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Review Article

Biochemistry of Free Radicals and Antioxidants

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Abstract: The biochemistry of reactive oxygen species (ROS) such as superoxide anion, hydrogen peroxide, hydroxyl radicals, and singlet oxygen is important in aerobic metabolism of the cell mostly reactive nitrogen species are well recognised for playing dual function as both dangerous and beneficial species. Overproduction of ROS from mitochondrial electron transport chain leakage or excessive stimulation of xanthine oxidase and other oxidative enzymes results in oxidative stress, a process that can be an important mediator of damage to cell structure and function, lipids, proteins, carbohydrates and DNA. In contrast, beneficial effects of ROS/RNS occur at very low concentrations and involve physiological roles in cellular responses in defence against infectious agents, gene expression, cellular growth, in the function of a number of cellular signalling pathways, hypoxia and respiratory burst. In the past and present years, progress has been made in the recognition and understanding of the roles of reactive oxygen species in many diseases. The body protects itself from the potential damages of reactive oxygen species, by utilizing antioxidant enzymes and non-antioxidant enzymes e.g superoxide dismutases, glutathione peroxidases, glutathione reductase and catalase. Scientists have indicated that antioxidant obtained from daily diets such as non-enzymatic antioxidants vitamin E, vitamin C, carotenoids and polyphenols can scavenge the reactive oxygen species. These compounds may also be required as cofactors for antioxidant enzymes or be used by cells for up-regulating enzymatic antioxidants.
Keywords: Oxidative stress, Reactive oxygen species, Nitric oxide, Antioxidants, Lipid peroxidation, antioxidant enzymes.

INTRODUCTION

Free radicals are continuously produced by the body's normal use of oxygen [1]. Oxygen is an element indispensable for life. When cells use oxygen to generate energy free radicals are produced by the mitochondria. These by-products are generally reactive oxygen species (ROS) as well as reactive nitrogen species (RNS) that result from the cellular redox process. The free radicals have a special affinity for lipids, proteins, carbohydrates and nucleic acids [2].

A free radical is any chemical species (capable of independent existence possessing one or more unpaired electrons, an unpaired electron being one that is alone in an orbital. The simplest radical is the hydrogen atom. Electrons are more stable when paired together in orbital: the two electrons in a pair have different directions of spin. Hence, radicals are generally less stable than non-radicals, although their reactivity varies. Free radicals are capable of reacting indiscriminately with any molecules with which they come in contact. Once radicals are formed they can either react with another radical or with another non-radical molecule by various interactions. If two radicals collide they can combine their unpaired electron, thus forming a covalent bond. However, most molecules found in vivo are non-radicals. In this case a radical might donate its unpaired electron to the other molecule, or might take one electron from it, thus transforming its radical character. At the same time a new radical is formed [3].

Historically, the triphenylmethyl radical studied by Gomberg [4] in 1900, is the first organic free radical discovered. The triphenylmethyl radical (Ph_3C') can be obtained by the reaction of triphenylmethyl halide with Ag metal.

ROS/RNS are present in the atmosphere as pollutants and can be generated (i) during UV light irradiation, by X-rays and gamma rays (ii) during metal catalyzed reactions (iii) by neutrophils, esinophils and macrophages during inflammatory cell activation [5,6] (iv) as by-products of mitochondrial catalyzed electron transport reactions, (v) by cytochrome P450 metabolism and the enzyme xanthine oxidase, which catalyzes the reaction of hypoxanthine to xanthine and xanthine to uric acid [7].

It has been established that ROS can be both harmful and beneficial in biological systems depending on the environment and concentration [8,9]. Beneficial effects of ROS involve, for example, the physiological roles in cellular responses to noxia such as defense against infectious agents, and in the function of a number of cellular signaling systems and gene expression. In contrast, at high concentrations, ROS can mediate damage to cell structures, including lipids and membranes, proteins and nucleic acids; this damage is often referred as "oxidative stress" [10].

