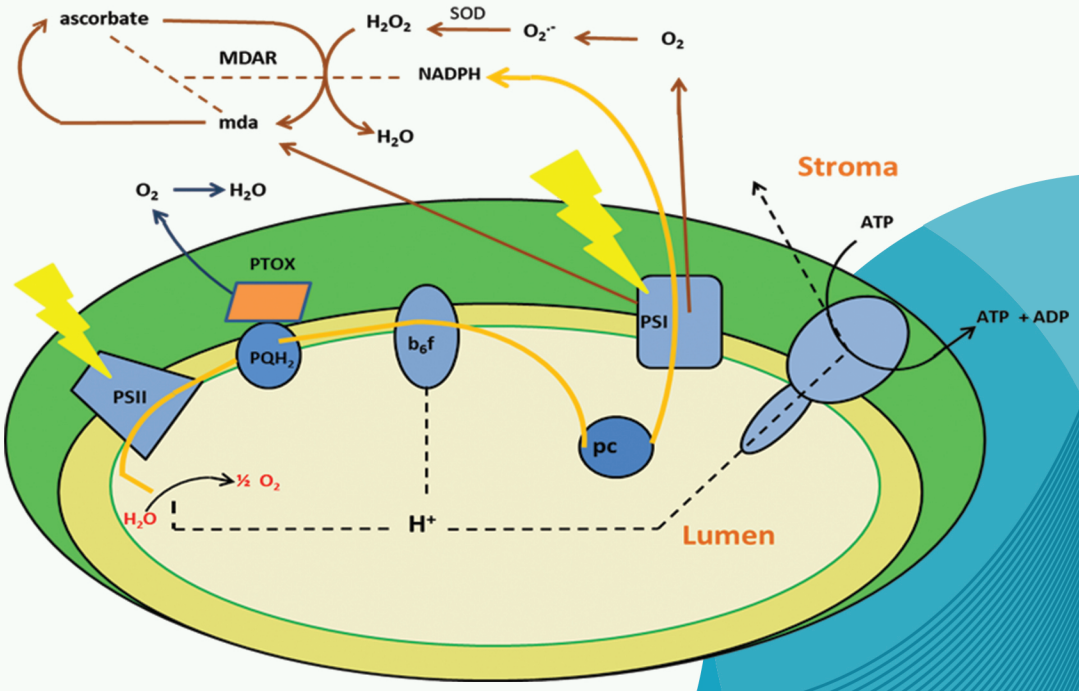
OXYGEN:

HIGH ENZYMATIC REACTIVITY OF REACTIVE OXYGEN SPECIES



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ISBN (Online): 978-981-5036-63-3

ISBN (Print): 978-981-5036-64-0

ISBN (Paperback): 978-981-5036-65-7

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PREFACE

Due to the importance of oxygen to conserve and maintain the life of organisms on earth, it is imperative to be conscious of the need for knowledge about this element, its physical, chemical and physicochemical properties, metabolism, and everything related to its behavior and its relationship with living organisms in different ecosystems and environments. Similarly, it is vital to know the causes and serious consequences caused by the incorrect management of natural resources on the levels and quality of this element in the biosphere.

This book presents and analyses evidence of the high enzymatic reactivity of reactive oxygen species, their production sources, chemical formation mechanisms, enzymatic oxidation, reaction centers, mechanisms involved in oxidation-reduction reactions, cell respiration chemistry, enzymatic kinetics, electron transport chain mitochondrial and chloroplast, oxidation-reduction potential, reaction constants, reaction velocity and reaction mechanisms involved, cellular cytotoxicity, antioxidant defense mechanisms in plants and animals, the response of plants to conditions of environmental stress, xenobiotics, heavy metals, paraquat, and the thermodynamics inherent to oxygen metabolism. Chapter 5 presents evidence and analyzes the action of flavonoids as promoters of reactive oxygen species. It is written as a paradoxical example of the high reactive affinity of reactive oxygen species for enzymes since during the whole metabolic process that presents flavonoids as trapping agents of reactive oxygen species or oxidants, in the end, and due to this high affinity and reaction rates, they become promoting agents of the same reactive oxygen speciesi-ROS.

Dioxide O_2 is not stored in the body. However ambient air (or water) if it is the immediate reservoir of dioxide. The ability to extract oxygen from the environment and carry it to each cell in complex multicellular organisms through just-in-time metabolism was one of the main developments of organisms during evolution. In human cells, there is an increase in reactive oxygen species under conditions of low levels of available oxygen-hypoxia.

The unfortunate experience in which we human beings currently live has alerted all of humanity to the need to take care of nature and the need to have an environment that is as unpolluted as possible since there is sufficient scientific evidence to show the decrease in oxygen levels in the terrestrial and aquatic environments and the devastating effects this has on the survival of organisms. Therefore, there is a need to form citizen conscience about the care of nature and the presence of this essential element for life on earth.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The author declares no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

Declared none.

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CHAPTER 1

Oxygen

Abstract: Earth's life depends mainly on the availability of oxygen in the terrestrial biosphere. Based on geochemical records of existing terrestrial oxides, oxygenic photosynthesis occurred in the cyanobacterial precursors approximately 2800 Ma ago. The oxygen level in the atmosphere is now 21%. The human cells use this oxygen to extract the necessary energy through mitochondrial respiration using the reactions of the redox system that involves the transfer of electrons, enzymatic agents, and reactive oxygen species, mainly superoxide radical ($O^{\cdot-}_2$), hydroxyl radical ($^{\cdot}OH$) and hydrogen peroxide (H_2O_2). The different reaction mechanisms from and to produce reactive oxygen species with their reaction constants in aquatic environments are presented here, as well as their production through the Fenton reaction. Oxidative stress is an imbalance between both normal oxygen-free radicals' production and the cell's ability to detoxify it.

Keywords: Enzymatic Reactivity, Great oxidation event-GOE, Hydrogen peroxide (H_2O_2), Hydroxyl radical ($^{\cdot}OH$), Lipid peroxidation, Oxygenic photosynthesis, Reactive oxygen species in aquatic environments, (ROS), ROS cytotoxicity, Superoxide anion ($O^{\cdot-}_2$).

