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ROLES OF REACTIVE OXYGEN SPECIES AND SELECTED ANTIOXIDANTS IN REGULATION OF CELLULAR METABOLISM

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Abstract
Reactive oxygen species (ROS) are essential for life of aerobic organisms. They are produced in normal cells and formed as a result of exposure to numerous factors, both chemical and physical. In normal cells, oxygen derivatives are neutralized or eliminated owing to the presence of a natural defense mechanism that involves enzymatic antioxidants (glutathione peroxidase, superoxide dismutase, catalase) and water or fat-soluble non-enzymatic antioxidants (vitamins C and E, glutathione, selenium). Under certain conditions, however, ROS production during cellular metabolism also stimulated by external agents may exceed the natural ability of cells to eliminate them from the organism. The disturbed balance leads to the state known as oxidative stress inducing damage of DNA, proteins, and lipids. An inefficient repair mechanism may finally trigger the process of neoplastic transformation or cell death. Reactive oxygen species are also an integral part of signal transduction essential for intercellular communication. The balance between pro- and antioxidative processes determines normal cellular metabolism manifested by genes activation and/or proteins expression in response to exo- and endogenous stimuli.

Key words:
Reactive oxygen species, Antioxidants, Selenoprotein, Signal transduction

INTRODUCTION

Production of reactive oxygen species (ROS) is a physiologic process, essential for life of aerobic organisms [1]. A number of environmental factors (e.g., UV radiation, ionizing radiation, xenobiotics, tobacco smoke) as well as an activation of superficial receptors of the cell may contribute to the increased production of ROS in those organisms [2,3]. 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) [1,3]. 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 [4,5].

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BIOLOGICAL ROLE IN GENERATION OF REACTIVE OXYGEN SPECIES

Under physiologic conditions or under the influence of external factors, the majority of generated ROS are produced in the respiratory chain in the mitochondria (Fig. 1). ROS are also generated by monoaminooxidases that metabolize catecholamines, lipoxygenases involved in the synthesis of prostaglandins, xanthin oxidase, and cytochrome P450 [6]. The electron transport chain present in endoplasmatic reticulum and nuclear membranes may be also the source of ROS [7].

In the respiratory chain, ROS are generated in complex I (oxidoreductase NADH: ubiquinon) and in complex III (oxidoreductase ubiquinon: cytochrome c reductase). Ubiquinon in complexes I and III is a natural mediator involved in the transport of electrons along the respiratory chain [8–10] (Fig. 1.).

Ubiquinon is bound to the internal membrane of the mitochondria. During electron transport, this compound is reduced to ubiquinol, which is responsible for electron transport to complex III. Ubiquinol exhibits an antioxidative effect by decreasing lipid peroxidation in the internal membrane of the mitochondria when supplying hydrogen atoms to lipid peroxyl radicals [11]. The yield of superoxide anion ($^{\bullet}O_2^-$) results from a single electron conversion into molecular oxygen caused by ubiquinon [7]. In this way about 2% of oxygen consumed by cells are converted to superoxide anion radical [12]. Although $^{\bullet}O_2^-$ is a radical of relatively low activity, it may be converted into more active forms, such as peroxyl (LOO$^{\bullet}$), alcoxyl (LO$^{\bullet}$), and hydroxyl radical ($^{\bullet}$HO) [13].

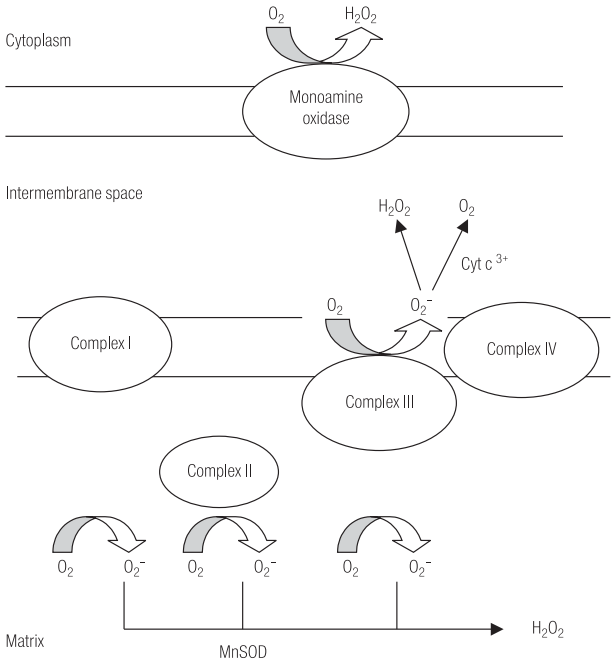

Fig. 1. Reaction of gradual reduction of molecular oxygen during oxidative phosphorylation [10].