Oxidative stress is defined as an imbalance between production of free radicals and reactive metabolites, so-called oxidants or reactive oxygen species, and their elimination by protective mechanisms, referred to as antioxidants. This imbalance leads to damage of important biomolecules and cells, with potential impact on the whole organism [11]. The harmful effects of ROS are balanced by the action of antioxidants, some of which are enzymes present in the body [12]. Despite the presence of the cell's antioxidant defense system to counteract oxidative damage from ROS, oxidative damage accumulates during the life cycle and has been implicated in diseases, aging and age dependent diseases such as cardiovascular disease, cancer, neurodegenerative disorders and other chronic conditions [13].

Reactive Oxygen Species

Reactive oxygen species can be classified into oxygen-centered radicals and oxygen-centered non radicals. Oxygen-centered radicals are superoxide anion ($\cdot O_2^-$), hydroxyl radical (·OH), alkoxyl radical (RO·), and peroxyl radical (ROO·). Other reactive species are nitrogen species such as nitric oxide (NO·), nitric dioxide ($NO_2\cdot$), and peroxynitrite (OONO–). Oxygen-centered non-radicals are hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$), Hypochlorous acid and Ozone [14, 15].

Superoxide anion

Superoxide anion is a reduced form of molecular oxygen created by receiving one electron. Superoxide anion is an initial free radical formed from mitochondrial electron transport systems. Mitochondria generate energy using 4 electron chain reactions, reducing oxygen to water. Some of the electrons escaping from the chain reaction of mitochondria directly react with oxygen and form superoxide anions [16]. The superoxide anion plays an important role in the formation of other reactive oxygen species such as hydrogen peroxide, hydroxyl radical, or singlet oxygen ($2\cdot O_2^- + 2H+ \rightarrow H_2O_2 + O_2$) in living systems [17]. The superoxide anion can react with nitric oxide (NO·) and form peroxynitrite ($ONOO^-$), which can generate toxic compounds such as hydroxyl radical and nitric dioxide ($ONOO– + H+ \rightarrow \cdot OH + \cdot NO_2$) [3].

The hydroxyl radical

•OH, is the neutral form of the hydroxide ion. The hydroxyl radical has a high reactivity, making it a very dangerous radical with a very short *in vivo* half-life of approximately, 10^{-9} s [18]. Thus when produced *in vivo* •OH reacts close to its site of formation. The redox state of the cell is largely linked to an iron (and copper) redox couple and is maintained within strict physiological limits. It has been suggested that iron regulation ensures that there is no free intracellular iron; however, in vivo, under stress conditions, an excess of superoxide releases "free iron" from iron-containing molecules. The release of iron by superoxide has been demonstrated for [4Fe–4S] cluster containing enzymes of the dehydratase-lyase family [19]. The released Fe^{2+} can participate in the Fenton reaction, generating highly reactive hydroxyl radical.

$$\mathbf{Fe^{+2} + H_2O_2^{\bullet-} \rightarrow Fe^{+3} + HO^- + HO^{\cdot}}$$

NO• is generated in biological tissues by specific nitric oxide synthases (NOSs), which metabolise arginine to citruline with the formation of NO• *via*a five electron oxidative reaction [20]. Nitric oxide (NO•) is an abundant reactive radical that acts as an important oxidative biological signalling molecule in a large variety of diverse physiological processes, including neurotransmission, blood pressure regulation, defence mechanisms, smooth muscle relaxation and immune regulation [21]. However, since it is soluble in both aqueous and lipid media, it readily diffuses through the cytoplasm and plasma membranes. NO• has effects on neuronal transmission as well as on synaptic plasticity in the central nervous system. In the extracellular milieu, NO• react with oxygen and water to form nitrate and nitrite anions.Overproduction of reactive nitrogen species is called **nitrosative stress**[22]. This may occur when the generation of reactive nitrogen species in a system exceeds the system's ability to neutralise and eliminate them. Nitrosative stress may lead to nitrosylation reactions that can alter the structure of proteins and so inhibit their normal function.