INTRODUCTION

According to the geochemical records of terrestrial oxides that exist, the accumulation of oxygen in the Earth's atmosphere was originated from evolutionary processes that took place in the precursors of cyanobacteria before about 2.8 billion years ago [1].

There is evidence of a permanent increase in O_2 concentrations in the atmosphere since 2400 and 2100 Ma. Evidence of the presence of oxygen in the atmosphere is the appearance of soils with an oxidized red color and the disappearance of the old stream beds of easily oxidizable minerals such as pyrite (FeS_2) [2]. Oxygen constitutes 21% of the current atmosphere.

In the search for oxygen levels in the atmosphere, Lyon *et al*., 2014, state that from the first photosynthetic production of oxygen and based on sulfur isotope records, after GOE, oxygen levels raised again and then decreased in the atmos-

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phere where they remained for more than 1000Ma with very low levels. This extended inactivity was possibly caused by biogeochemical feedbacks that generated a deep ocean without oxygen. This anoxygenic ocean, with large deposits of H_2S attracted concentrations of bio-essential elements that, together with the low availability of oxygen, unchained the evolutionary events that gave rise to eukaryotic organisms and animals diversity until the final oxygenation and life expansion.

The differentiation of biotic or abiotic oxidation pathways, which can occur with or without oxygen, is the main difficulty in reaching a consensus on the appearance of atmospheric oxygen, despite intensive research in recent times.

Biomarkers are fossil molecules derived from organic compounds that bind to specific biological products present at the moment in which the sediments were deposited. The presence of cyanobacteria and eukaryotes in rocks from 2700 Ma ago was recorded with a biomarker [4].

The oldest producers of O_2 through photosynthesis, which are still found today, are cyanobacteria. Oxygen identification can also be performed by the recognition of sterane biomarkers in Eukaryotes since oxygen is required for the biological synthesis of sterols. This implies that the production and accumulation of oxygen occurred approximately 300Ma before the Great Oxidation Event - GOE, which occurred approximately 3800 to 2350 Ma ago.

The GEO is a time interval in which the differences in oxygen concentrations in the biosphere would represent a balance between early oxygen production and carbon deposits.

The available evidence suggests that oxygenic photosynthesis is much older than 2500Ma and that the production of oxygen through photosynthesis did not accumulate permanently in the atmosphere, due to the balance between carbon deposition and compensatory buffering [5].

There is evidence that, under conditions such as low SO_4 sulfate content in the archaic ocean and low O_2 levels in both the ocean and the atmosphere, high levels of methane (CH_4) and hydrocarbons from its photochemistry, such as ethane (C_2H_6), were produced.

O_2 levels in the earth are mainly due to photosynthesis. In the ocean, most of this oxygen is consumed through aerobic microbial respiration. In nature, the most complex metabolic process is oxygenic photosynthesis. It consists of two reaction centers in which electrons produced in the first reaction center (PSII) are then

transferred to a second reducing center (PSI), through a cytochrome complex (Chapter 4).

A source of both light and electron reducing power is required for photosynthetic life. Considering that the electron donor for oxygenic photosynthesis is the surrounding water, it is possible that carbon fluxes through the biosphere were overloaded by oxygenic photosynthesis. This is supported because without an external source of carbon, H_2S-based photosynthesis is difficult to maintain, and organic matter deposits in archean reservoirs came from waters with Fe^{2+} levels.

It is unlikely that Fe^{2+} based photosynthesis would have occurred since this metabolism produces organic carbon particles and iron oxide minerals, which would disappear by microbial iron reduction. Likewise, H_2-based photosynthesis would also be unlikely to occur. Therefore, the most accurate explanation is that oxygenic photosynthesis was the origin of organic ponds in the pre-GOE ocean.

Oxygen present in nature has been originated by the fusion of ^4He atoms that occurs at high temperatures in stars, and the concentration of oxygen is approximately or higher than the concentration of carbon in the solar system. The electronic configuration of oxygen favors fast reactions with atoms and molecules to form radicals.

When oxygen reacts with a metal of groups I, II, III, IV, V, VI, corresponding oxides, such as H_2O, MgO, CaO, AlO, CO_2, SiO_2, NOx, PO_4, SOx are formed, and when it reacts with transition metals, such as Mn and Fe, it forms insoluble oxyhydroxides.

Redox reactions lead to oxygen reactivity and produce stable compounds such as H_2O, CO_2, HNO_3, H_2SO_4 and H_3PO_4 and intermediate unstable compounds, such as H_2O_2, NO, NO_2, CO, SO_2 are produced by abiotic oxygen reactions. Most oxygen reactions are exergonic.

Oxygen production from water oxidation is the most important reaction of oxygenic photosynthesis, in which Mn and Ca atoms are involved. By sequential electron transfer driven by a single photon, Mn atoms remove electrons releasing O_2 from the water molecule. Calcium stabilizes the intermediate oxygen until a second atom is released [6].

A complex five-step mechanism to remove four electrons and four protons (transition state S) is required to produce oxygen by water oxidation and is the most energy-demanding biological redox reaction [7].

CHAPTER 2

Biological Oxidation

Abstract: Biological oxidation is the mechanism by which oxygen is supplied to living organisms and energy is produced. These processes involve the participation of oxygen incorporated into the substrates by oxidoreductase enzymes. The mechanisms of reaction of enzymes involved in both oxidation-reduction processes and electron transport chain mitochondrial-ETC, and their relationship with reactive oxygen species (ROS) production, are presented here.

Keywords: Dehydrogenases, Hydroperoxidases, Mitochondrial Electron Transport Chain-ETC, Oxidases, Oxidoreductases, Oxygenases, Redox potential, ROS.

INTRODUCTION

The **oxidation** is a chemical process in which electron elimination occurs and **reduction** is the process by which electron gain occurs, so that oxidation is accompanied by the reduction of an electron. This oxidation-reduction principle allows us to understand the complexity of the biochemical nature of the metabolism of living organisms. However, some biological oxidations occur without the participation of molecular oxygen, such as hydrogenations. Earth life depends mainly on the supply of oxygen to carry out respiration processes, especially in higher organisms where cells produce energy in the form of ATP with water formation from oxygen-hydrogen reactions. Oxygen is incorporated into substrates by means of enzymes called *oxygenases*.

