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Review

Free radicals: Our enemies or friends?

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Abstract

Free radicals are atoms or groups of atoms containing at least one unpaired electron in their orbitals and can be formed when oxygen interacts with certain molecules. Once formed these highly reactive radicals can start a chain reaction, like dominoes. Their main danger comes from the damage they can do when they react with important cellular components such as DNA, or the cell membrane. Cells may function poorly or die if this occurs. On the other hand they are playing an important role in natural processes involved in cytotoxicity, defense against microorganisms and neurotransmission etc.
There are several enzyme systems within the body that neutralize free radicals. The defense system that prevents the body from free radicals' damage is called as antioxidants. Antioxidants are molecules which can safely interact with free radicals and terminate the chain reaction before vital molecules are damaged.
Nowadays most people are under the impression that the field of investigation into antioxidants and free radicals is a brand new phenomenon. The aim of this review is to clarify what is the role of free radicals in our metabolism.

Keywords: Free radicals, physiological functions, ROS, RNS

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Introduction

A common misconception amongst the general public is that 'natural' foods are always good for you. Due to the extending this logic to biological processes, there is an enormous media advertising campaigns for antioxidant foods or food extracts that belive to prevent free radicals' damage in the body.

Although essentially cancer and degenerative diseases are to major extent caused by damage done to our DNA by them, free radicals are also playing an important role in cell metabolism

The defense system of the body against free radicals is antioxidants. They interact with free radicals and stop them before vital molecules are damaged. Antioxidants are manufactured within the body and can also be extracted from the food humans eat such as fruits, vegetables, seeds, nuts, meats and oil. There is a balance with free radicals and antioxidant production in the body. Understanding the basic concepts of free radicals is important so that make judgments on free radicals whether our enemy or our friend. The aim of this paper is to clarify the importance of free radicals for our metabolism.

Physical features of free radicals

To understand what the free radicals and the antioxidants are and the way that free radicals and antioxidants interact, firstly, we have to explain terms of molecules and atoms. Molecules are the smallest particles which show all chemical and physical features of the matter. A molecule consists of atoms which are most stable in the ground state. What is the stable form of an atom?

An atom consists of an extremely small, positively charged nucleus surrounded by a cloud of negatively charged electrons. Nucleus of the atom basically consists of protons and neutrons. Protons and neutrons that held together by the so-called strong or nuclear force, theoretically, contains more than 99.9% of the mass of the atom. Protons are positively charged and neutrons are neutral electrically. Number of protons that determines atomic number is not changeable and is equal to number of electrons in stable form of atoms. Number of neutrons can change, and this situation causes to increase of nuclear mass and to change of physical properties of an atom.

Also every particle in the nucleus rotates on two directions: On its axis and around the center of nucleus. Rotating on its axis of a particle is called as spinning. If any particle rotates on right to left (counter-clockwise), it is defined as spin-up. If the particle rotates on left to right (clockwise), it is defined as spin-down.

Electrons are also turn two directions as nucleic particles protons and neutrons. They rotate on the orbitals that are energy strata around the nucleus of the atom. Electrons in every orbital must be paired and these paired electrons must spin in the opposite direction. It is called stable form of an atom when every electron in the outermost orbital of an atom has a complimentary electron that spins in the opposite direction (Halliday *et al.*,1992)

Electrons enter and fill orbitals according to four rules:

1. The Pauli exclusion principle states that an orbital can contain a maximum of two electrons which must be of opposite spin.
2. The Aufbau or buildup principle states that electrons enter and fill lower energy orbitals before higher energy orbitals.
3. Hund's rule states that every orbital in a subshell is singly occupied with one electron before any one orbital is doubly occupied, and all electrons in singly occupied orbitals have the same spin.
4. Madelung's rule states that the orbitals fill with electrons as $n + l$, where n is the principle quantum number and l is the subsidiary quantum number. This is the rule that results in the 4s orbital to have a lower energy than the 3rd orbital (Halliday *et al.*,1992)

The settlement of electrons on these orbitals (orbital number, shape, phase and electron occupancy) are described by four quantum numbers:

n principle quantum number

l subsidiary (azimuthal, angular momentum or orbital shape) quantum number (s,p,d, f; the letters s,p,d,f stand for sharp, principal, diffuse and fundamental which were early spectroscopic designations of spectral lines).

m_1 magnetic quantum number

m_s the electron spin quantum number (Halliwell, 1991, Halliday *et al.*,1992)

After all orbitals filled according to rules, at least one unpaired electron remains in the outermost orbital of unstable form of atoms or molecules.