Cells of the immune system produce both the superoxide anion and nitric oxide during the oxidative burst triggered during inflammatory processes. Under these conditions, nitric oxide and the superoxide anion may react together to produce significant amounts of a much more oxidatively active molecule, peroxynitrite anion (ONOO−), which is a potent oxidising agent that can cause DNA fragmentation and lipid oxidation [23]:$NO\bullet + O_2^{\bullet-} \rightarrow ONOO^-$ (1)Reaction (1) has one of the highest rate constants known for reactions of NO•, $7.0\times109\ M^{-1}\ s^{-1}$. Thus NO• toxicity is predominantly linked to its ability to combine with superoxide anions. Nitric oxide readily binds certain transition metal ions; in fact many physiological effects of NO• are exerted as a result of its initial binding to Fe^{2+} haem groups in the enzyme soluble guanylate cyclase (sGC) [24].

Nitric dioxide

(NO_2·) is formed from the reaction of peroxyl radical and NO, polluted air and smoking also produce this oxide. Nitric dioxide adds to double bonds and abstract labile hydrogen atoms initiating lipid peroxidation and production of free radicals. It also oxidizes ascorbic acid [25].

Peroxynitrite

Reaction of NO· and superoxide anion can generate peroxynitrite ($O_2^{\cdot-}$ + NO· → OONO–). Peroxynitrite is a cytotoxic species and causes tissue injury and oxidizes low-density lipoprotein (LDL) [3]. Peroxynitrite appears to be an important tissue-damaging species generated at the sites of inflammation [25] and has been shown to be involved in various neurodegenerative disorders and several kidney diseases [26].Peroxynitrite (OONO–) can cause direct protein oxidation and DNA base oxidation and modification acting as a "hydroxyl radical- like" oxidant. The significance of peroxynitrite as a biological oxidant comes from its high diffusibility across cell membranes [26]. Nitrotyrosine, which can be formed from peroxynitrite-mediated reactions with amino acids, has been found in age-associated tissues [26].

Peroxyl and Alkoxyl radicals

Peroxyl radicals (ROO·) are formed by a direct reaction of oxygen with alkyl radicals (R·), for example, the reaction between lipid radicals and oxygen. Decomposition of alkyl peroxides (ROOH) also results in peroxyl (ROO·) and alkoxyl (RO·) radicals. Irradiation of UV light or the presence of transition metal ions can cause homolysis of peroxides to produce peroxyl and alkoxyl radicals (ROOH → ROO·+ H·, ROOH + Fe^{3+} → ROO· + Fe^{2+} + H^+). Peroxyl and alkoxyl radicals are good oxidizing agents. They can abstract hydrogen from other molecules with lower standard reduction potential. This reaction is frequently observed in the propagation stage of lipid peroxidation. Very often the alkyl radical formed from this reaction can react with oxygen to form another peroxyl radical, resulting in chain reaction. Some peroxyl radicals break down to liberate superoxide anion or can react with each other to generate singlet oxygen [27]. Aromatic alkoxyl and peroxyl radicals are less reactive than respective open chain radicals because of the delocalization of electrons in the ring.

Singlet oxygen

Molecular oxygen in the ground state is a triplet (two electrons have the same spin). Energy transferred to ground state molecular oxygen from an excited sensitizer is used to promote the electron to the next energy level as well as a change of spin, thereby the name singlet oxygen.Singlet oxygen is a non-radical and excited status. The spin of one of the electrons of the two outer orbitals is inverted. Tayakama [28] reported that metastable phosphatidylcholine hydroperoxides present in the living organism produced singlet oxygen during their breakdown in the presence of Cu^{2+} in the dark. Singlet oxygen can be formed from hydrogen peroxide, which reacts with superoxide anion, or with HOCl or chloroamines in cells and tissues [17].

