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Review Article

OXIDATIVE STRESS AND ANTIOXIDANT ENZYMES

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ABSTRACT

Antioxidants are free radical scavengers which provide protection against free radicals that causes various pathological conditions such as ischemia, inflammation and aging process. There are some enzymatic and some non-enzymatic antioxidants present in the body which provide protection against oxidative damages caused by reactive oxygen species. Some enzymes which provide protection against oxidative stress produced by imbalance between free radicals and ability of body to counteract or detoxify their harmful effects through neutralization by antioxidant are Glutathione, Glutathione Reductase and Glutathione peroxidase.

Keywords: Antioxidant, Antioxidant enzymes, Oxidative stress, Reactive oxygen species.

INTRODUCTION

Oxidation is a basic part of the aerobic life and of our metabolism. The potentially reactive derivatives of oxygen species, attributed as reactive oxygen species (ROS). Generation of free radicals or reactive oxygen species (ROS) during metabolism of eukaryotic cells, which involve mitochondrial electrons transport, microsomal P_{450} and other activities beyond the antioxidant capacity of a biological system, gives rise to oxidative stress (Gacche *et al.*, 2010). Free radicals reactive oxygen species and reactive nitrogen species are generated by our body by various endogenous systems, exposure to different physiochemical conditions or pathological states. A free radical can be defined as any molecular species capable of independent existence that contains an unpaired electron in an atomic orbital. The presence of an unpaired electron results in certain common properties that are shared by most radicals. Many radicals are unstable and highly reactive. They can either donate an electron to or accept an electron from other molecules, therefore behaving as oxidants or reductants (Lobo *et al.*, 2010). In normal cells, permanently produced oxygen derivatives are neutralized or eliminated due to the presence of a natural defensive mechanism that involves enzymatic antioxidants (glutathione peroxidases, superoxide dismutase, catalase) water or fat soluble non-enzymatic antioxidants (vitamins C and E, glutathione, selenium). Their interactions determine normal functioning of cells in the oxygen environment.

Under certain conditions, however, the intensity of ROS production either during cellular metabolism or under the influence of external stimuli may exceed the natural ability of cells to eliminate them from the organism. The disturbed balance leads to the state known as oxidative stress responsible for damaged DNA, proteins, and lipids. An inefficient repair mechanism may finally trigger the process of neoplastic transformation or cell necrosis (Stańczyk *et al.*, 2005).

BIOLOGICAL ROLE IN GENERATION OF REACTIVE OXYGEN SPECIES

Reactive oxygen species (ROS) are universal products of aerobic metabolism, which can be also produced in stress conditions. In eukaryotic cells, mitochondria are the main source of ROS. The main mitochondrial sites of ROS formation are electron carriers of respiratory chain. However, there are also other enzymatic sites capable of ROS generation in different mitochondrial compartments. Reactive oxygen species can cause serious damage to many biological macromolecules, such as proteins, lipids and nucleic acids, which oxidation leads to a lost of their biological properties and eventually to a cell death. Mitochondria, which are also exposed to harmful ROS action, have a defense system that decreases ROS production (first line of defense) or removes generated ROS (second line of defense). Mitochondrial antioxidant system involves proteins that decrease ROS formation, enzymes that directly react with ROS, and non-enzymatic antioxidants that also remove ROS and other oxygen derivatives. Mitochondrial ROS can also act as signal messengers and modify operation of many routes in different cell compartments. Mitochondrial ROS are also important in execution of programmed cell death (Czarna M *et al.*, 2006).

DAMAGES CAUSED BY REACTIVE OXYGEN SPECIES

The biological targets for these highly reactive oxygen species are DNA, RNA, proteins and lipids. Much of the damage is caused by hydroxyl radicals generated from H_2O_2 via the Fenton reaction, which requires iron (or another divalent metal ion, such as copper) and a source of reducing equivalents (possibly NADH) to regenerate the metal. Lipids are major targets during oxidative stress. Free radicals can attack directly polyunsaturated fatty acids in membranes and initiate lipid peroxidation. A primary effect of lipid peroxidation is a decrease in membrane fluidity, which alters membrane properties and can disrupt membrane-bound proteins significantly. This effect acts as an amplifier, more radicals are formed, and polyunsaturated fatty acids are degraded to a variety of products. Some of them, such as aldehydes, are very reactive and can damage molecules such as proteins (Cabiscol *et al.*, 1999).

ACTIVITIES OF ANTIOXIDANTS

Damage to proteins, DNA and lipids (more particularly to polyunsaturated fatty acids) may result in loss of function, conformational changes and the formation of cytotoxic low molecular mass breakdown products. To deal with the potential dangers of ROS, a number of antioxidant defences have arisen. Their function is to maintain low steady-state levels of ROS and other radicals in the cell, a process involving precise regulation of their location and amount. The enzymes superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), selenium-dependent glutathione peroxidase (GPox), selenium-independent glutathione peroxidase (GSTPox) and the glutathione-*S*transferases (GST) are recognized as a key interacting line of defence against ROS and their products of attack. SOD dismutates superoxide anions directly but in so doing produces potentially toxic hydrogen peroxide, H_2O_2 (Joanisse *et al.*, 1996).