Unstable forms of atoms or molecules are tended to transform to stable forms. Many atoms or molecules transform to "unstable form" by different ways. (Halliwell, 1991, Valko *et al.*, 2007)

A free radical is also considered as unstable form of any atom or molecule capable of independent existence (hence the term 'free') that contains one or more unpaired electrons (Halliwell, 1991)

Free radicals could be produced in biological systems in many different ways such as;

1. Breaking of covalent bonds by homolitic way;
2. Losing an electron,
3. Gaining an electron (Halliwell, 2006, Valko *et al.*, 2007).

When a free radical formed during a chemical reaction in the body, this radical tries to transform stable state. The free radical's life is very short (10^{-10} sec) but this process continues as a chain reaction. Free radicals are showed as symbol of (·) in the papers.

There are numerous types of free radicals that can be formed within the body. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are major components of the "free radical" system. Except this two kinds of "free radicals"; atomic hydrogen, many heavy-transition- metals (as iron, copper, zinc and manganese), chlorine, many drugs, ionizing radiation, environmental wastes (as CO, asbestos, ozone, solvents etc.) are also behave like a source of free radical. It is possible to increase the samples.

ROS are first discovered free radicals in biological materials. ROS such as superoxide ($O_2^{\cdot -}$), hydroxyl ($OH^{\cdot}$) are derived from molecular oxygen during the normal oxygen metabolism. Molecular oxygen has two unpaired electrons that spin in the parallel direction and react with other radicals easily. It could be said, diradical molecular oxygen is the first step of the ROS formation and hydrogen peroxide (H_2O_2) that derived from molecular oxygen, is not a free radical but it is main source of other radicals. (Halliwell, 2006, Valko *et al.*, 2007) (Figure I).

In the same time, ROS cause different free radicals such as carbon-centered organic radicals ($R^\cdot$), peroxide radicals ($ROO^\cdot$), alcoxy radicals ($RO^\cdot$), thyil radicals ($RS^\cdot$), thyil peroxide ($RSO_2{}^\cdot$), sulphenyl radicals ($RSO^\cdot$) etc. (Halliwell,1991; Dröge, 2002; Valko *et al.*, 2006).

In the body, water is another source of free radicals. 80% of a cell is composed of water. There is no doubt that routine metabolic chemistry in each cell produces legions of free-radicals every hour, in the process of "just living" and there is no doubt that exposure to ionizing radiation also produces some extra free-radicals in irradiated cells. When cells are exposed to ionizing radiation, the damage may be caused by direct or indirect radiation. DNA is known to be the critical cellular target for the damaging effects of ionizing radiation. Direct effects involve the formation of ionic, radical and excited intermediates as a result of the deposition of energy within the DNA. Indirect effects involve the interaction of the DNA with water radiolysis products such as OH radicals, H-atoms or hydrated electrons (Figure 2).

As a result of the interaction of a photon of x- or -y-rays, or a charged particle such as an electron or proton, the water molecule may become ionized. This may be described by the equation: H_2O -> (H_2O+) + e- H_2O+ is an "ion radical." H_2O+ is both charged and has an unpaired electron; consequently, it is both an ion and a free radical. They decay to form free radicals, which are not charged, but which still have an unpaired electron. In the case of water, the ion radical reacts with another water molecule to form the highly reactive hydroxyl radical $OH^\cdot$: (H_2O+) + H_2O -> (H_3O+) + $OH^\cdot$ The $OH^\cdot$ possesses a total of nine electrons, and so one of them is unpaired. It is a highly reactive free radical and can diffuse a short distance to reach a critical target within a cell (Hall, 1978; Mundt *et al.*, 2003; Gridley *et al.*, 2007).

