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Free Radicals and Naturally Occurring Antioxidants

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Review Article

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ABSTRACT

Molecules in which one electron is unpaired or alone in its orbital are called free radicals. They have diverse roles to play in the life of organisms as a majority of the disease and disorders are mainly due to the imbalance between pro-oxidation and anti-oxidation. Free radicals that may cause damage or dysfunction in the living system could presumably be prevented from exerting their harmful effects. All organisms possess efficient antioxidant defense mechanism to scavenge the free radicals and protect themselves from destructive reactions. A regulated balance between ROS production and their destruction is required to maintain metabolic efficiency and functions under both optimal and stress conditions. The defense strategies of the antioxidants are always in high alert to scavenge or nullify the effect of ROS. Different antioxidants occur naturally in the body of organisms. The present review deals with free radicals and the natural antioxidant involved in the scavenging process.

INTRODUCTION

Antioxidants and reactive oxygen species have diverse roles to play in the life of organisms. It has been realized that a majority of the disease and disorders are mainly due to the imbalance between pro-oxidation and anti-oxidation homeostatic phenomenon in the body. Pro-oxidation conditions dominate either due to increased generation of free radicals and/or their poor quenching/scavenging into the body [1-3].

Oxygen is vital for aerobic processes. However, about 5% or more of the inhaled oxygen is converted to ROS i.e. reactive oxygen species [4]. Under normal conditions the ROS are balanced by the efficient antioxidant system of the body, but, when the balance is lost oxidative stress is produced, which through a series of events disrupts the cellular functions causing various physiological disfunctioning, like cardiovascular disfunctioning, cancer, neurological and other various diseases [5]. Antioxidants have the ability of protecting organismsfrom damage caused by free radical-induced oxidativestress [6].

The present review deals withfree radicals and the natural antioxidant involved in the scavenging process.

What are free radicals?

Stable molecules usually have paired electrons. However, there are some molecules in which one electron is unpaired or alone in its orbital, the species are called free radicals. Free radicals are produced whenever a covalent single bond between two atoms is cleaved in such a way as to leave at least one electron in an unpaired state. Such reactions are referred as homolysis [7]. They are designated by a single dot convention to indicate the unpaired electrons. Common examples of simple free radicals are chlorine atom, hydroxyl radical, superoxide anion, hydrogen peroxide, ground state oxygen molecule etc.

Consequences of generation of free radicals

Living things have colonized a wide range of habitats whose oxygen concentrations vary over the full range possible for the

earth's surface and near surface environments. Therefore, evolution has provided organisms with a range of defense mechanisms for existing in the hazardous environment. However, the defense systems are not perfect and damage to various constituents of the cell constantly occur, as well as accumulate during aging as the mechanism of damage control become less and less efficient [7]. Under such a condition free radicals attack vital cell components like polyunsaturated fatty acids, proteins and nucleic acids causing immense damage like altering the fluid mobility, ion transport, loss of enzyme activity, protein cross-linking, inhibition of protein synthesis, DNA damage etc. leading to cell death [4,8].

Lipid peroxidation

Lipids are highly reduced molecules whose structures all prominently feature aliphatic hydrocarbon moieties in some form or other [9]. Oxygen radicals catalyze the oxidative modification of lipids. The presence of double bond adjacent to a methylene group makes the methylene C-H bonds of polyunsaturated fatty acids (PUFA) weaker and therefore the hydrogen bonds more prone to abstraction [4]. Lipid peroxidations are initiated by peroxy radicals [10]. So, lipid peroxidation is a self perpetuating process since peroxy radicals are both reaction initiators as well as products of lipid peroxidation. Lipid peroxy radicals react with other lipids, proteins and nucleic acids; bring about the oxidation of the substrate by transfer of electrons [4]. The products formed by subsequent reactions are alcohols, hydroperoxides, ketones, epoxidesetc. Each of the compounds can undergo further reactions, leading to an increasingly complex mixture of products [7]. Cross-linking also occurs, either between lipid molecules or between lipid and proteins; and chain scissions of polypeptides also may result [11].Lipid peroxidation leads to membrane damage by altering the geometry of the alkyl chains and thereby disrupting the lipid bi-layer [7].

DNA damage

The DNA damage by free radicals has been the most extensively studied reaction. It has been found that both nuclear and mitochondrial DNA is attacked by free radicals. Among the free radicals generated in the cell, particularly hydroxyl radicals formed by radiolysis of water have been found to be most potential [12]. The main targets of oxidative damage of DNA chain include the purine and pyrimidine bases as well as the deoxyribose sugar moieties [7]. The nature of damage includes mainly base modifications, deoxyribose oxidation, strand breakage and DNA-protein cross-linking. The consequences of DNA damage include various mutagenic alterations of the molecule and interference of cell signaling resulting alterations in gene expressions.