GLUTATHIONE (GSH)

Glutathione (_-glutamyl-cysteinyl-glycine; GSH) is the most abundant low-molecular-weight thiol, and GSH/ glutathione disulfide is the major redox couple in animal cells. Glutathione plays important roles in antioxidant defense, nutrient metabolism, and regulation of cellular events (including gene expression, DNA and protein synthesis, cell proliferation and apoptosis, signal transduction, cytokine production and immune response, and protein glutathionylation). Glutathione deficiency contributes to oxidative stress, which plays a key role in aging and the pathogenesis of many diseases (including kwashiorkor, seizure, Alzheimer's disease, Parkinson's disease, liver disease, cystic fibrosis, sickle cell anemia, HIV, AIDS, cancer, heart attack, stroke, and diabetes). New knowledge of

the nutritional regulation of GSH metabolism is critical for the development of effective strategies to improve health and to treat these diseases (Guoyao *et al.*, 2003). Glutathione is synthesized and degraded in most cell types by a series of well characterized enzymatic reactions. It exists in both reduced form _usually abbreviated as GSH and an oxidized dimer GSSG form, the former by far the largest fraction in a ratio of approximately (98:2). GSH and GSSG are interconvertible by the action of two enzymes, GSH–Px and glutathione reductase (GSH–R). Reduced GSH strongly modulates the redox state (ratio of oxidizing to reducing equivalents) of the cell, a role which is critical for cell survival. GSSG is formed in antioxidant reactions that involve GSH, and can accumulate with increased oxidative processing in the cell. The ratio of GSSG/GSH serves as a sensitive index of oxidative stress. GSSG is reduced to regenerate GSH in a reaction catalyzed by GSH–R and requiring reduced nicotinamide adenine dinucleotide phosphate (NADPH) as a hydrogen donor (Bains *et al.*, 1997).

GSH SYNTHESIS

GSH synthesis requires two enzymatic steps involving ATP. Glutamate-cysteine ligase (GCL), also known as γ-glutamylcysteine synthetase, catalyzes the first, that is, rate-limiting enzymatic, step in GSH synthesis. GCL mediates the first reaction between glutamate and cysteine to form a dipeptide, γ-glutamylcysteine (γGluCys), which in turn reacts with glycine catalyzed by GSH synthetase (GS) to produce GSH. GSH regulates its own synthesis via feedback inhibition of GCL (Aoyama *et al.*, 2008).

ANTIOXIDANT FUNCTION OF GSH

All aerobic organisms are subject to a certain level of physiological oxidative stress from mitochondrial respiration. The intermediates that are formed, such as superoxide (O_2−•) and hydrogen peroxide (H_2O_2), can lead to the production of toxic oxygen radicals that can cause lipid peroxidation and cell injury. To prevent this, the endogenously produced hydrogen peroxide is reduced by GSH in the presence of selenium-dependent GSH peroxidase. In the process, GSH is oxidized to GSSG, which in turn is reduced back to GSH by GSSG reductase at the expense of NADPH, forming a redox cycle. Organic peroxides can be reduced by GSH peroxidase and GSH S-transferase. Hydrogen peroxide can also be reduced by catalase, which is present only in the peroxisome. In the mitochondria, GSH is particularly important because there is no catalase. Indeed, mitochondrial GSH is critical in defending against both physiologically and pathologically generated oxidative stress. Severe oxidative stress can overcome the ability of the cell to reduce GSSG to GSH leading to accumulation of GSSG. To protect the cell from a shift in the redox equilibrium, GSSG can be actively exported out of the cell or react with a protein sulfhydryl group leading to the formation of a mixed disulfide. Thus, severe oxidative stress depletes cellular GSH (Lu, 2009).

GLUTATHIONE REDUCTASE (GR)

Glutathione reductase is a ubiquitous FAD containing enzyme. It catalyzes the reaction

$$H^+ + NADPH + E \rightleftharpoons NADP^+ + EH_2$$

$$EH_2 + GSSG \rightleftharpoons 2GSH$$

where the intermediate EHZ is the stable 2-electron reduced form of the enzyme. The function of the enzyme is to keep the cellular concentration of the reduced form of glutathione (GSH) high and that of its oxidized form (GSSG) low. The enzyme uses NADPH as a source of reduction equivalents. Its substrate GSSG as well as its product GSH is important for a broad range of cellular functions, *e.g.* cell division, amino acid transport through membranes regulation of enzymatic activity, damage repair, drug metabolism and detoxication (Pai *et al.*, 1982).