The other major element of free radical system is reactive nitrogen species. One of common RNS, $NO^\cdot$ is a very important radical which acts as signaling molecule in different physiological and pathophysiological processes, including neurotransmission, blood pressure regulation, immune response, tissue damage. Nitric oxide (NO) is synthesized by mammalian cells from L-arginine through a complex oxidation reaction catalyzed by the flavo-hemoprotein NO synthase. NO is also produced with reducing of nitrits by nitrite reductase. Nitrite reductase activity in mammalian tissues has been linked to the mitochondrial electron transport system, protonation, deoxyhemoglobin, and xanthine oxidase (Dröge, 2002; Bryan, 2006; Valko *et al.* 2006).

All transition metals, except copper, contain one electron in their outermost orbital and can be considered free radicals. Iron has the ability to gain and lose electrons (i.e. Fe^{2+},Fe^{3+}) very easily. This property makes iron and copper two common catalysts of oxidation reactions. Owing to fact that iron is major component of red blood cells (RBC), during break down RBC, releasing free iron can be detrimental to cellular membranes because of the pro-oxidation effects it can have. Fenton Reaction can explain how iron makes a free radical as below.

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^\cdot + OH^- \text{ (Fenton Reaction)}$$

On the other hand, copper has a full outer orbital, but can lose and can gain electrons very easily to make itself a free radical as iron. In addition Zinc only exists in one valence (Zn^{2+}) and does not catalyze free radical formation. But Zinc may act to stop radical formation by displacing those metals that do have more than one valence.

Free radicals are continuously produced as intermediate products in vary enzymatic pathways in the cell. Sometimes these intermediate products leak from the active sides of enzymes and react with the molecular oxygen. Some important enzymes such as xanthine oxidase, aldehyde oxidase, dihydroorotate dehydrogenase, flavoprotein dehydrogenase, and aminoacid oxidase and triptophan dioxygenase play role in production of free radicals.

The major ROS source of normal cells is mitochondrial electron transport chain.

Endoplasmic reticulum and nuclear membrane produce free radicals by oxidizing cytochromes that bound membrane.

Peroxysomes are very important source of H_2O_2 in the cell. Such as D-amino acidoxidase, urate oxidase, L-hydroxil acid oxidase and fatty acid acyl-CoA oxidase in peroxisomes produce a large amount of H_2O_2.

Arachidonic acid and prostaglandin metabolisms are also great source of free radicals in the cell (Dröge, 2002; Bryan, 2006; Valko *et al.* 2006).

Physiological effects of free radicals in living organism

A great number of physiological functions are controlled by redox-responsive signalling pathways.These, for example involve: (1) redox regulated production of NO; (2) ROS production by phagocytic NAD(P)H oxidase (oxidative burst); (3) ROS production by NAD(P)H oxidases in nonphagocytic cells; (4) regulation of vascular tone and other regulatory functions of NO•; (5) ROS production as a sensor for changes of oxygen concentration; (6) redox regulation of cell adhesion; (7) redox regulation of immune responses; (8) ROS-induced apoptosis and other mechanisms (Dröge, 2002; Kamata and Hirata, 1999; Valko *et al.*, 2007).

Under normal conditions the antioxidant defense system within the body can easily handle free radicals that are produced. Antioxidants are effective because they are willing to give up their own electrons to free radicals. When a free radical gains the electron from an antioxidant it no longer needs to attack the cell and the chain reaction of oxidation is broken. After donating an electron an antioxidant becomes a free radical by definition. Antioxidants, in this state are not harmful because they have the ability to accommodate the change in electrons without becoming reactive. The human body has an elaborate antioxidant defense system. Antioxidants are manufactured within the body and can also be extracted from the food humans eat such as fruits, vegetables, seeds, nuts, meats and oil.

Pathological effects of free radicals

How do free radicals affect organism in molecular base?

Free radicals affect all important molecules of the cell such as lipids, proteins, DNA, carbohydrates.

Cellular components involving polyunsaturated fatty acid residues of phospholipids are highly sensitive to oxidation. A typical example of such a chain reaction is the process of lipid peroxidation that may be initiated by a radical (e.g.hydroxyl radical -OH$^{\cdot}$) in membranes of cytoplasm, mitochondria, nucleus and endoplasmic reticulum. The resulting, carbon-centered radical (L$^{\cdot}$) adds rapidly to O_2 to generate a lipid peroxyl radical (LOO$^{\cdot}$) that propagates the chain by reacting with a neighboring lipid molecule to generate another L$^{\cdot}$ and a lipid hydroperoxide. Peroxidation of lipid causes increasing of membrane permeability and at the end cellular damage. Lipid peroxidation can be catalysed by itself. (Dröge, 2002; Bryan, 2006; Valko *et al.*, 2007).