1. REDOX POTENTIAL

In oxido-reduction reactions, the free energy changes are related to the ability to donate or accept electrons by the reactants. The free energy change is expressed as $\Delta G^{0\prime}$ but can also be expressed as oxide-reduction potential or redox potential (E'_0). For biochemical systems, the redox potential (E'_0) is expressed at pH 7.0 where the hydrogen electrode potential is -0.42 volts, considering an E_0 systems redox potential referred to the hydrogen electrode potential (0.0 volts at pH 0.0).

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Biological oxidation-reduction processes involve enzymes called **oxido reductases** and are mainly classified into four groups: **oxidases**, **dehydrogenases**, **hydroperoxidases** and **oxygenases** [1].

1.1. Oxidases

Utilizing oxygen as a hydrogen acceptor, oxidases catalyze the removal of hydrogen from a substrate. Water or hydrogen peroxide is formed as a product of the reaction (Fig. **1**).

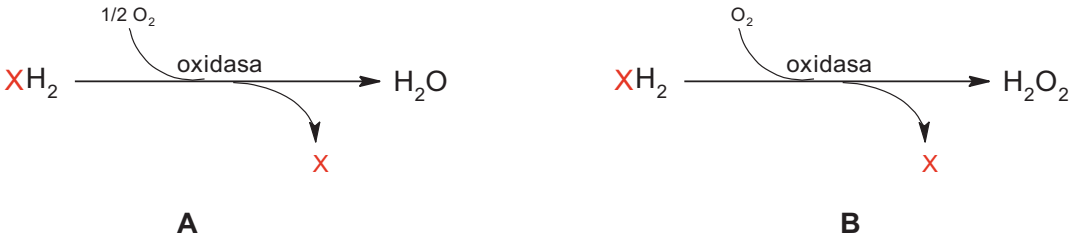

Fig. (1). Oxidase activity in metabolite oxidation **A.** Forming H_2O. **B.** Forming H_2O_2.

Cytochrome oxidase is found in hemoglobin, myoglobin, and other cytochromes. It is an oxidase containing copper and the typical *heme* prosthetic group. It is widely distributed in tissues and is the terminal component in the electron transport chain of mitochondria. The substrate is oxidized by hydrogenases and the electrons produced are transported by cytochrome oxidase to oxygen, which is the final electron acceptor. Carbon monoxide, hydrogen sulfide and cyanide inhibit the enzyme. This enzyme is also known as *cytochrome* a_3.

The following reaction is catalyzed by cytochrome *c* oxidase in solution:

$$4\text{ferrocytochrome } c + 4H^+ + O_2 \rightarrow 2H_2O + 4\text{ferrycytochrome } c \qquad \textbf{(1)}$$

For reaction 1, the average force is about 800-250 = 550 meV or ~12.7 kcal/mol per electron, *i.e.,* ~51 kcal/mol for the complete four-electron reaction [2]. The free energy exchange per electron for this reaction is 565 meV (~13 kcal/mol) and involves the release of 1 oxygen atom, which at 25°C corresponds to 1.2 mM O_2. Being a higher concentration than the O_2 concentration in mammalian tissues (0.005 - 0.025mM) and of the water-saturated atmosphere (0.258 mM at 25°C).

For the electron acceptor pair O_2/H_2O, a potential of 815 mV at pH = 7 ($E_{m.7}$) was determined. Cytochrome *c* oxidase is the 4-electron donor and the *c*-type cytochrome of some bacteria and mammalian cytochrome *c* has a potential of 250 mV at $E_{m.7}$.

Flavoproteins are oxidases that contain as their prosthetic group flavin mononucleotide (FMN) or flavin adenine dinucleotide (FAD). FMN and FAD, which are non-covalently bound to their respective apoenzymes, are formed in the organism from the vitamin riboflavin. Many riboflavin dehydrogenases are associated with electron transport or the respiratory chain. The metaloflavoproteins contain as cofactors one or more metals.

Flavoproteins include **xanthine oxidase** is important for the conversion of purine bases into uric acid, its structure contains molybdenum; an NMF-enzyme that has specificity for the oxidative deamination of L-amino acids is **L-amino acid oxidase** and is found in the kidneys; **aldehyde dehydrogenase** is a FAD enzyme that acts on aldehyde and *N*-heterocyclic sulfates, contains non-*heme* iron, molybdenum and is found in mammalian liver. By means of a two-step reaction, the oxidation and reduction processes of these enzymes are carried out. This mechanism is also present in ubiquinone, an electron transporting coenzyme (Fig. **2**).

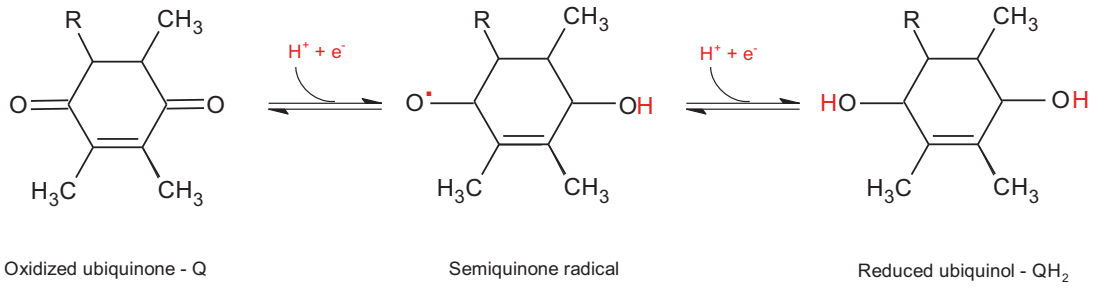

Fig. (2). Mechanism of oxidoreduction of ubiquinone *via* semiquinone-free radical.