Oxidative damage of protein

The amino acids that make up proteins are in general, more oxidized than lipids. They contain a highly oxidized carboxyl group and partially oxidized amino group. Several amino acids that occur in proteins have been shown to especially susceptible to oxidative damage. Whether the oxidant is ozone, hydroxyl radical or singlet oxygen, the most reactive species appears to be cysteine, histidine, tryptophan, methionine and phenylalanine [7,13-15]. Protein damage may occur independently or in conjugation with lipid damage. Peroxidizing lipid may damage proteins that are associated with them in membranes. It is also possible that protein damage could occur even in an environment where lipid peroxidation was fully protected [16].

Free radicals produced during mitochondrial electron transport chain stimulate protein degradation. Oxidative protein damage may be brought about by metabolic processes that degrade a damaged protein to promote synthesis of a new protein. In the process of cataractogenesis, oxidative modifications play a significant role in cross-linking of protein, leading to high molecular weight aggregates, loss of solubility and opacity [17]. The consequence of these events may include loss of enzyme activity, cytolysis and even cell death [18].

Autooxidation of carbohydrates

Carbohydrate autooxidation are usually initiated by electron oxidants such as transition metal ions (Cu^{++}or Fe^{+++}) or oxidizing free radicals(HO· or RO·) [19]. Intracellular reactions of carbohydrates are usually linked to metal ion promoted oxidation involving iron and copper. Stabilized free radical intermediates or oxidized products of sugars, including various dicarbonyl intermediates, react with and damage proteins by cross-linking and condensation reactions [20]

Autooxidation of vitamins

A few vitamins like C and E are well known antioxidants. Others like folic acid are degraded in the gastrointestinal tract under the acidic condition of stomach. Vitamin D is susceptible to photodecomposition, especially in the presence of photosensitizers, leading to an endoperoxide [21]. Ascorbate is able to provide significant protection against decomposition [22].

TYPES OF ANTIOXIDANTS

Free radicals natural by-products of metabolism. They are formed during normal metabolic processes involving energy transfer. Whenever, molecular oxygen is present in a system where free electrons are being formed, superoxides are generated [1,4,7,23].

Free radicals that may cause damage or dysfunction in either living or nonliving systems could presumably be prevented from exerting their harmful effects by several means. Either physical or chemical techniques could, in principle be employed to limit the potential damage.

Preventive antioxidation

Living organisms employ several such strategies. One approach is simply to avoid oxygen altogether. Many microorganisms live in environments that are either totally anoxic or limited in oxygen concentration. Other life forms eschew sunlight and occupy permanently dark environments, such as the ocean depths, subsurface layers of the soil, or caves. The surface of many animals that do live in presence of sunlight and oxygen are either dark in colour or highly reflective, or both, presumably at least in part because of the potentially toxic effects of light [1,4,7,23].

Chemical antioxidation

The most common and useful approach used is preventing damage to autooxidizable materials is to incorporate chemical additives in the formulation to deactivate the species that initiate or promote destructive oxidation reactions. Autooxidation reactions are normally initiated by species capable of producing free radicals, which then undergo rapid subsequent reactions with molecular oxygen leading to damage. Protective additives may be light absorbing compounds, metal ion complexing agents, free radical scavengers, peroxide destroying compounds or singlet oxygen quenchers [7].

Free radicals that may cause damage or dysfunction in the living system could presumably be prevented from exerting their harmful effects by several means. Both physical and chemical techniques could be employed to limit the potential damage [7]. All organisms possess efficient antioxidant defense mechanism to scavenge the ROS and protect themselves from destructive reactions. A regulated balance between ROS production and their destruction is required to maintain metabolic efficiency and functions under both optimal and stress conditions. The defense strategies of the antioxidants are always in high alert to scavenge or nullify the effect of ROS.

Naturally occurring antioxidants

The different antioxidants occurring naturally in the body of organisms are described below:

Alkaloids and related compounds: Alkaloids constitutes a wide variety of nitrogenous compounds. They are usually, but not always, of plant origin, heterocyclic and basic. A possible antioxidant role of alkaloids and related nitrogenous compounds could be as quenchers of singlet oxygen. Polyamines such as spermine, spermidine and putrescine have been shown to accumulate in some plants exposed to elevated levels of UV [24]. Boldine, an aporphine derivative, is the principle alkaloid found in the bark of the leaves of Peumusboldo, was found to inhibit autooxidation is several biological system [7]. Besides these several other derivatives are associated with antioxidant activity.

