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Oxidative Stress and Antioxidant Defense in Cells

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ABSTRACT

Oxidative stress is induced by the reactive oxygen species (ROS) produced in the cells as a result of environmental factors such as air pollutants, heavy metals etc. Reactive oxygen species are highly reactive and can damage cell structures. The main cellular components susceptible to damage by the reactive oxygen species are lipids, proteins, carbohydrates and nucleic acids. The formation of reactive oxygen species is prevented by the cellular antioxidant system which includes ROS- interacting enzymes such as Superoxide Dismutase, Catalase, Glutathione Peroxidase etc. and non- enzymatic antioxidant such as Ascorbic Acid, Tocopherol, Carotene, Glutathione etc. The antioxidants tend to maintain the redox state of the cell which is critical for the normal functioning of the cells.

KEYWORDS : Reactive Oxygen Species, Damage, Antioxidant System, Maintain, Redox State

Introduction

Generation of Reactive Oxygen Species (ROS) is an inevitable part of the normal cellular metabolism of living beings. Beside this, certain environmental stresses like air pollutants, UV-B radiations etc. also contributes towards the ROS generation in the different subcellular compartments. At low to moderate concentrations, the ROS assist in different physiological processes going on in the cells, but at high concentrations, they produce adverse modifications in cellular components such as lipids, proteins, DNA etc [1, 2]. Aerobic organisms have integrated antioxidant systems, which include enzymatic and non enzymatic antioxidants that are usually effective in blocking harmful effects of ROS. Under normal circumstances, the concentration of ROS and antioxidants in the cell maintain equilibrium to ensure the normal metabolic functioning of cells. However, excess ROS production may lead to a physiological stress condition which is technically termed as "Oxidative Stress". Oxidative stress is responsible for many pathological conditions, including cancer, neurological disorders [3], atherosclerosis, hypertension, ischemia/perfusion [4], diabetes, acute respiratory distress syndrome, idiopathic pulmonary fibrosis, chronic obstructive pulmonary disease and asthma [5]. This review focuses upon the endogenous and exogenous sources of ROS, their effects upon the different various cellular macromolecules and the antioxidant defense systems of the cell.

Sources of ROS

Along with the natural production of ROS in different cellular compartments, ROS generation is also influenced by certain environmental factors that bring about diversion in the normal cellular metabolism. These factors are termed as stress factors. Therefore two sources of ROS can be defined, endogenous and exogenous, which are discussed as under.

Endogenous sources of ROS

ROS production from molecular oxygen is inevitable and is a result of normal cellular metabolism. The complete reduction of oxygen to H_2O requires 4 steps and the generation of several free radicals and H_2O_2. The complete reduction of oxygen is summarized in the following equations:

$O_2 + e^- \rightarrow O_2^{\cdot-}$ superoxide radical [6]

$O_2^{\cdot-} + H_2O \rightarrow HO_2^{\cdot} + OH^-$ hydroperoxyl radical [7]

$HO_2^{\cdot} + e^- + H \rightarrow H_2O_2$ hydrogen peroxide [8]

$H_2O_2 + e^- \rightarrow \cdot OH + OH^-$ hydroxyl radical [9]

The important ROS formed during these serious of reactions are Superoxide radicals ($O_2^{\cdot-}$), Hydroperoxyl radicals ($HO_2^{\cdot}$), Hydroxyl radicals (•OH) and Hydrogen peroxide (H_2O_2). These oxygen-derived intermediates are highly reactive because of their unstable electron configurations which permit for the attraction of electrons from other molecules, resulting in production of another free radical, capable of reacting with yet another molecule. This chain reaction is thought to contribute to lipid peroxidation[10], DNA damage[11], and protein degradation[12] during oxidative stress.

$O_2^{\cdot-}$ is formed by the addition of 1 electron to the molecular oxygen [6]. This process is mediated by nicotine adenine dinucleotide phosphate [NAD(P)H] oxidase or xanthine oxidase or by mitochondrial electron transport system. The major site for producing superoxide radical is the mitochondria, the machinery of the cell to produce adenosine triphosphate. Normally, electrons are transferred through mitochondrial electron transport chain for reduction of oxygen to water, but approximately 1 to 3% of all electrons leak from the system and produce superoxide. The superoxide radical, unlike the other oxygen derived intermediates, can lead to the formation of additional reactive species [13]. In particular, the protonation of $O_2^{\cdot-}$ results in the formation of perhydroxyl radical $HO_2^{\cdot}$, a much stronger radical than $O_2^{\cdot-}$. $O_2^{\cdot-}$ also acts as a Bronsted base in aqueous solutions to shift the acid-base equilibrium to form a hydroperoxyl radical, thereby forming H_2O_2 in acidic environments [14]. Superoxide dismutase catalyzes the dismutation of the superoxide radical at neutral or acidic pH [14].