Dehydrogenase enzymes participate in hydrogen transfer in coupled oxidoreduction reactions, where hydrogenases utilize coenzymes or hydrogen transporters such as NAD^+, despite having substrate specificity. Considering that the reactions are reversible, then in the cell the equivalent reductants are freely transferred. Such reactions are useful for oxidative processes to occur in the absence of oxygen, as occurs in the glycolysis anaerobic phase where the substrate is oxidized at the expense of another.

Furthermore, hydrogenases are also components of the electron transport chain from substrate to oxygen (Fig. **3**).

CHAPTER 3

Reactive Oxygen Species Sources

Abstract: Reactive oxygen species -ROS are produced by the oxidation-reduction processes of some enzymes as a product of the energy metabolism of cells and organelles and by exogenous sources. The mechanisms of oxidation-reduction and the enzymatic kinetics of some ROS source enzymes are presented here, as well as the mechanisms related to ROS production in organelles and cells and exogenous sources such as Fe, Cd, Hg, Ni, Zn, among others and xenobiotics such as paraquat-PQ.

Keywords: *Cytochrome C*, Electron transfer, Enzymatic kinetics, Enzymatic oxidation, Galactose Oxidase, *Haber – Weiss* reactions, Heavy metal contamination, Microsomes, Paraquat, Reaction centre, Xanthine oxidase, Xenobiotics.

1. ENDOGENOUS SOURCES

Oxygen-free radicals can be produced in cells by various processes and reactions: Radiations on photosensitizers such as retinal, riboflavin, chlorophyll or bilirubin, redox reactions with transition metals or redox reactions catalyzed by enzymes.

1.1. Enzymes

Some enzymes generate oxygen free radicals during their catalytic cycle; therefore, regulating the activity of these enzymes can control the oxygen free radical concentration. Monoamine oxidase deaminates dopamine and forms H_2O_2 in neurons. Aldehyde oxidase oxidizes aldehydes in the liver and releases $O^{\cdot-}_2$. Cyclooxygenase and lipoxygenase are enzymes of the biosynthetic pathway of prostaglandins, thromboxanes and leukotrienes, also release oxygen-free radicals. These radicals can inactivate the enzymes that originate them and thus regulate the route in which they participate. On the other hand, it has been demonstrated that cyclooxygenase is also capable of metabolizing certain xenobiotics to more toxic species, which may react with molecular oxygen to give rise to new oxygen reactive species.

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In addition to the above, xanthine oxidase is an important source of reactive oxygen species -ROS. This enzyme, under normal conditions, presents dehydrogenase activity and oxidizes xanthine to uric acid utilizing NAD^+. When energy charge decreases for example, because of ischemia, the enzyme functions as oxidase, utilizes molecular oxygen to oxidize its substrate and produces superoxide radical and hydrogen peroxide. Calcium channel dysfunction during an ischemic period releases calcium ions from their reservoirs and these activate proteases catalyze the conversion of xanthine dehydrogenase to xanthine oxidase. Xanthine oxidase does not act as such until reoxygenation (Fig. **1**).

Another important source of oxygen free radical is NADPH oxidase. When foreign particles invade the body, the inflammatory response is triggered. During the process, macrophages, and neutrophils, activated by contact with the foreign substance, increase their O_2 consumption, which is transformed into $O^{\cdot-}_2$, which is then converted into hydroxyl radical and hydrogen peroxide.

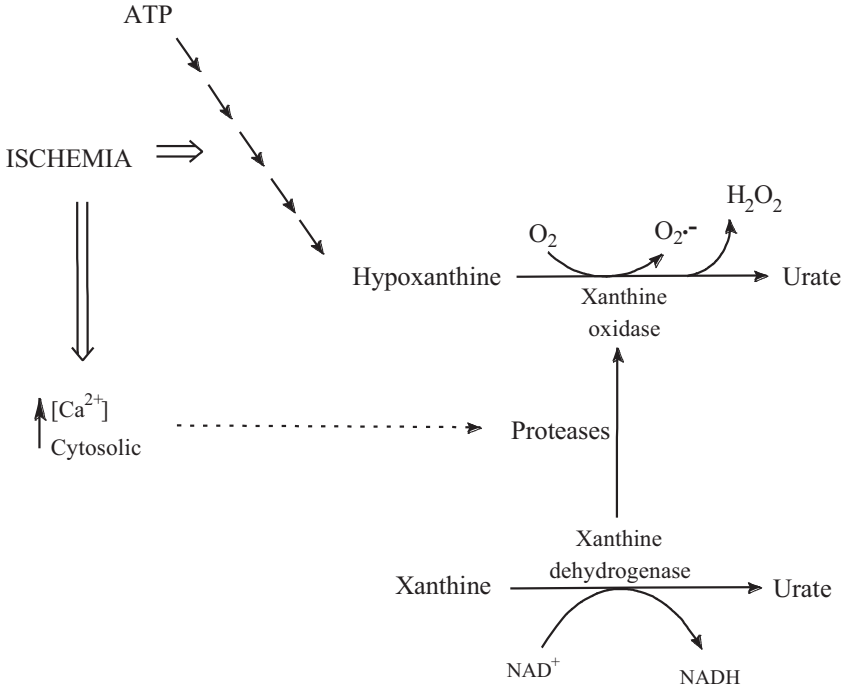

Fig. (1). Reactive oxygen species production during the ischemia process.

This respiratory flame is due to the enzyme complex located on the outer side of the plasma membrane and known as NADPH oxidase. NADPH oxidase contains adenindinucleotide flavine (FAD) and a cytochrome b_5 type with a sufficiently low redox potential to reduce O_2 to $O^{\cdot-}_2$, with NADPH electrons. In this way, foreign particles are exposed to oxygen free radical toxicity in the phagocytic

vacuole [1]. The lysosomal myeloperoxidase released in the vacuole forms hypochlorous acid in the presence of halides and H_2O_2 (Fig. **2**). This acid is very reactive and can oxidize different biological molecules; in also, it can react with $O^{\cdot}{}_2$ to produce $^{\cdot}OH$, or with H_2O_2 to produce singlet oxygen.