Amino acids and peptide derivatives

Amino acids have been variously reported to act as antioxidant. The indole containing aminoacids and derivatives like tryptophan, melatonin, and tryptamine are known to have antioxidant activities in some systems [25,26].

Beta carotene

The carotenes are a class of terpenoid hydrocarbons found in almost all higher plants. The most abundant of this hydrocarbons beta carotene has a structure featuring two substituted cyclohexene rings linked by a 22 carbon polyene chain. It is almost entirely insoluble in water but readily soluble in hydrophobic environments and non polar solvents [7].Carotenoids particularly beta carotene scavenge free radicals under some conditions. Many studies have demonstrated that beta carotene inhibits autooxidation of lipids in biological tissues and in food products. Peroxy radicals in particular have been shown either to add to long the chain of conjugated double bonds present in beta carotene and other carotenoids, or to take part in electron transfer reactions giving rise to carbon-centered beta carbonyl free radicals [27].

Carnosine: It is sulphur containing peptide that has been suggested to possess antioxidant activity. It is found in muscle tissue at levels from 1-60mM. Physiologistshave believed that carnosines present at such high levels could be related to its buffer activity; however, it also appears to be a potential antioxidant. Addition of carnosine to meat products, leads to greatly improved storage, stability due to inhibition of lipid oxidation27.Several investigators have shown that carnosine has relatively high activity with hydroxyl peroxide and other free radicals [28].

Chalcones and catechins: Chalcones are natural polyphenolic precursors of flavonoids. They occur in plants and have shown antioxidant activity in several investigations. Butein has shown surprisingly high activity of antioxidant activity [29]. Catechins or flavans, a tricyclic polyphenol related to flavonoids and condensed tannins from tea is a potential anticancer agent [30-33]. Epicatechin, a relative of catechin were found to be similar in antioxidant activity.

Curcumin and derivatives: The rhizomes of tropical gingers and turmeric are rich in *curcumins*, their derivatives and other potential antioxidants. They have the ability to scavenge almost all free radicals generated in the cell [34]. *Curcumin* is a very interesting substance because it generates phototoxic oxidizing species, including HO· and H_2O_2, when exposed to light, but it also protects lipid peroxidation as a radical scavenger [34-36].

Ergothioneine: The aminoacid derivative ergothionine is a major sulphur containing constituent of some fungi. Itis not by

mammals but when ingested it is assimilates and its concentration maintained to the point where it can approach concentrations in the liver, bone marrow, erythrocytes, and other tissues. Evidence suggests that is likely to provide protection against several varieties of oxidative stress like lipid peroxidation.

Flavonoids: Flavonoids represent a large and diverse group of phenolic compounds derived from higher plants. Derivatives of flavone display a wide range of substitution patterns and oxidation states including flavonols, flavonones, flavans or catechins [7]. The compounds appear to possess a variety of mechanisms of action which include free radical scavenging and metal ion complexation. The reaction of flavonoid derivatives with superoxide has been thoroughly investigated. Reactions of flavonoids with singlet oxygen have been studied. It has been found that favonols such as quercetin and fisetin, which quench singlet oxygen by chemical reaction, were generally more reactive than those of other types such as flavones [33]. Anthocyanins are cationic polyphenols and are considered as a class of flavonoids. Several researchers have found positive responses regarding the antioxidant activity of this group. Polyphenols, occur naturally in many fruits, account for the majority of antioxidant activity [37]. However, polyphenols can undergovarious reactions in the course of food processing and storage, which affect their stability [38]. Atpresent, the probable toxicity of synthetic antioxidants has been condemned and thus there is a shift towards the use of natural antioxidants [39]. It is strongly believed that regular consumption of plant-derived phytochemicals may drift the balance towards an adequate antioxidant status in the body [40].

Free phenolic acids: Metabolites of the shikimic acid pathway, and in particular compounds derived from the C_6-C_3phenylpropanoid unit, are virtually universal in plant tissues and are especially abundant in seeds and barks. The basic structural unit undergoes many alterations in the biosynthesis of phenylalanine, tyrosine, tannins, flavonoids, lignin and lignins. In plants, the free phenolic acids occur as substituted benzoic and cinnamic types. Most tests of the antioxidant effectiveness of these compounds have shown that the cinnamic derivatives are superior than benzoic derivatives [41]. Antioxidant activities of caffeic and ferulic acids are also positive in their responses [42,43].