H_2O_2 although not a free radical by definition, is considered as an important ROS because of its ability to generate the hydroxyl radical, whose oxidative efficiency is much higher [15]. Further, because of its nonionized and low charged state, H_2O_2 is able to diffuse through hydrophobic membranes, as seen with the leakage of H_2O_2 from mitochondria [14]. Hydrogen peroxide is also produced by xanthine oxidase, amino acid oxidase, and NAD(P)H oxidase [16] and in peroxisomes by consumption of molecular oxygen in metabolic reactions. In a succession of reactions called Haber–Weiss and Fenton reactions, H_2O_2 can breakdown to •OH in the presence of Fe^{2+} or Cu^{2+} [17]. $O_2^{\cdot-}$ can also react with H_2O_2 and generate OH^- [18]. The ability of the hydroxyl radical to remove or add hydrogen molecules to unsaturated hydrogen bonds of organic lipids makes it potentially one of the most reactive oxidants in biological systems. Its very short half-life ($1x10^{-9}$ at 37°C), however, restricts its diffusion capability and its potency [19].

Exogenous sources of ROS

1. Ozone exposure:

One of the most important consequences of ozone exposure is the increased membrane lipid peroxidation which results in enhanced ROS generation. Membranes are believed to be the initial sites of ozone injury [20]. Any change in the membrane leads to some membrane leakage [21], or shifts in signal transduction proteins within the membrane [22, 23]. PUFA, the main components of membrane lipids are susceptible to peroxidation [24]. Short-term exposure to ozone also causes the release of inflammatory mediators, such as MPO, eosinophil cationic proteins and also lactate dehydrogenase and albumin [25]. Even in healthy subjects, ozone exposure causes a reduction in pulmonary functions [26].

2. Hyperoxia:

Hyperoxia refers to conditions of higher oxygen levels than normal partial pressure of oxygen in the lungs or other body tissues. It leads to greater production of reactive oxygen and nitrogen species [27].

3. Ionizing radiations:
Ionizing radiation, in the presence of O_2, converts hydroxyl radical, superoxide, and organic radicals to hydrogen peroxide and organic hydroperoxides. These hydroperoxide species react with redox active metal ions, such as Fe and Cu, via Fenton reactions and thus induce oxidative stress [28]. Narayanan et al. [29] showed that fibroblasts that were exposed to alpha particles had significant increases in intracellular $O_2^{\cdot-}$ and H_2O_2 production via plasma membrane-bound NADPH oxidase [29]. After exposure to ionizing radiation, intracellular level of glutathione (GSH) decreases for a short term but then increases again [30].

4. Heavy metal ions:
Heavy metal ions, such as iron, copper, cadmium, mercury, nickel, lead, and arsenic, can stimulate generation of reactive radicals and cause cellular damage via diminution of enzyme activities through lipid peroxidation and reaction with nuclear proteins and DNA [31]. One of the most important mechanisms of metal mediated free radical generation is via a Fenton-type reaction. Superoxide ion and hydrogen peroxide can interact with transition metals, such as iron and copper to form OH radicals. Certain metal ions can react directly with cellular molecules to generate free radicals, such as thiol radicals, or induce cell signaling pathways. These radicals may also react with other thiol molecules to generate $O_2^{\cdot-}$. $O_2^{\cdot-}$ is converted to H_2O_2, which causes additional oxygen radical generation [32].