Proteins are less sensitive than lipids. However, effects of free radicals on protein can be harmful at least as lipids. Many amino acids which have sulphur and unsaturated bonds can be affected from free radicals. Espescially cysteine and methionine residues of proteins are sensitive to oxidation by the action of ROS/RNS. Oxidation of cysteine residues may lead to the reversible formation of mixed disulphides between protein thiol groups (–SH) and low molecular weight thiols, in particular GSH (*S*-glutathiolation). Because of

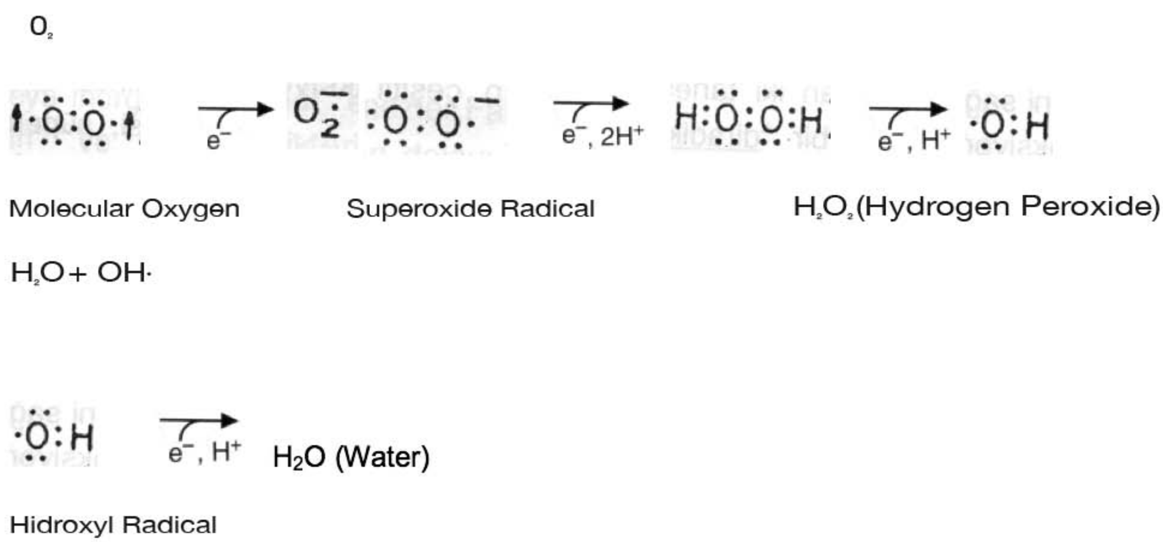

Figure 1. Formation of ROS.