$$\mathbf{HOCl + H_2O_2^- \rightarrow Cl^- + H_2O + H^+ + {}^1O_2}$$

Compared with other reactive oxygen species, singlet oxygen is rather mild and nontoxic for mammalian tissue [17]. However, singlet oxygen has been known to be involved in cholesterol oxidation [29]. Oxidation of cholesterol by singlet oxygen results in formation of 5α–OOH (3β–hydroxy– 5α–cholest–6–ene–5–hydroperoxide), [30]. Oxidation and degradation of cholesterol by singlet oxygen was observed to be accelerated by the co-presence of fatty acid methyl ester. In the human organism, singlet oxygen is both a signal and a weapon, with therapeutic potency against various pathogens such as microbes, viruses, and cancer cells [17].

Hydrogen peroxide (H_2O_2)

Hydrogen peroxide can be generated through a dismutation reaction from superoxide anion by superoxide dismutase. Enzymes such as amino acid oxidase and xanthine oxidase also produce hydrogen peroxide from superoxide anion. Hydrogen peroxide is highly diffusible and crosses the plasma membrane easily.Hydrogen peroxide is the least reactive molecule among reactive oxygen species and is stable under physiological pH and temperature in the absence of metal ions. Hydrogen peroxide is a weak oxidizing and reducing agent and is thus regarded as being poorly reactive. Hydrogen peroxide can generate the hydroxyl radical in the presence of metal ions and superoxide anion ($\cdot O_2^- + H_2O_2 \rightarrow \cdot OH + OH^- + O_2$) [3]. Hydrogen peroxide can produce singlet oxygen through reaction with superoxide anion or with HOCl or chloroamines in living systems. Hydrogen peroxide can degrade certain heme proteins, such as hemoglobin, to release iron ions.

Ozone

This pale blue gas, which is not produced in vivo, serves as an important protective shield against solar radiation in the atmosphere. Close to the earth's surface. Ozone is an unwanted oxidant and is often regarded as the most toxic air pollutant [31]. Ozone can form in laboratory equipment that has high energy ultraviolet (UV)-producing lamps and in urban air as a result of photochemical reactions and pollution. The tissue most susceptible to damage upon exposure to ozone is the lung. The biological effect of ozone is often attributed to its ability to cause oxidation or peroxidation of biomolecules either directly or via free-radical mechanisms [32].

Hypochlorous Acid

HOCl is produced by the neutrophil derived enzyme myeloperoxidase at sites of inflammation and when activated neutrophils infiltrate reoxygenated tissue [33]. The enzyme oxidizes chloride ions in the presence of H_2O_2. HOCl is not a free radical, but it is a potent chlorinating and oxidizing agent. It has been suggested that the formation of chlorohydrins could disrupt cell membranes and lead to cell lysis and death [34]. On the basis of this observation, the cholesterol chlorohydrins have been suggested to be potential biomarkers for oxidative damage associated with neutrophil/monocyte activation [34]. HOCl can attack the proteolytic inhibitor, alpha-1-antiproteinase (α_1AP) is the major inhibitor in human plasma of proteolytic enzymes such as elastase. Thus its inactivation by HOCl might greatly potentiate tissue damage because elastase is also released from activated neutrophils. HOCl attacks primarily amines and sulfhydryl groups in proteins and chlorinates purine bases in DNA [35].

Antioxidants

The term "antioxidant" refers to any molecule capable of stabilizing or deactivating free radicals before they attack cells. Humans have evolved highly complex antioxidant systems (enzymatic and nonenzymatic), which work synergistically, and in combination with each other to protect the cells and organ systems of the body against free radical damage. The antioxidants can be endogenous or obtained exogenously e.g, as a part of a diet or as dietary supplements. Some dietary compounds that do not neutralize free radicals, but enhance endogenous activity may also be classified as antioxidants.

