronic inflammatory diseases, such as rheumatoid arthritis.

- **Cellular Defences Against Oxygen Toxicity**
- Cells **protect** themselves against damage by ROS and other radicals through 1- Antioxidant defence enzymes, 2- Dietary antioxidants, 3- Cellular compartmentation, → every organell is a compartment
- 4- Metal sequestration , and 5- Repair of damaged cellular components.
- Defence through compartmentation refers to separation of species and sites involved in ROS generation from the rest of the cell. For example, many of the enzymes that produce hydrogen peroxide are found in peroxisomes with a high content of antioxidant enzymes.
- Metals are bound to a wide range of proteins within the blood and in cells, preventing their participation in free radical production. Iron, for example, is tightly bound to its storage protein, ferritin and cannot react with hydrogen peroxide.
- Repair mechanisms for DNA and other component are available to the cell. Oxidized amino acids on proteins are continuously repaired through protein degradation and resynthesis of new proteins.

Antioxidant Enzymes

Enzyme	Function	Location	Mechanism
Superoxide Dismutase (SOD)	Primary defense against oxidative stress	Nucleus, mitochondria, extracellular	Converts superoxide anion to H_2O_2 and O_2 using metal ions (Cu, Zn, Mn)
Catalase	Reduces H_2O_2 to water	Peroxisomes, cytosol (kidney, liver)	Prevents hydroxyl radical formation
Glutathione Peroxidase	Major H_2O_2 removal outside peroxisomes	Throughout cell	Reduces H_2O_2 to water, oxidizes glutathione to GSSG
Glutathione Reductase	Regenerates reduced glutathione	Throughout cell	Transfers electrons from NADPH to GSSG disulfide bond