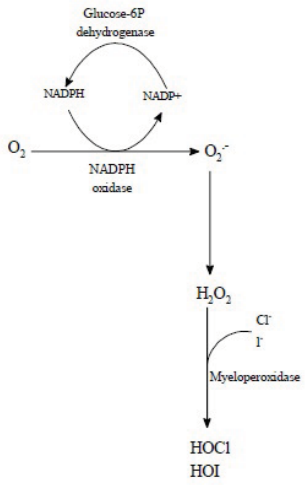

Fig. (2). Reactive oxygen species production during respiratory flame.

1.1.1. Cytochrome c – Enzymatic Oxidation

Cytochrome *c* (Cyt *c*) is found in the mitochondrial inner membrane, specifically on its outer surface. Cyt *c* is a 12kDa globular protein with a *heme* group, protoporphyrins IX and Fe^{3+}, covalently linked to two cysteine residues Cys-14 and Cys-17, coordinated to His-18 and Met-80. In mammals, there are 104 amino acid residues. Cyt *c* reacts with the hydroperoxides of *α*-linolic acid, leading to its decomposition by homolytic cleavage and producing singlet oxygen.

Cytochrome *c* and phospholipids are major targets of $^{\cdot}OH$, hydroxyl and $O^{\cdot}{}_2$ superoxide radicals during oxidative stress. Protoporphyrin IX and amino acid chain residues of Cyt *c* that are hydroxylated or carboxylated are oxidized during hydroxyl- and superoxide radical-mediated oxidation of Cyt *c*. Among these amino acid residues is Met-80, which is oxidized to methionine sulfide, becoming the main ROS target. The derangement from the coordinated Met-80/iron *haeminic* bond leads Cyt *c* from being an electron transporter to being a peroxidase.

The free radicals formed in enzymatic oxidation have high reactivity and act as reductants or oxidants. These reaction rates can be measured by electron spin resonance -ESR, a spectrophotometer and flow equipment. Thus, Ohnish *et al*.,

CHAPTER 4

Antioxidant Defense Systems

Abstract: The enzymatic and non-enzymatic reaction mechanisms of primary and secondary defense systems developed by cells to diminish the effects caused by overproduction of reactive oxygen species-ROS as a metabolic response to the damaging effects from endogenous and environmental factors are presented here. Enzymatic reaction mechanisms developed by plants as an antioxidant defense system are also presented.

Keywords: Antioxidants, Catalase, Coenzyme Q-CoQ, Cu-ZnSOD, Glutathione, HNE, Plant antioxidant defense, Superoxide dismutase-SOD, Trapping free radicals, UCPs, Vitamin C, Vitamin E.

INTRODUCTION

To counteract the effect of oxygen-free radicals, the biological systems contain molecules of both enzymatic and non-enzymatic nature that constitute the so-called *antioxidant defense system.* These antioxidant defense systems function very efficiently in a coordinated manner and is responsible for the maintenance of cellular homeostasis against oxidative stress generated from reactive oxygen species-ROS and other radicals originated during oxygen metabolism.

A distinction is made between primary or preventive antioxidant defense systems and secondary or chain-breaking antioxidant defense systems. The primary defense system directly reacts with the reactive oxygen species -ROS and thus reduce the initiation rate of free radical reactivations. The secondary defenses trap the propagating radicals, stopping their deleterious effect in the initial stages.

1. PRIMARY ANTIOXIDANT DEFENSE SYSTEMS

1.1. Enzymes

There are several enzymes whose primary function is to decrease intracellular and intercellular concentrations of reactive oxygen species. These include superoxide dismutases, catalase, glutathione peroxidase, glutathione reductase, glucose

-6-phosphate dehydrogenase, among others. Fig. (**1**) summarizes the concerted action of some of these intracellular enzymes.

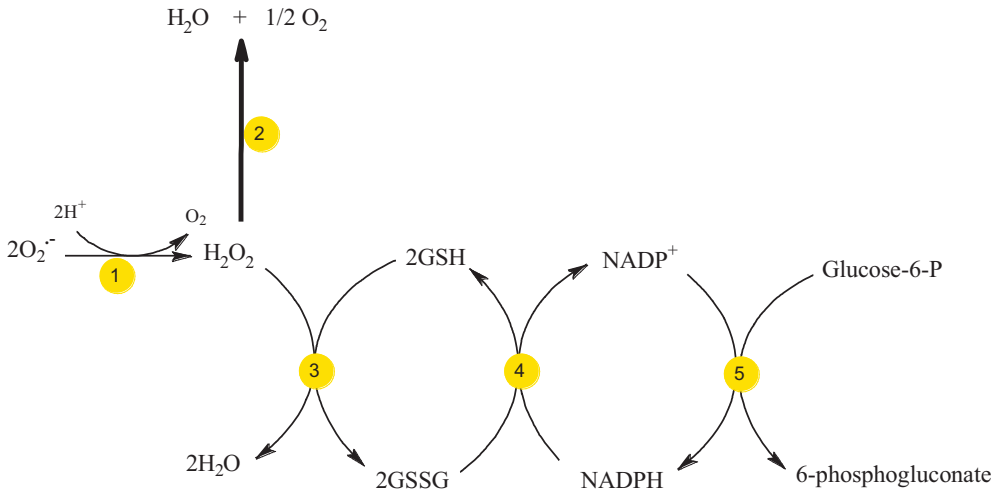

Fig. (1). Antioxidant Defense Enzyme Systems. **1.** SOD Superoxide dismutase, **2.** Catalase, **3.** GSH peroxidase, **4.** GSH reductase, **5.** Glucose 6-P-dehydrogenase.

1.1.1. Superoxide Dismutase-SOD

Superoxide dismutase (SOD) is a metalloenzymes family, which are found in almost all oxygen-exposed organisms and catalyzes the $O^{\cdot-}_2$ dismutation reaction to produce H_2O_2 and O_2 (reaction 1). The spontaneous dismutation rate at physiological pH is much lower, about 10^4 times, than the dismutation rate of the reaction catalyzed by SOD. The SOD catalytic action was discovered by McCord and Fridovich. The classification of SOD depends on the great variety of prosthetic groups they possess.

$$O^{\cdot-}_2 + O^{\cdot-}_2 + 2H^+ \rightarrow H_2O_2 + O_2 \qquad \textbf{(1)}$$

Enzymes such as catalases and peroxidases transform H_2O_2 into a stable aqueous product.