An ideal antioxidant should be readily absorbed and quench free radicals, and chelate redox metals at physiologically relevant levels. It should also work in both aqueous and/or membrane domains and effect gene expression in a positive way. Endogenous antioxidants play a crucial role in maintaining optimal cellular functions and thus systemic health and well-being. However, under conditions, which promote oxidative stress, endogenous antioxidants may not be sufficient and dietary antioxidants may be required to maintain optimal cellular functions. The most efficient enzymatic antioxidants involve glutathione peroxidase, catalase and superoxide dismutase [36]. Non-enzymatic antioxidants include Vitamin E and C, thiol antioxidants (glutathione, thioredoxin and lipoic acid), melatonin, carotenoids, natural flavonoids, and other compounds [37]. Some antioxidants can interact with other antioxidants regenerating their original properties; this mechanism is often referred to as the "antioxidant network" [38]. There is growing evidence to support a link between increased levels of ROS and disturbed activities of enzymatic and nonenzymatic antioxidants in diseases associated with aging.

Enzymatic antioxidants

Superoxide dismutase (SOD), (EC 1.15.1.1) it is one of the most effective intracellular enzymatic antioxidants and it catalyzes the conversion of superoxide anions to dioxygen and hydrogen peroxide. Superoxide dismutase exists in several isoforms, which differ in the nature of active metal centre, amino acid composition, co-factors and other features. There are three forms of SOD present in humans: cytosolic Cu, Zn-SOD, mitochondrial Mn-SOD, and extra cellular-SOD [39]. Superoxide dismutase neutralizes superoxide ions by going through successive oxidative and reductive cycles of transition metal ions at its active site [40]. Cu, Zn-SOD has two identical subunits with a molecular weight of 32 kDa [36] and each of the subunit contains as the active site, a dinulcear metal cluster constituted by copper and zinc ions, and it specifically catalyzes the dismutation of the superoxide anion to oxygen and water. The mitochondrial Mn-SOD is a homotetramer with a molecular weight of 96 kDa and contains one manganese atom per subunit [36], and it cycles from Mn(III) to Mn(II), and back to Mn(III) during the two-step dismutation of superoxide. Extra cellular superoxide dismutase contains copper and zinc, and is a tetrameric secretary glycoprotein having a high affinity for certain glycosaminoglycans such as heparin and heparin sulphate [36] however, its regulation in mammalian tissues occurs primarily in a manner coordinated by cytokines, rather than as a response to oxidative stress.

Catalase (EC1.11.1.6)

This enzyme is present in the peroxisome of aerobic cells and is very efficient in promoting the conversion of hydrogen peroxide to water and molecular oxygen. Catalase has one of the highest turnover rates for all enzymes: one molecule of catalase can convert approximately 6 million molecules of hydrogen peroxide to water and oxygen each minute [36].

Glutathione peroxidise

It has two forms of this enzyme, one which is selenium-dependent (GPx, EC1.11.1.19) and the other, which is selenium-independent (glutathione-S-transferase, GST, EC2.5.1.18) [36]. The differences are due to the number of subunits, catalytic mechanism, and the binding of selenium at the active centre, and glutathione metabolism is one of the most important antioxidative defense mechanisms present in the cells. There are four different Se-dependent glutathione peroxidases present in humans [40] and these are known to add two electrons to reduce peroxides by forming selenoles (Se-OH) and the antioxidant properties of these seleno-enzymes allow them to eliminate peroxides as potential substrates for the Fenton reaction. Selenium-dependent glutathione peroxidase acts in association with tripeptide glutathione (GSH), which is present in high concentrations in cells and catalyzes the conversion of

hydrogen peroxide or organic peroxide to water or alcohol while simultaneously oxidizing GSH. It also competes with catalase for hydrogen peroxide as a substrate and is the major source of protection against low levels of oxidative stress [40]. However, the most important H_20_2-removing enzymes in human cells are glutathione peroxidises (GSHPX), enzymes that require selenium (has selenocysteine at the active site) for their action. GSHPX enzymes remove H_20_2 by using it to oxidize reduced glutathione (GSH) to oxidized glutathione (GSSG). Glutathione reductase, an FAD-containing enzyme, regenerates GSH from GSSG, with NADPH as a source of reducing power [41].

