

METABOLISM OF OXYGEN

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Contents

1. Introduction
 2. Oxygen chemistry
 - 2.1. Oxygen molecule
 - 2.2. Singlet oxygen
 - 2.3. Superoxide, hydrogen peroxide and hydroxyl radical
 - 2.4. Carbon dioxide and monoxide
 - 2.5. Sulfuric acid-sulfur dioxide
 - 2.6. Nitrogen and its oxides
 3. Mitochondria and oxygen
 - 3.1. Oxidative phosphorylation
 - 3.2. Mitochondrial superoxide synthesis and its elimination
 4. Oxygen activation by cytochrome P450
 5. Peroxisomes
 6. Vascular endothelium and xanthine oxidase
 7. Reactive metabolites as bullets of phagocytes
 - 7.1. NADPH oxidase
 - 7.2. Hypochloric acid
 8. Oxygen damages of Biomolecules
 - 8.1. Protein oxidation
 - 8.2. Lipid peroxidation
 - 8.3. DNA oxidation
 - 8.4. Tissue anoxia
 - 8.5. Anoxic necrosis
 9. Sensing oxygen levels
 - 9.1. Chemoreceptors
 - 9.2. Glutathione cycle
 10. Oxygen and genome regulation
- Glossary
Bibliography
Biographical Sketch

Summary

Oxygen is highly toxic at too high concentrations and dangerous due to fires. Oxygen on the other hand is a vitally critical element for animals and even plants which themselves generate oxygen in their photosynthesis. ATP is required in most energy-

consuming steps in intermediary metabolism as well as in maintaining proper ion gradients in cells and extracellular fluids. Aerobic metabolism provides the energy needed for ATP synthesis. There are numerous regulatory mechanisms which help in the maintenance of appropriate oxygen levels in tissues. There are, however, diseases which provoke poor levels of oxygen and cause chronic problems like leg ulcers due to poor oxygen levels. Oxygen reduction takes place step by step in mitochondria and final products are water and carbon dioxide. Some oxygen escapes, however, from the ordered process and is released as reactive oxygen species like superoxide and hydroxyl radicals. Again the cells have several mechanisms to control their harmful effects. On the other hand the oxygen metabolites give signals which activate antioxidative defences. In this defence both endogenous enzymes as well as nutritional factors are needed. Failure to keep the balance of the action of radicals and the defence may cause problems. For example, chronic cardiovascular and neurological diseases seem to be attributable to oxidative stress factors having the upper hand in the tuning of the balance.

1. Introduction

Oxygen is the most abundant element on this planet. The Earth's crust consists of 46.6% oxygen by weight, the oceans 86% oxygen also by weight, and the atmosphere contains 21% oxygen by volume. Some 65% of the adult human body is made up of oxygen.

Approximately 2×10^9 year ago gaseous O_2 was introduced into earth's atmosphere via evolution of O_2 releasing photosynthetic organisms.

The name *oxygen* comes from the Greek stems *oxys*, "acid" and *gennan* "to form or generate". Thus, oxygen literally means "acid former". Molecular oxygen (dioxygen, O_2) is essential for the survival of all aerobic organisms. High concentration of oxygen is, however, toxic. But already the oxygen levels found in cells cause the formation of toxic reactive intermediates of the oxygen metabolism. Reactive oxygen species are constantly formed, for example, in the human body and removed by antioxidant defences.

Oxidative metabolism provides an enormous metabolic advantage to aerobic organisms by allowing the complete combustion of glucose to yield carbon dioxide, water and 38 moles of high energy adenosine triphosphate (ATP) per mole of glucose consumed. Aerobic metabolism is dependent on oxidative phosphorylation (OXPHOS), a process by which the oxidoreduction energy of mitochondrial electron transport (via a multi-component NADH dehydrogenase enzyme complex) is converted to the high-energy phosphate bond in the synthesis of ATP. O_2 serves as the final electron acceptor for cytochrome-c-oxidase, the terminal enzyme component of this mitochondrial complex, that catalysis the four-electron reduction of O_2 to H_2O . About 90% of O_2 is consumed in the mitochondria. O_2 is also consumed in fatty acid metabolism in the peroxisomes, and in the detoxification of xenobiotics, lipid-soluble drugs and e.g. steroid hormones in the endoplasmic reticulum and nuclear membranes by cytochrome P-450. This enzyme can also oxidize unsaturated fatty acids. It reduces molecular O_2 to produce O_2^- and/or H_2O_2 .