The effects of oxidative stress in cells
During oxidative stress, the excess of ROS generated interact with many cellular macromolecules such as lipids, DNA, proteins etc. bringing about certain modifications in their structure. These interactions include protein degradation, DNA strand breakage and damage to other genomic structures. These reactive species affect lipids which disturbs the homeostatic environment of the cell. The effect of oxidative stress on certain cellular components is discussed as under:

1. Oxidation of nucleic acid/DNA by ROS:
ROS break the DNA strands, forms DNA adduct which is characterized by deletion, mutation and causes genetic effects. Sugars and base moieties are degraded by ROS and causes oxidation of bases and cross linking to protein. Metals such as iron, cadmium, chrome, and arsenic, are also involved in DNA damage by generating free radicals or binding with thiol groups. Formation of 8-OH-G is the best known DNA damage occurring via oxidative stress and is a potential biomarker for carcinogenesis. 8-hydroxyguanine and hydroxyl methyl urea are the important products of oxidation of nucleic acid bases. Polyadenosine diphosphate ribose synthesis occurs in the nuclei due to DNA oxidation resulting in extensive depletion of cellular NADH pools in the cells. DNA-MDA adducts is the most characteristic feature of nucleic acid oxidation. Single-stranded DNA breaks caused by oxidant injury can easily be tolerated by cells; double-stranded DNA breaks induced by ionizing radiation can be a significant threat for the cell survival [33].

2. Protein oxidation by ROS:
Reactive oxygen species interacts on protein molecules at the specific amino acid side chain and form the modification in protein structure resulting in fragmentation of the peptide chain, alteration in electrical charges, increase in accumulation of peroxynitrite nitrate protein, thus increasing the proteolysis[34]. Cysteine and methionine residues in proteins are particularly more susceptible to oxidation [35]. Oxidation of sulfhydryl groups or methionine residues of proteins cause conformational changes, protein unfolding, and degradation [36]. Enzymes that have metals on or close to their active sites are especially more sensitive to metal catalyzed oxidation which inhibits their activities [37]. Garrison [38] has found that active oxygen has potential to react with amino acid side groups and cleaving the polypeptide chain, thus resulting in the formation of reactive carbonyl groups which acts as biomarkers of oxidative stress in proteins [39]. Gamma rays, metal-catalyzed oxidation, HOCl, and ozone can cause formation of carbonyl groups [40].

3. Carbohydrate oxidation by ROS:
Free carbon and hydrogen of deoxy sugars are attributed to the oxidation of carbohydrates, e.g. mannitol and glucose. The free radicals binds with these carbohydrates and forms carbon centered radicals. These carbon centered radicals interacts with other carbohydrates, and thus series of autocatalytic chain reaction commence resulting in the destruction of the cells. Ketoamines and ketoaldehydes are the most common oxidative products of carbohydrates [38].

4. Lipid peroxidation by ROS:
ROS can induce lipid peroxidation and disrupt the membrane lipid bilayer arrangement that may inactivate membrane-bound receptors and enzymes and increase tissue permeability [41]. Peroxidation of lipids is particularly more damaging because the formation of lipid peroxidation products leads to a simplistic proliferation of free radical reactions. The interaction between free radicals and lipids involves 3 processes: initiation, propagation, and termination. During initiation, conjugated dienes are formed through the abstraction of a hydrogen atom from a backbone methylene group of a lipid [42]. This allows for the interaction of molecular oxygen with carbon centered free radicals to form lipid hydroperoxides, also called propagation. The resultant decomposition of these lipid hydroperoxides produces alkoxyl or peroxyl radicals that continue the process of propagation [13]. Polyunsaturated fatty acids (such as those found in biological membranes) are particularly vulnerable to this process of initiation and propagation because of the multiple unsaturation points found along their backbone. Oxidative damage of membranes results in increased membrane fluidity, compromised integrity, and inactivation of membrane-bound receptors and enzymes [41].

Thus, a series of oxidation processes occurring in the cells detoxifies the cellular environment from the oxidants. The failure in the neutralization events of oxidative status results in oxidative stress in cells which leads to cell death by lipid peroxidation, carbohydrates oxidation and nucleic acid oxidation. Cells, however, posses an efficient defense system which serves to deactivate the oxidative stress produced as a result of certain environmental factors.

Cellular antioxidant system
Antioxidants are chemical compounds which contain monohydroxy/polyhydroxy phenol which work to slow down the lipid peroxidation [43]. Due to their low activation energy, they cannot initiate the formation of the second free radical. The human body is equipped with a variety of antioxidants that serve to balance the effect of the ROS produced under stress conditions. Antioxidant system contains endogenous and exogenous antioxidants.