Hydroxen peroxide (H_2O_2), produced due to spontaneous or enzymatic conversion of $^{\bullet}O_2^-$, is in turn an oxidant of relatively low activity and remains neutral to all molecules present in the cell [14]. H_2O_2 is produced by many types of cells in response to various extracellular stimuli (e.g., cytokines, signaling cascade factors, growth factors or hormones). Under physiologic conditions, H_2O_2 is *inter alia* essential for biosynthesis of the thyroid hormone, activation of nuclear transcriptional factors, thyrosine cascade activation, and phospholipases activity [14].

Hydroxen peroxide plays a crucial role in the regulation of programed cell death (apoptosis). It has been evidenced that exposure to low doses of H_2O_2 mediates apoptosis in numerous types of cells [15]. The mechanism of this process involves the release of cytochrome c from the mitochondria and activation of caspases [16]. This suggests that H_2O_2 may be essential for modification of permeability of mitochondrial membranes [17].

In the presence of Fe^{2+} or Cu^+, H_2O_2 is converted into hydroxyl radical ($^{\bullet}$HO), one of the most reactive compounds. Reaction of $^{\bullet}$OH with purines and pirymidynes generates over 100 types of modified nucleotides in DNA [18]. Hydroxyl radicals can also activate certain oncogenes (e.g., K-ras), which leads to activation of neoplastic promoters [19]. Although metal ions-dependent generation of $^{\bullet}$OH occurs under *in vivo* conditions, physiological significance of this process has not yet been fully elucidated [20].

DAMAGES INDUCED BY REACTIVE OXYGEN SPECIES

Reactive oxygen species attack cysteine and aromatic aminoacids in polypeptyde chains, purine and pirymidine bases of nucleic acids and polyunsaturated fatty acids [21].

Sulfhydryl (-SH) groups in cysteine are especially vulnerable to chemical modification by H_2O_2 [14]. Oxidized -SH forms cross bonds with other thiols [7]. Conversion of -SH groups to disulfides and/or other oxidized forms is one of the earliest changes observed during protein oxidation [13,22]. Oxidative modification of aminoacids may lead to aggregation or fragmentation of polypeptide chains. ROS-modified proteins activate proteolitic enzymes (e.g., macroxyproteinase complex) and thus easily undergo proteolysis [23].

Polyunsaturated fatty acids present among others in membrane phospholipids are another important target for ROS in the cell [24]. ROS react with unsaturated fatty acids leading to the production of lipid radicals ($^{\bullet}$LH) and conjugated dienes [25]. Oxidation of lipid radicals generates peroxyl radicals (LOO$^{\bullet}$), which reacting with unsaturated fatty acids initiate lipid peroxidation. This process results in the increased oxidation of unsaturated fatty acids, cholesterol, and other lipid compounds. *In vivo* lipid peroxidation is particularly intensive in macrophages, endothelium, and smooth muscle cells. Oxidized lipids exhibit immunogenic, cytotoxic, and chemotactic properties for monocytes and smooth muscle cells, and enhance monocyte adhesion. They directly or indirectly stimulate smooth muscles to produce cytokines (Il-1, Il-8), monocyte chemotactic protein 1 (MCP-1), colony-stimulating factor (CSF), and inhibitor of plasminogen activation. Oxidized lipids also activate T lymphocytes, inhibit generation of tumor necrosis factor α (TNFα), and influence the production of nitrogen oxide [26]. Lipid peroxidation terminates when substrates are exhausted, or such derivatives as alkanes, alcohols, ketones, aldehydes, and carboxylic acids are formed, or intermolecular bonds, e.g., with proteins are established. Some of the indirect products of lipid peroxidation, e.g., malonyldialdehyde, may react with purines or pirymidynes so that exocyclic DNA adducts are formed [27].

4-Hydroxynonenal (HNE) is the most reactive aldehyde produced during oxidation of arachidonic or linolenic acid. Under normal conditions, HNE is conjugated with glutathione (GSH) [28]. Influenced by H_2O_2 or fatty acid hydroperoxides, HNE is oxidized to epoxide intermediate. This intermediate attacks nitrogen atom in DNA bases, generating ethene derivatives of cytydine, adenosine and guanosine [29]. Numerous studies indicate that HNE can inactivate Na^+-K^+-ATPase and adenine nucleotide translocator (ANT) as well as inhibit transcription processes [25]. It also influences cell cycling and differentiation of granulocytes and modifies monocyte migration and chemotactic response [25].