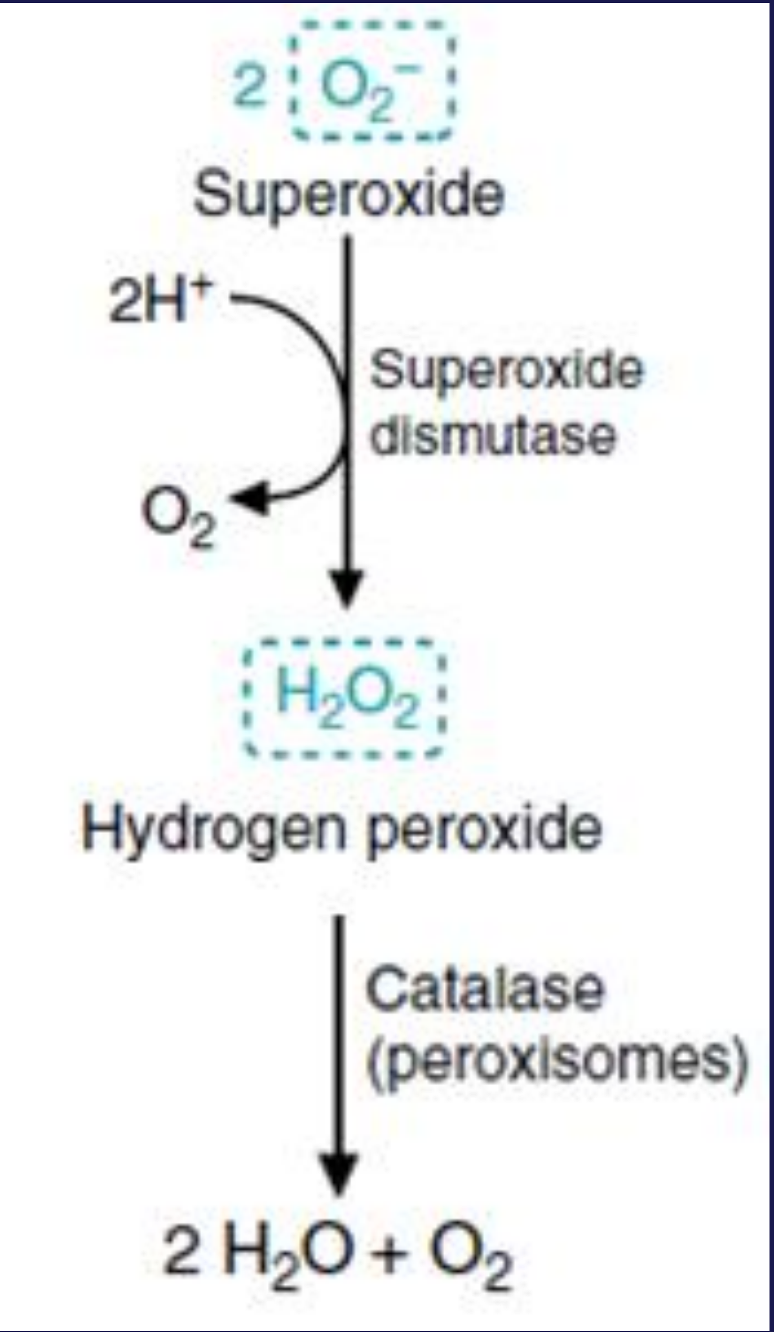
Dietary Antioxidants

Antioxidant	Function	Properties	Food Sources
Vitamin E (α-tocopherol)	Protects against lipid peroxidation in membranes; reacts with lipid radicals to stop chain reactions	Lipid-soluble, widely distributed in nature	Olive oil, margarine, almonds, peanuts, meats, dairy, leafy greens
Ascorbic Acid (Vitamin C)	Regenerates reduced vitamin E by donating electrons to oxidized vitamin E	Water-soluble, circulates unbound in blood and extracellular fluid	
Carotenoids (β-carotene)	Chain-breaking antioxidants; may slow cancer and degenerative disease progression	Vitamin A precursor	Fruits and vegetables

- **A. Antioxidant Enzymes**

Primary defence

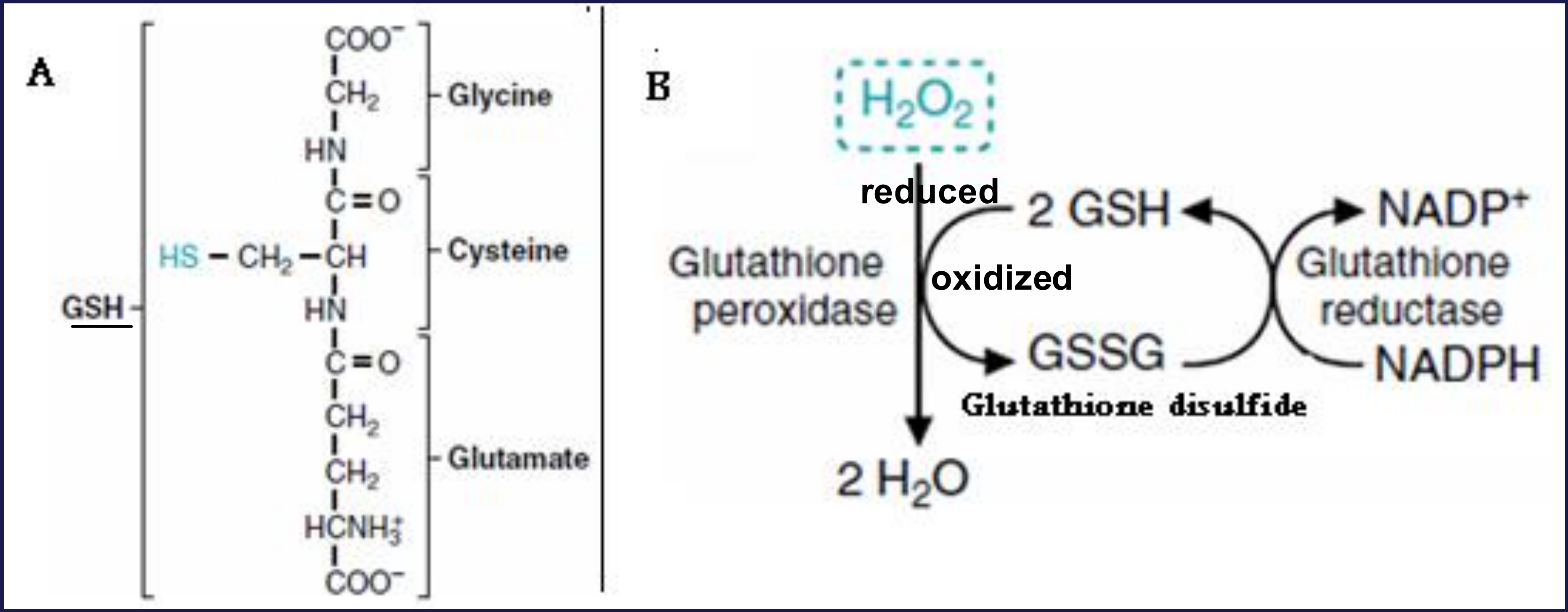
- **1. Superoxide Dismutase (SOD)**
- Found in different parts inside cells like nucleus and mitochondria and also found extracellularly.
- Function: conversion of superoxide anion to hydrogen peroxide and O_2 which is often called the primary defence against oxidative stress.
- Superoxide dismutase utilizes different positively charged metal ions (copper and zinc and manganese) to neutralize the negatively charged superoxide radical.
- (**Oxidative stress occurs when the rate of ROS and RNOS production overbalances the rate of their removal by cellular defence mechanisms)**
- **2. Catalase**
- Hydrogen peroxide, once formed, must be reduced to water to prevent it from forming the hydroxyl radical. One of the enzymes capable of reducing hydrogen peroxide is catalase. Catalase is found principally in peroxisomes, and to a lesser extent in the cytosol. The highest activities are found in tissues with a high peroxisomal content (kidney and liver).