Nonenzymatic antioxidants

Tocopherols and tocotrienols

Vitamin E

This is a fat-soluble vitamin existing in eight different forms. In humans, α-tocopherol is the most active form, and is the major powerful membrane bound antioxidant employed by the cell [42]. The main function of Vitamin E is to protect against lipid peroxidation [43], and there is also evidence to suggest that α-tocopherol and ascorbic acid function together in a cyclic-type of process. During the antioxidant reaction, α-tocopherol is converted to an α-tocopherol radical by the donation of a labile hydrogen to a lipid or lipid peroxyl radical, and the α-tocopherol radical can therefore be reduced to the original α-tocopherol form by ascorbic acid [44].

Tocopherols consist of a chroman ring and a long, saturated phytyl chain. Tocols are 2–methyl–2(4', 8', 12',–trimethyltridecyl) chroman–6–ols, and tocotrienols have 3 double bonds at position 3', 7', and 11' of the side chain in tocols. The α, β, ϒ, and σ–tocopherols and tocotrienols differ in the number and position of methyl groups attached to the 5, 7, and 8 of the ring structure [45]. Tocopherols and tocotrienols are very nonpolar and exist in lipid phase. Tocopherols are natural constituents of biological membranes. Tocotrienols are found mainly in palm oil, cereal grains, and kale [46].

Antioxidant mechanisms of tocopherols include the transfer of a hydrogen atom at 6–hydroxyl group on the chroman ring, and scavenging of singlet oxygen and other reactive species. Tocopherols are regenerated in the presence of ascorbic acids. Phytyl chain in tocopherols can be fit in the membrane bilayer while active chroman ring is closely positioned to the surface. This unique structure enables tocopherols to act as effective antioxidants and to be regenerated through reaction with other antioxidants such as ascorbic acid [25]. α–Tocopherol has higher vitamin E activity and singlet oxygen-quenching ability than β, ϒ and σ–tocopherols, whereas ϒ–tocopherol has better nitrogen dioxde and peroxynitrite radical-scavenging ability than α–tocopherols [45].

Ascorbic acid

L–Ascorbic acid is a 6-carbon lactone ring structure with 2,3–enediol moiety. The antioxidant activity of ascorbic acid comes from 2,3–enediol. L–Ascorbic acid first changes to semi-dehydroascorbic acid through donating 1 hydrogen atom and electron, and then L–dihydroascorbic acid by donating a 2nd hydrogen atom and electron. Both L–ascorbic acid and L–dihydroascorbic acid retain the vitamin C activity. Ascorbic acid is highly susceptible to oxidation in the presence of metal ions such as Cu^{2+} and Fe^{3+}. Oxidation of ascorbic acid is also influenced by heat, light exposure, pH, oxygen concentration, and water activity [45].

The antioxidant mechanisms of ascorbic acid are based on hydrogen atom donation to lipid radicals, quenching of singlet oxygen, and removal of molecular oxygen. Scavenging aqueous radicals and regeneration of α–tocopherol from the tocopheroxyl radical species are also well known antioxidant mechanisms of ascorbic acid. Ascorbic acid is an excellent electron donor because of the low standard 1-electron reduction potential (282 mV), the generation of relatively stable semi-dehydroascorbic acid, and the easy conversion of dehydroascorbic acid to ascorbic acid [47].

Carotenoids

Carotenoids are a group of tetraterpenoids. The basic carotenoid structural backbone consists of isoprenoid units formed either by head-to-tail or by tail-to-tail biosynthesis. There are primarily 2 classes of carotenoids: carotenes and xanthophylls. Carotenes are hydrocarbon carotenoids and xanthophylls contain oxygen in the form of hydroxyl, methoxyl, carboxyl, keto, or epoxy groups. Lycopene and β–carotenes are typical carotenes whereas lutein in green leaves and zeaxanthin in corn are typical xanthophylls. The structures of carotenoids are acyclic, monocyclic, or bicyclic. For example, lycopene is acyclic, ϒ–carotene is monocyclic, and α– and β–carotenes are bicyclic carotenoids [48]. Double bonds in carotenoids are conjugated and trans forms of carotenoids are found in plant tissues.