Protein adducts generated in the reaction with aldehydes inhibit activity of some signaling proteins (e.g., adenyl cyclase) and enzymes (hexokinases, aldolases, lactic dehydrogenases, DNA polymerase), and also modify certain metabolic pathways (DNA replication, RNA transcription, protein synthesis, functions of mitochondria, glycolysis) [30].

REACTIVE OXYGEN SPECIES AND SIGNAL TRANSDUCTION

In normal cells, ROS form one of the links in the signal transduction of intracellular communication. The most recent studies provide evidence that pathways of intracellular signaling are regulated by intracellular redox potential. Under normal conditions, ROS generated by specified membrane oxidases transmit information to growth factors and cytokines. ROS also generated by agents originated from other cellular transformations, e.g., xenobiotic detoxification intermediates, activate signal transduction cascade [31]. Hydroperoxides, including those of unsaturated fatty acids, present in cellular membranes, may initiate signal transduction through triggering protein kinases cascade [32]. Mechanism by which signal is transmitted by ROS involves the change in cell redox potential and oxidative modifications of proteins [33].

Redox potential of the cell can be modified by reactive oxygen and/or nitrogen species, as well as by inflammatory mediators, e.g., TNFα. To maintain all its functions, the cell must "respond" to changes in redox potential, for example, by producing new proteins sensitive to this signal, or by changing concentration or confirmation of regulatory proteins, which initiate transcription of relevant genes [34]. In the majority of cells, two nuclear transcriptional

factors, nuclear factor κB (NF-κB) and protein activator 1 (AP-1) are sensitive to redox potential. Hydrogen peroxide, HCIO, cytokines, and over 100 already known xenobiotics generating directly or indirectly ROS, activate translocation of NF-κB to the cell nucleus and its binding to DNA. NF-κB activation involves the change in protein confirmation and binding to promoter regions of numerous genes, e.g., cytokines. ROS directly activate the process of phosphorylation of subunit I-κB of NF-κB molecule [35].

Proteins activated by ROS usually contain numerous cysteines, and thus cysteine oxidation, nitrosylation or formations of disulfides are most essential processes in redox-dependent signal transduction. Oxidation of thiols in transmitter proteins induces their structural modification finally leading to their activation [36]. Cysteine is indispensable to maintain the activation of many transcriptional factors and thioredoxin (Trx) plays a central role in this process [31]. Oxidative stress induced by chemical or physical agents leads to Trx translocation to cell nucleus. Trx translocation enhances binding of NF-κB and AP-1 to DNA. This process occurs through reduction of intracellular disulfide (-S – S-) primarily in regions of nuclear factors binding to DNA [37].

Numerous ROS-generating xenobiotics influence gene expression, for example, of enzymes involved in phase II of detoxification (S-glutathione transferase, quinone reductase) [38]. ROS also activate expression of protein genes participating in protection of cells against oxidative stress. Gene expression of such enzymes as glutathione peroxidase (GSH-Px), quinone reductase, catalase, superoxide dismutase, heme oxygenase, transferrin, ferrityn, thioredoxin reductase, metalothioneine, cyclooxygenase, and γ-glutamylcysteine synthase is also involved in this process. The aim of gene expression is not only to directly protect cells against ROS action, but also to participate in intracellular signal transduction [39].

Protein expression depends on physiological signals, e.g., transmitted by cells released hormones or cytokines, but it is also stimulated by external agents (xenobiotics or physical factors) [29]. ROS generated as a result of oxidative stress stimulate cellular signaling as well. They are known as a "second messenger" for many cytokines and growth factors [31].

ACTIVITIES OF ANTIOXIDANTS

Scavenging of ROS involves antioxidative enzymes, frequently occurring with metal ions in their active sites, directly participating in redox reactions. In the protection of cellular components against ROS, two cellular antioxidative systems play an important role: 1) glutathione system composed of glutathione (reduced and oxidised forms), glutathione reductase, and peroxidase; and 2) thioredoxin system comprising thioredoxin (reduced and oxidised form), thioredoxin reductase, and peroxidase [40]. Among enzymes active in both systems, two selenoproteins, glutathione peroxidases, and thioredoxin reductases in particular are most essential [41].