Most biologic molecules are nonradicals, containing only paired electrons. The diatomic molecule oxygen contains two uncoupled electrons and can therefore undergo reduction, yielding several different oxygen metabolites, which are collectively called Reactive Oxygen Species or ROS. They are invariably produced in aerobic environments through a variety of mechanism, which include electron “leakage” during biologic oxidations, action of flavin dehydrogenases, and specific membrane associated secretion, as well as by physical activation of oxygen by irradiation, e.g. ultra violet sun-light.

The most common ROS are: superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical (HO^\bullet), since all these metabolites are highly reactive and affect almost every kind of organisms, either directly or through conversion into other derivatives, notably nitric oxide-derived radical (NO) or Reactive Nitrogen Species (RNS). Depending on their tissue concentration ROS can either exert beneficial physiologic effects (control of gene expression and mitogenesis) or damage cell structures, including lipids in membranes, proteins and nucleic acid, leading even to cell death. The inappropriate metabolism of oxygen may thus be toxic to cells and tissues and contribute to the etiology of a wide variety of diseases. At present cardiovascular and neuronal diseases are possibly linked to the imbalance of radical formation and antioxidant defense. ROS contribute to the inflammation responses and they are used in the control of bacterial invasions.

Organisms have developed efficient protective mechanisms against excessive accumulation of ROS.

This article discusses the different forms of oxygen in mammalian tissues. In addition to the usage of oxygen in energy release from nutrients, some forms of oxygen have specialized functions, e.g. in the defence against invading microbes. As these forms are also toxic, the cells have mechanisms to defend themselves. The provision of oxygen to tissues has been discussed more in depth in this volume in chapters concerned with respiration, circulation, metabolism and gene regulation.

2. Oxygen chemistry

2.1. Oxygen molecule

Oxygen is in the periodic table with symbol **O** and has atomic number 8. At standard temperature and pressure, oxygen is predominantly found as a diatomic gas (the chemical formula O_2). Oxygen has three stable isotopes and ten known radioactive isotopes. The radioisotopes all have half lives of less than three minutes.

O_2 itself has two energetic forms: the low-energy, predominant single-bonded diradical triplet oxygen, and the high-energy double-bonded molecule singlet oxygen (1O_2).

Because oxygen is one of the major substances required for chemical reactions in the metabolism, it is fortunate that the human body has special control mechanisms which maintain almost constant oxygen concentration in the extracellular fluid. This mechanism depends principally on the chemical characteristics of hemoglobin present

in red blood cells. Oxygen diffuses from lung alveoli into the pulmonary blood and is transported principally in combination with hemoglobin to the capillaries, where it is released and diffuses through the cell membranes for metabolic use. In tissues the cells are seldom more than 50 micrometers away from a capillary, and oxygen can diffuse readily enough from capillary to cell to supply the required amounts of oxygen for metabolism.

Molecular oxygen may, however, also be harmful to human health. This idea was postulated in the late nineteenth century by Paul Bert (1878). Methods aimed at increasing tissue oxygenation, like hyperbaric oxygen therapy (HBO), have a high therapeutic potential. In addition, oxygen is an important mediator of, for example, wound healing. The skin of the lower extremities is frequently the site of chronic ulceration due to the poor perfusion of its vasculature. Oxygen availability can limit the healing rate. By locally increasing the oxygen pressure, e.g. in a plastic boot around the problem area, promotes the healing of chronic ulcers.

2.2. Singlet oxygen

Singlet oxygen ($^1\text{O}_2$) was first observed in 1924. Singlet oxygen is a very short-lived and reactive form of molecular oxygen in which the outer electrons are raised to a higher energy state. It can be formed by a variety of mechanisms, e.g. the Haber-Weiss reaction ($\text{O}_2^- + \text{H}_2\text{O}_2 + \text{Fe}^{2+} \rightarrow \text{O}_2 + \text{OH}^- + \text{HO}^\bullet + \text{Fe}^{3+}$).