Epidemiological studies have revealed that an increased consumption of a diet rich in carotenoids is correlated with a lower risk of age-related diseases. Carotenoids contain conjugated double bonds and their antioxidant activity arises due to the ability of these to delocalize unpaired electrons [49]. This is also responsible for the ability of carotenoids to physically quench singlet oxygen without degradation and for the chemical reactivity of carotenoids with free radicals. The efficacy of carotenoids for physical quenching is related to the number of conjugated double bonds present in the molecule.

Polyphenols

Phenolic compounds or polyphenols are ubiquitous in plants with more than 8000 structures reported [50]. Flavonoids, the most important single polyphenol group, are glycosides with a benzopyrone nucleus. The flavonoids including flavones, flavonols, flavanones, flavanonols, and anthocyanins. The flavones have a double bond between C2 and C3, whereas the flavanones have a saturated C2–C3. Flavononols have an additional hydroxyl group at the C3 position, and flavanonols are saturated between C2 and C3 with a hydroxyl group at the C3 position. The most ubiquitous flavonoid is quercetin, 3, 5, 7, 3', 4'–pentahydroxy flavone. Each flavonoid group is different, depending on the number of hydroxyl, methoxyl, and other substituents on the 2 benzene rings.

Antioxidant mechanisms of polyphenolic compounds are based on hydrogen donation abilities and chelating metal ions [50]. After donating a hydrogen atom, phenolic compounds become resonance-stabilized radicals, which do not easily participate in other radical reactions. However, phenolic compounds act as prooxidants under certain conditions, such as high concentrations of phenolic compounds or metal ions, and high pH. Chemical structures also affect the antioxidant activities.

Thiol antioxidants

The major thiol antioxidant is the tripeptide glutathione (GSH), which is a multifunctional intracellular antioxidant and is considered to be the major thiol-disulphide redox buffer of the cell [51]. It is abundant in cytosol, nuclei, and mitochondria, and is the major soluble antioxidant in these cell compartments [51]. The reduced form of glutathione is GSH, glutathione, whilst the oxidized form is GSSG, glutathione disulphide. The antioxidant capacity of thiol compounds is due to the sulphur atom, which can easily accommodate the loss of a single electron [52]. Oxidized glutathione (GSSG) is accumulated inside the cells and the ratio of GSH/GSSG is a good measure of oxidative stress of an organism [53]. The main protective roles of glutathione against oxidative stress are that it can act as a co-factor for several detoxifying enzymes, participate in amino acid transport across plasma membrane, scavenge hydroxyl radical and singlet oxygen directly, and regenerate Vitamins C and E back to their active forms [51].

Another thiol antioxidant is the thioredoxin (TRX) system; these are proteins with oxidoreductase activity and are ubiquitous in both mammalian and prokaryotic cells [54]. It also contains a disulphide and possesses two redox-active cysteins within a conserved active site (Cys-Gly-Pro-Cys) [55]. Thioredoxin contains two adjacent –SH groups in its reduced form that are converted to a disulphide unit in oxidized TRX when it undergoes redox reactions with multiple proteins. Thioredoxin levels are much less than GSH, however, TRX and GSH may have overlapping as well as compartmentalized functions in the activation and regulation of transcription factors [56].