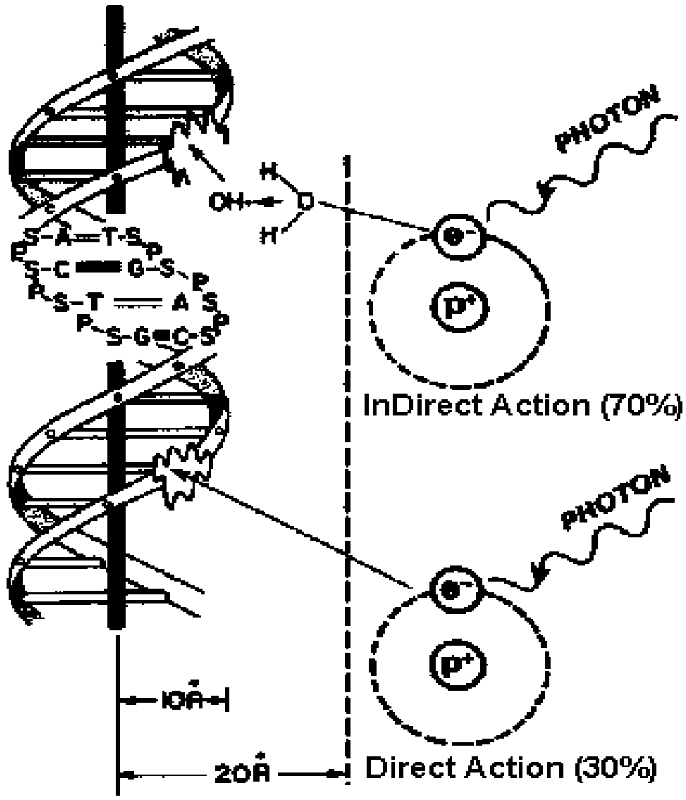

Figure 2. Illustrating the direct and indirect actions of radiation (Hall, 1978).

proteins' functional importance in the body, effecting of many proteins such as IgG, albumine, haemoglobine can be vital (Valko *et al.*, 2007).

Nucleic acids are also sensitive to hazardous effects of free radicals. Oxygen radicals that generated during reduction of O_2 can attack DNA bases or deoxyribose residues. The highly reactive hydroxyl radical (OH.) reacts with DNA by addition to double bonds of DNA bases and by abstraction of an H atom from the methyl group of thymine and each of the C-H bonds of 2-deoxyribose. Double-strand breaks in the sugar-phosphate backbone of DNA are especially important, since they are frequently not repaired or are repaired inaccurately. The interaction of oxygen radicals with DNA can also lead to single-strand breaks, changes in structure or loss of bases and DNA-DNA or DNA-protein cross-linkages (Dugan and Choi, 1999; Martnett, 2000; Cooke *et al.*, 2003; Stocker and Keaney, 2005).

Effects of free radicals on carbohydrates are not well-known yet. But many studies support this hypothesis: *in vitro* studies and in animal models, antioxidants have been shown to improve insulin sensitivity. Several clinical trials have demonstrated that treatment with vitamin E, vitamin C, or glutathione improves insulin sensitivity in insulin-resistant individuals, although there is evidence from molecular biology studies to support the possibility that oxidative stress alters the intracellular signaling pathway inducing insulin resistance. The recent finding that insulin resistance is associated in humans with reduced intracellular antioxidant defense also supports this hypothesis. Recent studies demonstrate that a single hyperglycemia induced process of overproduction of superoxide by the mitochondrial electron transport chain seems to be the first and key event in the activation of all other pathways involved in the pathogenesis of endothelial dysfunction in the case of hyperglycemia (Bonnefont-Rousselot, 2002; Ceriello and Motz, 2004).

Conclusion

Free radicals can be defined as molecules or molecular fragments containing one or more unpaired electrons in atomic or molecular orbital. There are numerous types of free radicals that can be formed within the body. These radicals make their functions by loosing or gaining electrons. Their effects can be deleterious or beneficial for the body.

ROS and RNS are major components of the "free radical" system. Free radicals are continuously produced as intermediate products in vary enzymatic and non-enzymatic pathways in the cell. Beside the internally generated free radicals, there are also some externally free radicals sources such as cigarette smoke, environmental pollutants, radiation, ultraviolet light, certain drugs, pesticides, anaesthetics and industrial solvents. (Dugan and Choi, 1999; Martnett, 2000; Bonnefont-Rousselot, 2002; Cooke *et al.*, 2003; Ceriello and Motz, 2004; Stocker and Keaney, 2005; Dongmei *et al.*, 2006; Reddy *et al.*, 2007).

The antioxidant defense system within the body can easily handle free radicals that are internally produced. Antioxidants are manufactured within the body and can also be extracted from the food humans eat such as fruits, vegetables, seeds, nuts, meats and oil. The principle micronutrient (vitamin) antioxidants are vitamin E, beta-carotene, and vitamin C. Additionally, selenium, a trace metal that is required for proper function of one of the body's antioxidant enzyme systems, is sometimes included in this category. The body cannot manufacture these micronutrients so they must be supplied in the diet. There is no doubt that antioxidants are necessary components for our health but we do not forget that the body's finely tuned mechanisms are carefully balanced to withstand a variety of insults. Taking chemicals without a complete understanding of all of their effects may disrupt this balance. In order to take antioxidants, reducing externally free radical sources in our life may be the better solution for our health.

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