➢ **Endogenous Antioxidants:**
It can be categorized into primary antioxidants and secondary antioxidants. SOD, Catalase and Glutathione peroxidase are the primary antioxidant enzymes which inactivate the ROS into intermediates [44]. Besides the antioxidant enzymes, primary antioxidants are water soluble and lipid soluble. Ascorbate, glutathione etc. are water soluble whereas tocopherols, ubiquinols and carotenoids, etc. are lipid soluble. Secondary antioxidants like Glutathione reductase, glutathione-S-transferase and ubiquinone work directly to detoxify ROS by decreasing the peroxides level and continuously supplying the NADPH and glutathione for primary antioxidant enzymes to maintain their proper functioning [43].

➢ **Exogenous Antioxidants:**
These are mainly derived from food and other dietary sources. Several herbs, spices, vitamins, vegetables etc. include antioxidative properties. Flavonoids, anthocyanins, lignans, catechins etc. are important antioxidants found in plants which form a part of dietary intake.

Antioxidants may be enzymatic and non- enzymatic. Enzymatic system directly/indirectly contributes to defense against ROS. Catalase (CAT), Superoxide Dismutase (SOD), Glutathione Peroxidase (GTPx), Glutathione Reductase (GTR), Thioredoxin (TRX) etc. demonstrates biological significance. Non enzymatic antioxidants are actually the scavengers of ROS and these include Glutathione, Ascorbic Acid, Tocopherol, Carotenoids, Bilirubin, Melatonin etc. The different antioxidants of the cellular defense system are tabulated in Table 1.

Enzymatic antioxidants
Since $O_2^{\cdot-}$ is the primary ROS produced its dismutation by SOD is of primary importance for each cell. Several common forms of SOD exist which are cofactored with copper and zinc, or manganese, iron, or nickel. Thus, there are three major families of superoxide dismutase, depending on the metal cofactor: Cu/Zn (which binds both copper and zinc), Fe and Mn types (which bind either iron or manganese),

and the Ni type, which binds nickel. Three forms of superoxide dismutase are present in humans, in all other mammals, and most chordates. SOD1 is located in the cytoplasm, SOD2 in the mitochondria, and SOD3 is extracellular. The first is a dimer (consists of two units), whereas the others are tetramers (four subunits). SOD1 and SOD3 contain copper and zinc, whereas SOD2, the mitochondrial enzyme, has manganese in its reactive centre. CuZn- SOD and Mn- SOD are generally thought to act as bulk scavengers of superoxide radicals.

H_2O_2 that is produced by the action of SODs or the action of oxidases, such as xanthine oxidase, is reduced to water by CAT and the GTPx. CAT exists as a tetramer composed of 4 identical monomers, each of which contains a heme group at the active site. Degradation of H_2O_2 is accomplished via the conversion between 2 conformations of catalase-ferricatalase (iron coordinated to water) and compound I (iron complexed with an oxygen atom). CAT also binds NADPH as a reducing equivalent to prevent oxidative inactivation of the enzyme (formation of compound II) by H_2O_2 as it is reduced to water [45].

Beside CAT, another enzyme responsible for the reduction of H_2O_2 and lipid hydroperoxides (generated as a result of membrane lipid peroxidation) includes the GTPxs [46]. The GTPxs are a family of tetrameric enzymes that contain the unique amino acid selenocysteine within the active sites and use low-molecular-weight thiols, such as GSH, to reduce H_2O_2 and lipid peroxides to their corresponding alcohols. Four types of GTPxs are described in the cells which are encoded by different genes. Cellular GTPx (GTPx-1) is ubiquitous and reduces H_2O_2 and fatty acid peroxides, but not esterified peroxyl lipids [47]. Esterified lipids are reduced by membrane-bound GTPx-4 (phospholipid hydroperoxide GTPx), which utilizes some low-molecular-weight thiols as reducing equivalents. GTPx-2 (gastrointestinal GTPx) is localized in gastrointestinal epithelial cells where it serves to reduce dietary peroxides [48]. GTPx-3 (extracellular GTPx) is different from the other members of GTPx family as it is localized in the extracellular compartment and is believed to be one of the most important extracellular antioxidant enzyme in mammals [49].