It is specific for oxidised glutathione (GSSG) but shows a low activity with NADH as electron donor. The pH optimum for NADH-dependent GSSG reduction is lower than that for NADPH-dependent reduction. The enzyme has a low affinity for reduced glutathione (GSH) and for $NADP^+$, but GSH-dependent $NADP^+$ reduction is stimulated by addition of dithiothreitol (Halliwell *et al.*, 1978).

REGULATION OF GR DURING STRESS

The molecular biology and biochemistry of GR, it is clear that regulation of expression of GR in different organisms under stress could occur at a number of levels. These are the regulation of enzyme activity, the regulation of the amounts of individual GRs in a population of isoforms and of posttranslational processes, the steady-state levels of the enzyme and its transcripts, and the regulation of transcription of the GR gene(s) (Mullineaux, 2009). It has been known that GSH is present in almost all organisms and serves as a reductant in numerous biochemical reactions, including counteraction of oxidative events and protection of the thiol groups of intracellular proteins. Accordingly, the reduction of GSSG is of fundamental importance in the metabolic function of GSH. Glutathione reductase catalyses the reduction of GSSG by NADPH (Shigeoka *et al.*, 1987).

GLUTATHIONE PEROXIDASE (GPx)

Glutathione peroxidase (GSHPx) is a critical intracellular enzyme involved in detoxification of hydrogen peroxide (H_2O_2). (Klivenyi *et al.*, 2000) Glutathione peroxidase (GPX), which is the major enzyme in the antioxidative defense mechanism depending on glutathione. (Jurkovič *et al.*, 2008) Glutathione peroxidase (GPx), an enzyme dependent on the micronutrient selenium (Se), plays a critical role in the reduction of lipid and hydrogen peroxides. If GPx activity is decreased, more hydrogen peroxide is present, which leads to direct tissue damage and activation of nuclear factor-κB–related inflammatory pathways (Espinoza *et al.*, 2008).

There are four known GPXs which contain selenocysteine at the active site (Arthur *et al.*, 2000)

a) GPx1; Cellular glutathione peroxidase (cGSHPx, e.g. red cell GSHPx)
b) GPx2; Gastrointestinal glutathione peroxidase (giGSHPx)
c) GPx3; Extracellular glutathione peroxidase (eGSHPx, e.g. plasma GSHPx)
d) GPx4; Phospholipid hydroperoxide glutathione peroxidase (phGSHPx)
e) GPx5; Epididymis-specific secretory GSHSPx (Espinoza *et al.*, 2008).

BIOSYNTHESIS OF GLUTATHIONE PEROXIDASES

Biosynthesis of glutathione peroxidases is similar to biosynthesis of all selenoproteins which depends on the availability of selenium (Se) (Jurkovič *et al.*, 2008). Biosynthesis of selenoproteins, of course, depends on the availability of selenium. Selenium is incorporated as selenocysteine into the growing polypeptide chain. Unexpectedly, selenium is evenly used for the biosynthesis of the selenoproteins only at optimum selenium supply (Brigelius-Floh´e *et al.*, 1999).

FUNCTIONS OF GLUTATHIONE PEROXIDASE

Many of the pathological consequences of selenium deficiency were originally associated with what was hypothesized to be oxidative damage to tissues. Thus, it was considered that loss of GPX-1 activity, particularly when vitamin E levels were low, would result in peroxide-induced membrane damage and pathogenesis. There is much less information on the functions of GPX-2 in mammals. However, its unusual distribution in the epithelium of the gastrointestinal tract suggests a specific function in metabolizing the ingested lipid hydroperoxides. Regulation of the mRNA for GPX-2 in cell lines in comparison with other GPXs indicates that it is one of the proteins that is well preserved in selenium deficiency. GPX-3 is found in plasma and extracellular fluids and is glycosylated to improve stability; it is thought to act as an antioxidant, perhaps protecting cell membranes. The functions of GPX-4 are clearly associated with its ability to metabolize phospholipid hydroperoxides. In addition, GPX-4 represents at least 50% of the capsule material that embeds the helix of sperm mitochondria. The protein is enzymatically inactive and has been cross-linked by an oxidative process (Arthur, 2000).

GLUTATHIONE PEROXIDASE AND ANTIOXIDANT ACTIVITY

Glutathione peroxidase is an important enzyme in destroying H_2O_2 and organic hydroperoxides such as lipid hydroperoxides. It therefore guards against oxidative damage to the cell membranes and other oxidant-sensitive sites in the cell. While this selenium-dependent system destroys lipid hydroperoxides and other peroxides, vitamin E is believed to protect against oxidant damage to membranes by preventing the formation of lipid hydroperoxides (Hoekstra, 1975).

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