2 O2−
Superoxide
2H+
Superoxide
dismutase
O2
H2O2
Hydrogen peroxide
Catalase
(peroxisomes)
2 H2O + O2

3. Glutathione Peroxidase and Glutathione Reductase

- Glutathione is a tripeptide composed of glutamate, cysteine, and glycine.
- The cysteine provides an exposed free sulphydryl group (SH) that is very reactive and provide electrons, providing an abundant target for radical attack.
- **Glutathione peroxidases** are the major means for removing H_2O_2 produced outside of peroxisomes.
- Glutathione peroxidases reduces hydrogen peroxide to water.
- In these reactions, two glutathione molecules are oxidized to form a single oxidized glutathione molecule the glutathione disulfide (GSSG).
- **Glutathione reductase** reduces GSSG to glutathione form
- Glutathione reductase catalyzes transfer of electrons from NADPH to the disulfide bond of GSSG.

Glutathione Peroxidase and Glutathione Reductase



The species that loses an electron is said to be oxidized
The species that gain electron is said to be reduced

- **B. Dietary antioxidants**
- Most Nonenzymatic antioxidants neutralize free radicals by donating a hydrogen atom (with its one electron) to the radical.
- Antioxidants, therefore, reduce free radicals and are themselves oxidized in the reaction.