Singlet oxygen is generated by input of energy, e.g. radiation. Photosensitization reactions also produce much $^1\text{O}_2$. Singlet oxygen is a nonradical reactive oxygen species often associated with oxygen free radicals that has strong oxidising activity. Singlet oxygen is an electronically excited and mutagenic form of oxygen.

Singlet oxygen can also be generated enzymatically by the action of peroxidases or lipoxygenases or by the reaction of hydrogen peroxide with hypochlorite or peroxynitrite.

Singlet oxygen is responsible for much of the physiological damage caused by reactive oxygen species. It can react with biological molecules such as proteins (especially with amino acids e.g. histidine and methione), lipids and DNA. The nucleic acid modification takes place through a selective reaction with deoxyguanosine. These are discussed more in detail later in this chapter.

2.3. Superoxide, hydrogen peroxide and hydroxyl radical

Donation of single electron to molecular oxygen results in the formation of the superoxide radical (O_2^-). Superoxide is not particularly stable, and it spontaneously decomposes into hydrogen peroxide and oxygen (e.g. $\text{HO}_2^\bullet + \text{O}_2^- \rightarrow \text{HO}_2^- (\text{H}_2\text{O}_2) + \text{O}_2$).

Most superoxide is formed in mitochondria, and this is discussed later in this chapter.

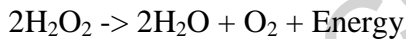
It is estimated that about 5% of oxygen consumed escapes the normal routes to water and other products. The main generation of radicals in the body takes place during physical activity in skeletal muscle mitochondria. At rest the share is great in the brain, heart and kidneys as the relaxed muscles consume only as much as the brain.

Donation of second electron yields peroxide, which then undergoes protonation to yield hydrogen peroxide (H_2O_2) and donation of a third electron, such as occurs in the Fenton reaction ($\text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \text{OH}^- + \text{HO}^\bullet$), resulting in production of the highly reactive hydroxyl radical (HO^\bullet).

The first report on H_2O_2 generation in tissues as a by-product of regular mitochondrial respiration was published more than 30 years ago by Chance and co-workers. They found that in the absence of ADP, the mitochondria released H_2O_2 that was derived from superoxide radicals. There are, however, also significant extra mitochondrial sources of H_2O_2 from a variety of oxidases, such as peroxisomal oxidases, plasma membrane NADH oxidases, e.g. in the neutrophils of the blood and xanthine oxidase associated with endothelial cells.

Hydrogen peroxide (H_2O_2) is freely miscible with water. It is apparently able to cross cell membranes readily, high (usually 50+ μM) levels being cytotoxic. It is therefore widely thought that H_2O_2 is very toxic *in vivo* and must be rapidly eliminated. Enzymes such as catalases, peroxidases (especially glutathione peroxidases) and thioredoxin-linked systems are employed.

Hydrogen peroxide also spontaneously decomposes exothermically into water and oxygen gas:



2.4. Carbon dioxide and monoxide

Carbon dioxide (CO_2) is an atmospheric gas composed of one carbon and two oxygen atoms. It is the most voluminous waste product of metabolism, and is exhaled in respiration. Carbon dioxide has significant regulatory functions, too.

A small amount of carbon monoxide exists in air. It is one of the byproducts of combustion. It is very poisonous as it blocks the oxygen-carrying sites of haemoglobin in red blood cells. Therefore smokers should remain outdoors or in very well ventilated spaces when smoking to avoid causing harm to neighbours. Passive smoking is a serious problem. One should not keep car engines running in garages as released carbon monoxide can kill the people there as usually ventilation has not been built effectively enough to cope with such amounts.

Carbon dioxide content in fresh air is less than 1% while in exhaled air the concentration is some 4.5%. When inhaled in high concentrations of CO_2 (about 5% by volume), it is toxic to humans and other animals.

In clinical therapy up to 5% of carbon dioxide is added to pure oxygen used, to stimulate breathing after apnea and to stabilize the O_2/CO_2 balance in blood.