Lipoic acid, and dihydrolipoic acid, have shown antioxidant activities. The chemical structure of lipoic acid is 1,2–dithilane–3–pentanoic acid. Lipoic acid is present in meat, liver, and heart [57]. Lipoic acids can prevent oxidative damages of proteins. Antioxidant activity of lipoic acid can help to reduce diabetic late complication, which can be developed through oxidative stress. Lipoic acid plays an important role in reducing blood glucose concentration. Lipoic acid regenerates GSH in liver, kidney, and lung tissue and also regenerates vitamins C and E. A dietary study of lipoic acid showed a decrease in age-related decline in oxygen consumption and radical formation, improvement of mitochondrial membrane potential, and increases of ascorbic acid and GSH levels [58]. Lipoic acid may improve age-related decline in memory and cognitive function and brain related ailments, including Alzheimer's disease and Parkinson's disease [59].

Reduced (dihydrolipoic acid) and oxidized forms of lipoic acid both act as antioxidants and scavenge reactive oxygen species. Lipoic acids are excellent antioxidants, showing abilities for radical scavenging, metal chelating, interaction with other antioxidants, metabolic regeneration, and gene regulation [57].

The standard 1-reduction potential of lipoic acid/dihydroxy lipoic acid is –320 mV, which is significantly lower than that of GSSG/GSH and dehydroascorbic acid/ascorbic acid, 250 and 282 mV, respectively.

Bilirubin

Biliverdin and bilirubin are powerful scavengers of different oxidants in vitro, although several factors need to be considered when attempting to extrapolate this activity to a potential *in vivo* antioxidant property of the pigments [60]. In mammals, for example, biliverdin does not usually accumulate to an appreciable concentration because of its rapid conversion to bilirubin by biliverdin reductase. For this reason, the following discussion will be limited to bilirubin. In human plasma, normal bilirubin concentrations are 5–20 mM, and essentially all of the pigment is bound by albumin. Albumin bound bilirubin can synergize with lipoprotein associated a-tocopherol and, by doing so, effectively inhibit LDL lipidoxidation, particularly if the latter process is caused by lipid soluble radical oxidants [61]. Bilirubin is also an effective inhibitor of protein oxidation [62].

Melatonin (N-acetyl-5-methoxytryptamine)

This is an indoleamine neurohormone that is synthesized mainly in the pineal gland and has many effects on a wide range of physiopathological functions. One major function of melatonin is to scavenge free radicals in oxygen metabolism, thereby potentially protecting against free radical-induced damage to DNA, proteins and membranes, thus it has the potential to play an important role in the reduction of free radical mediated diseases [63].

COENZYME Q

CoQ or ubiquinone is a redox-active and lipophilic substance present in most cellular membranes. It consists of a quinone head attached to a chain of isoprene units numbering 9 or 10 (CoQ9 or CoQ10) in different mammal species. CoQ10 has a fundamental role in cellular bioenergetics as a co-factor in the mitochondrial electron transport chain (respiratory chain) and is therefore essential for the production of ATP [64]. CoQ10 functions as a mobile redox agent shuttling electrons and protons in the electron transport chain. CoQ is highly efficient in preventing lipid, protein and DNA oxidation and it is continuously regenerated by intracellular reduction systems. CoQ10 in its reduced form as the hydroquinone (ubiquinol) is a potent lipophilic antioxidant and is capable of recycling and regenerating other antioxidants such as tocopherol and ascorbate [64, 65]. In some pathologic processes when tissue CoQ content is decreased it may be advantageous to supplement CoQ by dietary administration.

CONCLUSION

Biological systems are under a continuous influence of oxidative stress because of excessive generation of ROS. Although biological systems are affected in different ways by OS, there are sufficient antioxidant protections that can decrease the progression of the damage. However, when an imbalance exists between levels of ROS and the natural antioxidant defenses, various measures can be used to protect humans against the OS-induced injury. Diet forms an important component of the antioxidant protection system; it supplies the major antioxidants such vitamin C, vitamin E, and carotenoids. Therefore, food rich in these elements should form a part of the daily diet. For those patients who are suspected to have high levels of ROS, antioxidant supplements can be considered. Nevertheless, further studies are required to validate their use in this group of patients. In certain cases, it is also essential to modify certain lifestyle behaviors because many habits and environmental factors increase the production of ROS and affect the body.

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