The existence of SOD is common to all life forms. Their production and evolution as an antioxidant enzyme are linked to O_2 production by photosynthetic organisms approximately 2.000 Ma ago, through oxygen metabolism. In the SOD family, different metal centers have been detected, mainly Cu, Zn-, Fe-, Mn- and Ni-SODs. However, in prokaryotes two forms Cu,Zn SODs and Fe SODs/Mn SODs appear independently.

In chloroplasts, eukaryotes and bacteria mainly Cu,Zn SOD is found. Two different forms of Cu,ZnSOD such as cytosolic SOD1 and extracellular SOD/SOD3 are found in animals, where SOD1 differs from SOD3 in terms of amino acid composition and molecular weight. In turn, MnSOD is found in mitochondria of eukaryotes and prokaryotes. In addition to chloroplasts and mitochondria SOD accumulates in cytosol, extracellular matrix, glyoxysomes, peroxisomes and microsomes or any compartment where O_2 can be activated. Since $O^{\cdot-}_2$ cannot cross the membrane, it can be eliminated at the site where it is produced.

The following reaction sequence describes the SOD catalytic mechanism:

$$M^{3+} + O^{\cdot-}_2 + H^+ \rightarrow M^{2+}(H^+) + O_2 \quad \textbf{(2)}$$

$$M^{2+}(H^+) + H^+ + O^{\cdot-}_2 \rightarrow M^{3+} + H_2O_2 \quad \textbf{(3)}$$

Where M, represents a metallic cofactor.

The stepwise sequence in which the SOD reaction proceeds presents several thermodynamic advantages: 1) With only one molecule at a time, the reactant overcomes the repulsion of the electrostatic potential between the two $O^{\cdot-}_2$ anions. 2) The positively charged metals mediate specific binding with the negatively charged $O^{\cdot-}_2$ at the active site. 3) The capture of a proton in the second step, through the reduction of metal ions, preserves the electrostatic attraction of the active sites. Considering the neutrality of the disproportionation products and that by this mechanism they do not bind and 4) The energy released in the first (thermodynamically favorable) half-reaction is utilized for $O^{\cdot-}_2$ reduction of the second step [1].

SOD presents interesting properties such as: 1) The reaction rate ($M^{-1}s^{-1}$) of Mn-SOD is 6.8×10^8 and 6.6×10^8 for Fe-SOD in *Escherichia coli*. respectively and for erythrocyte Cu,ZnSOD is 6.4×10^9. The electron transfer between the substrate and the active site usually reaches the desired value and the catalytic rate reaches the diffusion limit. 2) SODs present high stability to factors such as free thawing, high temperatures, urea, and unfavorable pH.

Cu,Zn SODs are homodimeric, each monomer containing one atom of Cu and Zn and a molecular weight of 14-33 KDa. Their catalytic activity is resistant to cleavage by the enzyme proteinase K; to physical treatments such as heat, thawing and cold cycles; and to chemical treatments such as 4% SDS and 8M urea. They are inhibited by H_2O_2, amide, diethyldithiocarbamate and cyanide. Due to their homotetrameric nature, Cu,Zn SODs are different between humans and other mammals. Extracellular SODs have some differences from cytoplasmic SODs:

CHAPTER 5

Flavonoids as Reactive Oxygen Species Promotors

Abstract: The high enzymatic reactivity of reactive oxygen species can be seen in the metabolism of flavonoids, since throughout the metabolic process that presents this type of chemical compounds as trapping agents of reactive oxygen species or antioxidants, finally and due to the speed of reaction they themselves become promoting agents of the same reactive oxygen species. The flavonoids are organic molecules that, due to their chemical nature and their low redox potential ($0.23 < E7 < 0.75$ V), can easily react with oxygen-free radicals, inhibiting both the action of the radicals and the molecules producing them; through antioxidant action mechanisms; some of which are explained by physicochemical and molecular parameters such as heat formation (ΔH_f), Ionization potential and (IP Bond dissociation energy (BDE). However, after bioavailability and absorption, the flavonoids promote oxygen free radical's production acting as prooxidants mainly through hydrogen atom transfer HAT or simple electron transfer-SET mechanisms, properties and mechanisms of antioxidant and prooxidant reaction.

Keywords: Antioxidants, Bioavailability, Bond Dissociation Energy – BDE, Flavonoids, Formation heat of flavonoids radical -ΔH_f, Hydrogen atom transfer -HAT, Measurement -ROS, Prooxidant enzymes mechanism, Pro-oxidant mechanisms of flavonoids, Quantitative Structure-Activity Relationship-QSAR, Radical trapping, Redox chemistry, Single electron transfer -SET, Structure-Activity Relationship-SAR.

INTRODUCTION

Polyphenols are compounds produced by plants as a product of their secondary metabolism and represent the most abundant and widely distributed groups of substances in plants. Polyphenols are biosynthesized by two main pathways: the shikimic acid pathway and the malonic acid pathway [1]. They are involved in plant reproduction, growth, mechanical support, and resistance to pathogens and predators, harvest protection and seed storage, absorption of prejudicial ultraviolet radiation and allelopathic mechanisms [2], among others.

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1. FLAVONOID CHEMICAL STRUCTURE

Polyphenols can be classified into phenolic acids, flavonoids, stilbenes and lignans, considering the number of phenolic rings they contain, and the substituent groups present on these rings. Flavonoids are one of the main groups of phenolic compounds. The chemical nature of flavonoids depends on their structure, degree of hydroxylation, substitutions, conjugations, and degree of polymerization.