Carbon dioxide passes easily across cell membranes, and travels in the blood from the tissues to the lungs where it is exhaled. Hemoglobin, the main molecule in red blood cells, binds both oxygen and carbon dioxide.

Carbon dioxide is a metabolic waste product in organisms that obtain energy from breaking down sugars, fats and amino acids with oxygen as part of their metabolism. In aerobic cell respiration, when oxygen is present, most metabolism will undergo two more steps after glycolysis—Krebs Cycle and Electron Transport, to produce their ATP. In eukaryotes, these processes occur in the mitochondria, while in prokaryotes they occur in the cytoplasm. In Krebs cycle the carbon dioxide is released as an end (and thus waste) product. A small amount of carbon monoxide is formed also in the human body in the catabolism of heme compounds.

2.5. Sulfuric acid-sulfurdioxide

Sulfur is a major inorganic element, essential for the entire biological system because of its incorporation into amino acids, proteins, enzymes, vitamins and other biomolecules. Sulfur, like oxygen, is a typical non-metal element belonging to group 6A in the periodic table of elements. Sulfur dioxide is a highly water-soluble gas that is rapidly hydrated, forming sulfite (SO_3^-) which in reaction with superoxide forms a very reactive bisulfite radical (SO_3^\bullet) and hydrogen peroxide (H_2O_2).

SO_3^\bullet participates in a radical chain process such as lipid peroxidation. Sulfur dioxide and its salts are commonly used inhibitors for enzymatic and non-enzymatic browning. In the body, sulfur is present as a part of the amino acids, methione, cystine and taurine. It occurs also in the iron-sulfur proteins of the Q-cytochrome c reductase complex of the respiratory chain and in the iron-containing flavoenzymes such as succinate dehydrogenase and NADH reduced dehydrogenase.

In addition, sulfur has antioxidative properties. Inter-conversion between disulfide and sulfur groups in oxidation-reduction reactions are used to eliminate H_2O_2 before it can cause cellular destruction (see 9.2 Glutathione Cycle). Natural sulfur-containing compounds such as cysteine, glutathione and lipoic acid protect against oxidative stress in biological systems through the scavenging and reduction of various oxidants.

2.6. Nitrogen and its oxides

Nitrogen is the most abundant element in the air (almost 80%). It is inert in the human body. In diving accidents it may, however, physically cause serious problems due to bubbles in the blood. Its biological fixation is a complicated process. Nitrogen compounds are essential for life. Amino acids, their derivatives and proteins as well as nucleic acids contain nitrogen. Nitric oxide (NO) is synthesized in the body from the amino acid L-arginine and it contributes to the physiological regulation of, for example, vessel wall tone in circulation.

Nitric oxide is not very reactive as a free radical. However, overproduction of NO is associated with chronic inflammatory diseases like rheumatoid arthritis and inflammatory bowel disease and neurodegenerative disease. The production of NO and

its reaction with superoxide radical can generate peroxynitrite ($O_2^- + NO \rightarrow ONOO^-$). This is a cytotoxic compound and oxidizes low-density lipoproteins (LDL) leading to developments of atherogenic lesions. *In vivo* the nitration of protein tyrosine to 3-nitrotyrosine is a finger-print left by peroxynitrite (Figure 1). Peroxynitrite is capable of causing direct protein oxidation and DNA base oxidation. Polluted air and smoking can cause nitric dioxide formation (NO_2^\bullet). The interaction of NO_2^\bullet and plasma, can lead to depletion of many antioxidants as: ascorbic acid, uric acid and α -tocopherol.

3. Mitochondria and oxygen

The typical human cell has several hundred mitochondria. They are cytoplasmic organelles that convert chemical energy to forms that can directly be used to drive cellular reactions (Figure 2). Without them cells would be dependent on anaerobic glycolysis for all their adenosine triphosphate (ATP) synthesis like erythrocytes.