1. VITAMIN E:

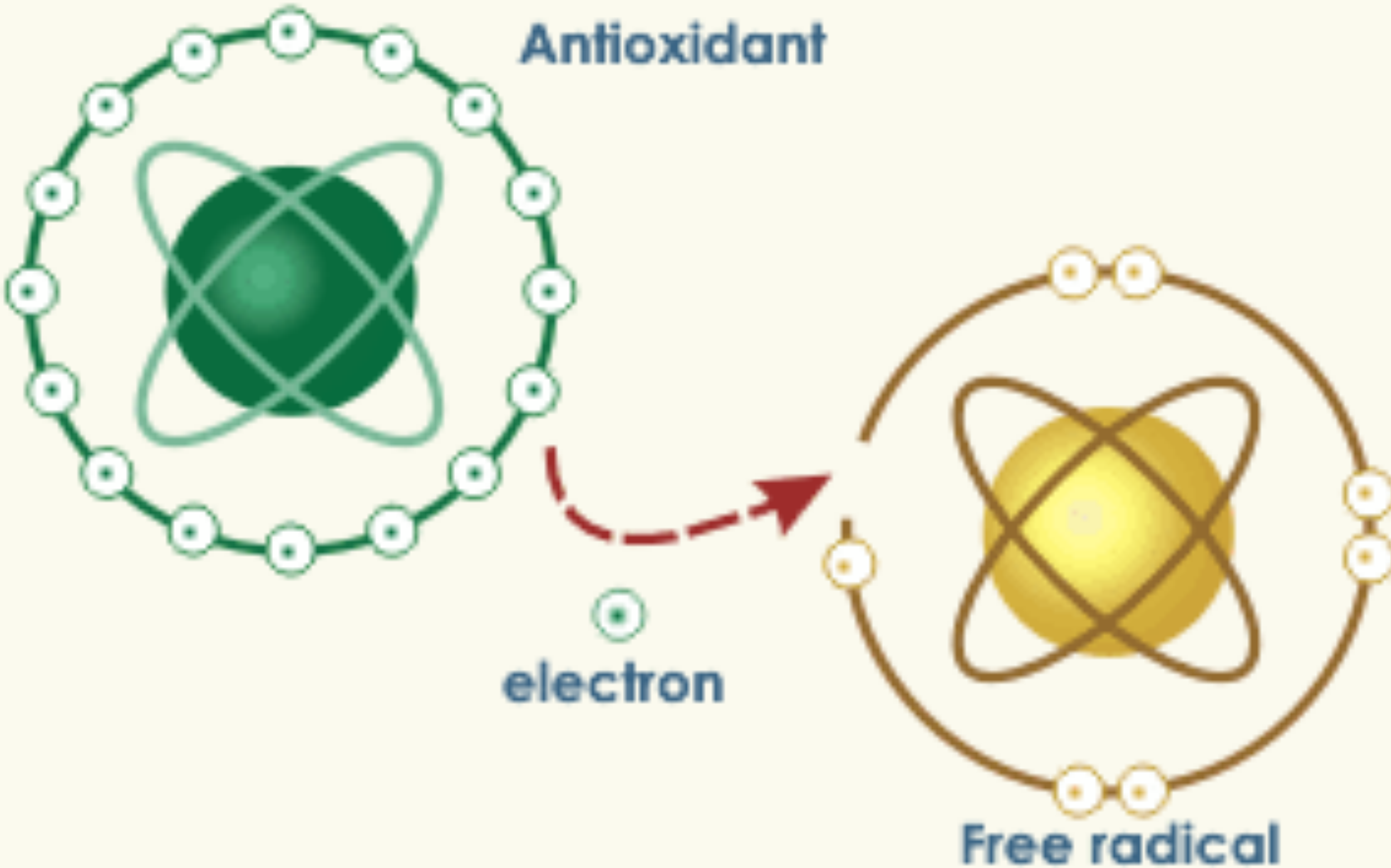
- Is also known as α-tocopherol.
- Is the most widely distributed antioxidant in nature.
- Is a lipid-soluble antioxidant that functions principally to protect against lipid peroxidation in membranes.
- Vitamin E reacts with lipid radicals produced in the lipid peroxidation chain reaction (peroxidation: oxidative degradation of lipids).
- This would remove the free radical intermediates and prevent the oxidation reaction from continuing and thus protects cell membranes from oxidation.
- Foods rich in vitamin E include olive oil, margarine, almonds, peanuts, meats, dairy, and leafy greens.

2. ASCORBIC ACID

- Ascorbate can regenerate the reduced form of vitamin E through donating electrons to the oxidised vitamin E.
- It is water-soluble and circulates unbound in blood and extracellular fluid, where it has access to the lipid-soluble vitamin E present in membranes and lipoprotein particles.

3. CAROTENOIDS

- Carotenoids is a term applied to β-carotene (the precursor of vitamin A) and similar compounds.
- Epidemiologic studies have shown a correlation between diets high in fruits and vegetables and health benefits, leading to the hypothesis that carotenoids might slow the progression of cancer and other degenerative diseases by acting as chain-breaking antioxidants.



Antioxidant neutralizing a free radical