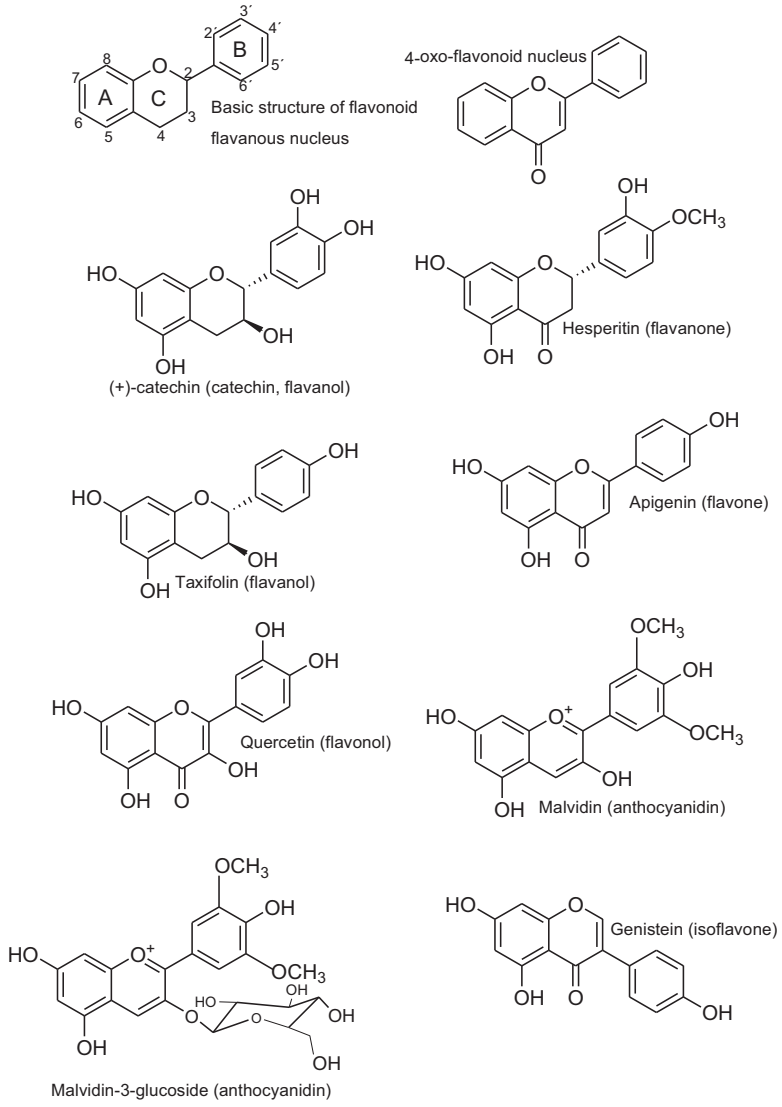

Fig. (1). Flavonoids. Basic structure and types.

They are constituted structurally by a basic skeleton of 15 carbons organized in two aromatic rings (A and B) connected by three carbon atoms that form an oxygenated heterocycle (C ring). The A ring, originated by condensation of three units of acetate pathway malonic acid and the B ring together with the three carbon atoms, constitute a unit of phenylpropanoid, biosynthesized by the pathway of shikimic acid.

Theoretically it is possible to obtain a very large number of different flavonoid structures if 10 carbons of the basic flavonoid backbone are substituted by different groups such as methyl, methoxyl, hydroxyl, isoprenyl and benzyl. Additionally, each hydroxyl group and some carbons can be substituted by one or more sugars and in turn each sugar can be alcyled by several phenolic or aliphatic acids. According to Williams & Grayer, 2004 over 9000 different flavonoids of plant origin have been reported [3], [4].

Generally, the classification of flavonoids is based on the degree of oxidation of the three-carbon bridge and on the presence and way the hydroxyl and methyl groups are attached to the basic molecule. Depending on their structural characteristics (Fig. **1**) flavonoids can be classified into:

1. Flavanols as catechin, with a group−OH in position 3 of ring C.
2. Flavonols, represented by quercetin possessing a carbonyl group in position 4 and a−OH group in position 3 of the C ring.
3. Anthocyanidins have the−OH group attached to the 3 position and in the C ring a double bond between carbons 3 and 4.
4. Flavones, such as diosmethine, have a carbonyl group at position 4 of the C-ring and at position C3 it lacks the hydroxyl group.
5. Flavanones, they lack the hydroxyl group in position C3.
6. Isoflavonoids vary from the previous ones since the B ring is attached to the C3 of the C ring instead of the C2 ring.

In plants flavonoids are present mainly as glycosides. Flavonols and flavones are bound to sugars, preferably to the C3 position and less frequently to the C7 of ring A, so that these compounds are generally found as *O*-glycosides, where the most frequent sugar residue is D-glucose. There are also sugar residues such as D-galactose, L-arabinose, L-rhamnose, D-xylose and D-glucuronic acid. The structure of the flavonoid molecule without sugar is called an aglycone. Glycosides are more soluble in water and less reactive in respect to free radicals than their aglycone or respective flavonoid [5].

CHAPTER 6

Oxygen Availability

Abstract: The ability to extract oxygen from the environment and deliver it to each cell of the multicellular organism through metabolism in time was the main development of organisms during evolution. The life of living organisms is absolutely dependent on oxygen supplementation for respiration, a process by which cells produce ATP to obtain energy energy from controlled reactions of hydrogen with oxygen to produce water. Depending on cell type, function and biological state, cells have a broad range in oxygen utilization. Here there is evidence on the considerable increase of reactive oxygen species-ROS caused by metabolic alterations, as response the low levels of oxygen available-hypoxia that occur in human cells.

Keywords: Glucose Regulating Proteins (GRPs), Heat Shock Proteins (HSPs), Hipoxic Conditions, Hypoxia-Associated Proteins (HAPS) and Oxygen Regulating Proteins (ORPs), Oxygen Consumption Rates (OCRs), Oxygen Levels in the Blood, Respiratory Insufficiency.

1. REACTIVE OXYGEN SPECIES-ROS IN HYPOXIA

The human body utilizes oxygen to extract approximately 2,550 calories from food to provide for daily energy needs. For example, 10.4 MJ are required for a 20-year-old person and 70 kg, this consumption requires approximately 22 moles of O_2 per day, or 2.5 x 10^{-4} mol s^{-1}. Thus, the oxygen consumption rate for the 70 kg subject is 3.6 x 10^{-9} mol s^{-1} g^{-1}. Furthermore, if this 70 kg human contains 1 x 10^{14} cells, the oxygen utilization rate per cell would be 2.5 x 10^{-18} mol $cell^{-1}s^{-1}$.