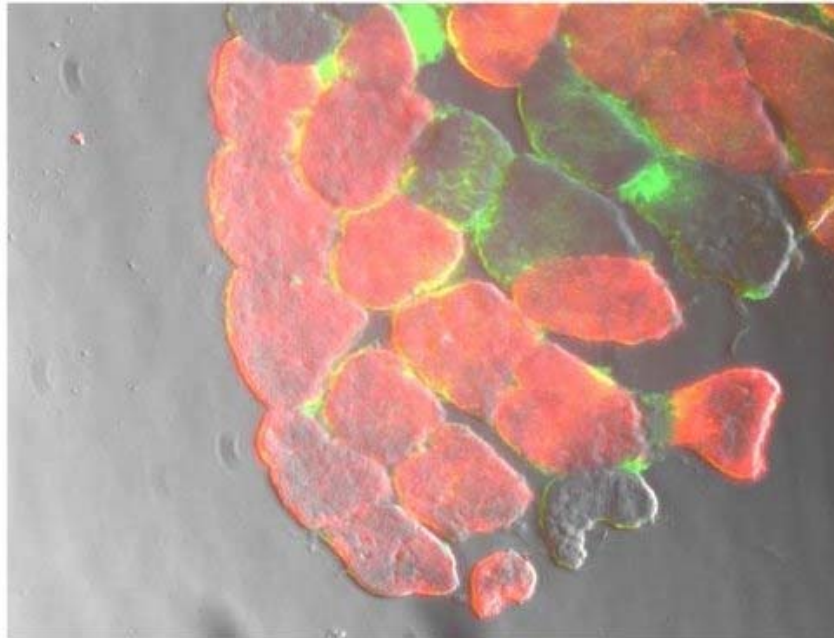


Figure 1. Nitrotyrosine; a finger-print of peroxynitrite *in vivo*, can be visualized with immunofluorescence staining. A differential interference contrast (DIC) image of the frozen section from *m. vastus lateralis* of the type 2 diabetic subject has been double-stained with slow-myosin heavy chain antibody (red) and anti-nitrotyrosine antibody (green). 20 x magnification. Zeiss Axiovert 200M. M. Venojärvi.

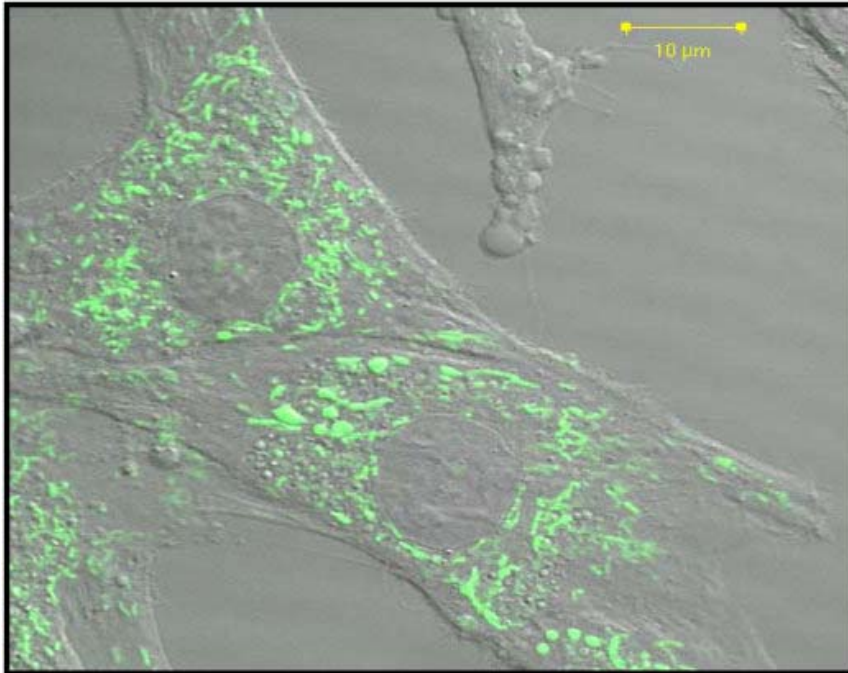


Figure 2. Mitochondria can be visualized in a living cell with specific dyes. Rat L6-skeletal muscle cells which have been stained with dihydrorhodamine DHR123-probe. The oxidized product, rhodamine 123, localized primarily to the mitochondria (green) 63x magnification. Zeiss LSM 510 META. M. Venojärvi.

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Biographical Sketch

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