H_2O_2 degradation in the cells is also brought about by some thiol-containing enzymes such as thioredoxins (TRXs), thioredoxin reductases (TRRs), thioredoxin peroxidases (PRXs), and glutaredoxins [50]. Two TRXs and TRRs and six different PRXs have been found in human cells, differing in their ultrastructural compartmentalization [51].

Common to these antioxidants is the requirement of NADPH as a reducing equivalent. NADPH maintains CAT in the active form and is used as a cofactor by TRX and GSH reductase, which converts GSSG to GSH, a co-substrate for the GTPxs [52].

GSTs, another antioxidant enzyme, inactivate secondary metabolites, such as unsaturated aldehydes, epoxides, and hydroperoxides. Three major families of GSTs have been described: cytosolic GST, mitochondrial GST and membrane-associated microsomal GST [53].

Non enzymatic antioxidants

1. Ascorbic acid (Vitamin C):

Water-soluble vitamin C (ascorbic acid) is present in the aqueous phase in both intracellular and extracellular compartments and depicts its antioxidative nature primarily by scavenging oxygen free radicals. It converts Vitamin E free radicals back to active Vitamin E molecules [54].

2. Tocopherol (Vitamin E):

Lipid soluble Vitamin E is concentrated in the hydrophobic interior site of cell membrane. It protects against lipid peroxidation by acting directly with a variety of oxygen radicals including singlet oxygen, lipid peroxide products and superoxide radicals to form a relatively harmless tocopherol radical [55]. α-Tocopherol is the most active form of vitamin E and the major membrane-bound antioxidant in cell.

3. Carotenoids (β-carotene):

β-carotene, the major carotenoid precursor of Vitamin A is the most efficient "quencher" of singlet oxygen [56]. Primarily, b-carotene has been found to react with peroxyl (ROO•), hydroxyl (•OH), and superoxide ($O_2^{-\cdot}$) radicals [57]. Carotenoids show their antioxidant effects in low oxygen partial pressure but may have pro-oxidant effects at higher oxygen concentrations [58].

4. Glutathione:

GSH is oxidized to GSSG in cells in response to an increase in free radicals. GSSG efflux from cells into to the plasma is considered indicative of oxidative stress [7]. GSH shows its antioxidant nature in several other ways [59]. It detoxifies hydrogen peroxide and lipid peroxides via action of GTPx. GSH donates its electron to H_2O_2 to reduce it into H_2O and O_2. GSSG is again reduced into GSH by GSH reductase that uses NAD(P)H as the electron donor. GTPxs are also important for the protection of cell membrane from lipid peroxidation. Reduced glutathione donates protons to membrane lipids and protects them from oxidant attacks [59]. GSH also acts as a cofactor for several detoxifying enzymes, such as GTPx and GST. It has a role in converting vitamin C and E back to their active forms [59].

Conclusion

Production of ROS in plants is an important feature of life as these reactive metabolites play essential biological functions in a controlled environment. However, overproduction of these ROS disturbs the homeostasis of the cell and leads to a condition called oxidative stress. This state results in damage to several biological macromolecules in the cell, such as nucleic acids, proteins, carbohydrates, lipids etc. The antioxidants found in the cells serve to control the overproduced ROS thus tending to maintain the cellular redox balance. The antioxidants may be enzymatic or non enzymatic; produced within the cell (endogenous) or supplemented with the dietary intake (exogenous). The quenching ability of the cellular antioxidants keeps a check on the unusual ROS generation due to certain external stress conditions. Regulation of redox state is critical for cell viability, activation, proliferation and organ function. However, it is worth important to note that increased antioxidants may interrupt the normal biological oxidant processes, thereby inhibiting the ROS to perform their normal biological functions.

Table 1: Enzymatic and Non Enzymatic Scavengers of Antioxidant Defense

	Enzymatic Scavengers	
	Name	Acronym
1.	Superoxide dismutase	SOD
2.	Catalase	CAT
3.	Glutathione Peroxidase	GTPx
4.	Glutathione Reductase	GTR
5.	Glutathione Transferase	GST
6.	Thioredoxins	TRX
7.	Thioredoxin Reductases	TRRs
8.	Thioredoxin Peroxidases	PRXs
Non Enzymatic Scavengers		
	Name	Acronym
1.	Ascorbic Acid	Vitamin C
2.	α-Tocopherol	Vitamin A
3.	β-Carotene	-
4.	Glutathione	GSH

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