Oxygen utilization rate by cells depends on cell type, function, and biological state. Thus, the oxygen utilization rate by a red blood cell without mitochondria, which depends entirely on glycolysis to supply its energy needs instead of respiration, is very different from the oxygen utilization rate by a hepatocyte containing a number of mitochondria of the order 10^3.

The majority of the O_2 utilized in mitochondrial respiration presents a four-electron reduction to produce water (reaction 2). However, O^{-}_2 superoxide is also

formed by the one-electron reduction of a small fraction of this oxygen, corresponding to ≈ 1% or less of the Oxygen Consumption Rate-OCR. Therefore, in the electron transport of mitochondria *In vivo*, this O_2 reduction could be less than this.

$O^{\cdot-}_2$ superoxide and H_2O_2 hydrogen peroxide can initiate or contribute to the generation of different pathologies. However, they are species that contribute to the organism's redox biology by establishing a suitable reducing environment in cells and tissues. Oxygen consumption rate-OCR studies are indispensable for understanding the mechanisms by which reactive oxygen species affect redox biology of cells and tissues. For partially reduced species such as superoxide and hydrogen peroxide, this rate corresponds to the absolute upper limit of their potential flux.

Wagner *et al*., 2011, found that the oxygen consumption rate-OCR depends on the size of the cell and the number of proteins it contains. In addition, to maintain an adequate redox environment in normal and pathological conditions with different oxygen requirements, the cell develops different strategies. In addition, the surface area-volume relationship varies from cell to cell as the volume of the cell also varies. Therefore, different responses are expected when comparing the effect of exposure of a very small bacterium and a large mammalian cell to hydrogen peroxide.

In vitro cells may have different oxygen consumption rates (OCRs) depending on the state of growth and metabolic demand, such as the oxygen consumption is different between cells in growth phase and quiescent cells or differentiated cells. Cells in logarithmic phase may consume oxygen at higher rates than when they are in exponential phase, because when the redox state of extracellular thiols is adjusted in the logarithmic phase, through the pentose cycle a considerable flow of oxygen and a high ATP requirement is indispensable.

In reaction 3 chapter 1, where the NADPH-oxide electrons transfer from a two-electron reducer, NADPH, to oxygen to produce superoxide, they transfer electrons through the membrane *e.g.* when neutrophils are activated, OCR is increased by superoxide produced by Nox. In some cases, most of the oxygen consumed is associated with superoxide production, as occurs in phagocytic cells with a Nox. However, less than 1% of oxygen utilization produces $O^{\cdot-}_2$ superoxide and H_2O_2 hydrogen peroxide, in ATP-producing metabolic processes. Thus, when the OCR is 20 zmol $cell^{-1}s^{-1}$, the rate of $O^{\cdot-}_2$ production would be 200 zmol $cell^{-1}$ s^{-1}. In addition, the rate of H_2O_2 production would be 100 zmol $cell^{-1}s^{-1}$ when $O^{\cdot-}_2$ removal is performed by a dismutation catalyzed by SOD.

In most human cells in the resting state, 5% of oxygen pressure is reached.

However, in the mitochondria, oxygen is consumed by *cytochrome c* oxidase, resulting in an oxygen concentration lower than normal, unlike the concentration in the extracellular environment, thus generating an oxygen gradient [2]. Under these low mitochondrial oxygen conditions, most tissues are in hypoxic conditions [3]. This leads to normal development or to pathophysiological conditions in which a reduced oxygen supply caused by respiratory failure or vascular defects can lead to diabetes, inflammatory diseases, cardiovascular or cerebral ischemic disorders and solid tumors.

The living cells, possess the ability to detect low-oxygen levels in the environment and to activate intracellular and extracellular response mechanisms to regulate normal oxygen levels or adapt to new hypoxic conditions is essential. When an organism detects a decrease in oxygen, one of the first mechanisms it develops is the hyperventilation reflex, caused by glomus type I cells activity in the carotid body, which perceive the oxygen level reduction (15%) and activate the response mechanism to hypoxia. However, these cells are insensitive to oxygen levels in the blood.

In the immediate response to low oxygen levels, there is inhibition of K^+ channels, depolarization of the membrane, calcium influx and from synaptic vesicles the release of neurotransmitters. Carotid body sensitivity to hypoxia is modulated by Dopamine, by stimulating the D_2 receptors. Oxygen levels are related with Dopamine metabolism.

When hypoxic conditions are prolonged, compensatory mechanisms such as polycythemia are activated, *i.e.* an increase in the number red blood cells which results in increased blood oxygen levels, that is carried to all tissues. This mechanism is carried out by the glycoprotein EPO-erythropoietin whose expression in conditions of normal oxygen levels is minimal, but in conditions of hypoxia its production is increased mainly in fibroblasts, in interstitial type I cells from the kidney cortex and outer medulla [4].

Glycolysis rate increases while gluconeogenesis and oxidative phosphorylation levels decrease, during the first hours of hypoxia [5]. To stimulate glycolysis, the activity of the insulin-independent glucose transporter type I (GLUT-1) increases. At the same time, ATP deficiency is compensated by increased gene expression and enzymatic activity of glycolytic enzymes.

The expression of specific-stress proteins is another mechanism of adaptation to low oxygen levels in cells: Glucose regulatory proteins (GRP), Hypoxia-associated proteins (HAPS), Oxygen regulatory proteins (ORP) and Heat shock proteins (HSP).

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Dr. Espindola has an academic and professional background that has allowed her to work as an outstanding professor in several public and private universities in Colombia, at graduate and postgraduate levels in the areas of Biology, Biochemistry, Chemistry, Physiochemistry and Nanomedicine. Dr. Espindola has received training at the doctoral level in several universities in Spain in the areas of Biochemistry and Chemistry and has doctoral studies in Chemistry. She has been director of the Biology Program and National Coordinator of the elaboration of the Higher Education Quality Examinations and works in research and training of Ph.D. students. Dr. Espindola has advised research projects on the conservation of medicinal plants of the native flora of the countries of the Andrés Bello Agreement. She has presented at several international congresses on reactive oxygen species -ROS and flavonoids.