Glutathione: The cysteine containing tripeptide glutathione is one of the most important biological antioxidant. It occurs in high concentrations in the cytosol of many types of cells including human blood plasma. In addition is also present in organelles like chloroplasts. It is the key component of a variety of cellular mechanisms including detoxification of foreign metabolites, maintenance of growth rates and protection against gamma radiation damage [44,45]. Glutathioneis poorly absorbed if ingested, and most animals synthesize it within their body. The antioxidant biochemistry of glutathione has been summarized [46]. Like other antioxidants it is readily oxidized, thiols such as GSH, in particular react rapidly with many one-electron oxidants to form thiyl radicals.

Hydroquinones and quinines: Arbutin is a simple naturally occurring hydroquinone derivative found in some plants and has shown to have antioxidant property. Ubiquinol, another derivative of hydroquinone is found in heart, kidney and liver. The compound is a good inhibitor of free radical generators and scavenger of the same in lipid systems [7]. Quinines are known to react with superoxide to remove them [47]. Ubiquinone is able to react readily with peroxy radicals generated from lipids [48]and singlet oxygen [45].

Isoflavonoids: Isoflavonoids are of restricted distribution in plant kingdom. In fact, only one family Leguminosae commonly contains them. It is less effective than the flavonoids in its antioxidant activity. Genistein isolated from soyabean has been reported to inhibit the activities of a number of enzymes, and also to promote the synthesis of antioxidant enzymes like catalase [49].

Lignans: C_6-C_3 dimers of varying degrees of complexity have been found to be antioxidants. Their activity tends to be correlated with the number of phenoxy or alkoxy substituents in the compound7. Kadsurin, isolates from Kadsuraheteroclita was found to inhibit lipid peroxidation [50].

Lipoic acid: This compound is synthesized from linoleic acid and occurs naturally in many organisms like micro organisms, plants and animals. It acts as an important coenzyme and growth factor7. It sometimes occurs as amide derivative. It is a potential antioxidant, so it is used in liver disorder and as an antidote for poisoning.

Ovothiol: The non protein aminoacid ovothiol is a thiol derivative of histidine that is found in marine animals and parasitic protozoa [51].The combinations of readily oxidized functional groups in this compound make it an extremely effective antioxidant.

Renitol and derivatives: They share many of the structural features of carotenoids and the assumption has been that they could also exhibit antioxidant activities [7].

Tetrapyrroles: Bilirubin is a linear tetrapyrrolic bile pigment found in blood inhibits a number of free-radical induced oxidation reactions, probably because of its reactivity with peroxy radicals [52]. They are also well known sensitizers of singlet oxygen formation and highly effective physical quenchers. It also reacts with superoxide [47] Chlorophyll-a, chlorophyll-b and their related compounds are considered as potential antioxidants, of which Chlorophyll-a, chlorophyll-b shows maximum scavenging potential.

Uric acid and other purines: Uric acid is the most-studied and apparently the most active antioxidant having a purine structure [7]. Uric acid occurs in high concentrations in excretory products of many animals and was considered a total waste product with no biological functions. But researchers have found that uric acid is an effective antioxidant in biological systems containing DNA and lipids [53-55]. Uric acid is a potential scavenger of Hydroxyl and peroxy radical [56,57]. It is also effective in reducing blood plasma concentration of ozone [58].

Vitamin C: Vitamin C is found in some fruits, aqueous fractions of animal tissues including the spinal cord, lung, eye, blood plasma etc. Although most organisms are able to synthesize it, a few have to obtain it in their diets. Ascorbic acid is a potential antioxidant against hydroxyl radical, peroxy radical and singlet oxygen [59,60]. However, the reaction efficiency of ascorbate is partially ameliorated by the ability of it to produce superoxide upon its own oxidation by molecular oxygen [7].

Vitamin E and related compounds: Tocopherols and related substances are compounds found in high concentrations in certain vegetable oils, grains and other plant products and at much lower concentrations in animal tissues. This most potent antioxidant agent is very important to maintain cell membrane and other cell parts. The concentration of vitamin E, even if low is adequate to prevent most instances of autooxidative damage in normally functioning cells. There are several isomers of tocopherol, of which α, β, γ and δ are most abundant. The order of antioxidant activity among the tocopherols is ordinarily α→β→γ→δ. Vitamin E analogues like prunusols A and B are also good antioxidants [61].

CONCLUSION

With increase in the impetus on research in medical science and more particularly in finding remedies by plants, studies in antioxidants have been increased tremendously. Medicinal plants are now not only discussed by herbalists but chemists are more attracted to their chemical constituents. This bridge between herbalists and chemists has open up new dimensions in phytochemistry, for which antioxidants have become more relevant with new sources of antioxidants being discovered almost every day.

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