Glutathione (γ-glutamylcysteinylglicyne) is a key antioxidant involved in the protection of mammal cells against ROS [42,43]. Although it is synthesized by all cells, the liver is the major place of its production [44]. In all cells, GSH occurs in relatively high concentrations (1–10 mM). Almost 15% of cytosol GSH are transported to the mitochondria with participation of specific mitochondrial transporters. Cysteine in GSH molecule is its most essential functional component, being responsible for supplying active thiol groups [44] directly reacting with ROS or establishing reversible bonds with protein thiol groups [13]. GSH is also involved in accumulation and transport of cysteine, apoptosis, regulation of gene expression, immune response, DNA and protein syntheses, regulation of cell cycle and differentiation [45]. GSH is the major non-protein compound that contains thiol groups, therefore its presence is essential for maintaining the reduction intracellular environment indispensable for optimum activity of the majority of enzymes and other cellular macromolecules [43,46]. Moreover, in the regulation of the redox potential of the cell, the GSH/GSSG ratio, not GSH concentration plays a significant role [43,47].

Glutathione is also responsible for regulation of apoptosis [48]. It is rapidly released to intracellular areas by cells, which undergo the process of apoptosis. This mechanism

as well as its physiologic significance have not yet been elucidated. It is likely that this process initiates apoptosis and it is stimulated by caspeses [46].

Glutathione peroxidases are essential elements of the antioxidative system in cells [49–51]. These enzymes catalyze hydroperoxides reduction of polyunsaturated fatty acids and hydrogen peroxide to less toxic alcohols and water, respectively. In the process of scavenging potential oxidants, GSH-Px combines its efforts with glutathione and glutathione reductase. Four different selenium-dependent proteins with glutathione peroxidase activity, acting in different cell compartments, have already been described [50]:

- cytosolic (classic GSH-Px, cGSH-Px),
- gastrointestinal (GI-GSH-Px),
- extracellular (plasma, eGSH-Px),
- phospholipid hydroperoxide (membrane, PH-GSH-Px).

Cytosolic GSH-Px, occurring in cell cytosol, is a protein composed of four identical subunits, and each of them contains one of selenocysteine. It is also present in all tissues, however, in different concentrations; its highest concentration is found in the liver and erythrocytes, and the lowest in the brain and muscles [52].

The -SeH group, present in the enzyme active site, is sensitive to the effect of metabolites with oxidative properties. Xenobiotics or metabolites, e.g., fructose, dicarbonyl derivatives of hydrocarbons, nitric oxide (NO) and its derivatives, aldehydes derived from decay of fatty acids, generate oxidative stress in the cell through cGSH-Px inactivation, however, they also inhibit expression of mRNA cGSH-Px [53,54]. It has been revealed that the increased concentration of peroxides is a signal that activates the expression of cGSH-Px gene in cells of the rat kidney [55]. This results in the enhanced expression of mRNA, protein concentration, and GSH-Px activity. Activation of signal transduction is concomitant with inhibition of apoptosis [56].

Extracellular GSH-Px is a glicoprotein composed of four subunits with molecular mass of 23 kDa, mostly synthesized in kidneys and then released to extracellular fluids [57,58]. Biological significance of eGSH-Px still remains to be clarified. Nevertheless it is assumed that this enzyme may play a local role in the protection of an external part of cellular membrane from the attack of ROS [50].

A recently described GSH-Px occurs in tissues, taking the form coupled with cellular membranes. Contrary to other enzymes of this family, PH-GSH-Px is a monomer with molecular mass of 22–23 kDa. This enzyme is characterized by its ability to decompose not only H_2O_2 and free fatty acids hydroperoxides, but also phospholipid hydroperoxides. Owing to this ability it supports vitamin E in protecting cellular membranes from lipid peroxidation [47,59]. The results of numerous studies indicate that PH-GSH-Px also participates in redox regulation, inflammation, and apoptosis [50,52]. It has been evidenced that overexpression of this enzyme in different cellular lines may influence cytochrome c release from the mitochondria, DNA fragmentation, and NF-κB inhibition. PH-GSH-Px also induces thymidine peroxide reductase, which suggests that it may play a role in DNA repair [60].

In *in vitro* studies carried out with human fibroblasts exposed to pure oxygen or nitrofurantoine, Michiels et al. [61] showed that GSH-Px is the most efficient enzyme in the protection of cells from oxidative stress. GSH-Px has a higher than catalase affinity for H_2O_2. It is thought that GSH-Px decomposes about 70% of hydroxen peroxide generated in activated neutrofils. The efficiency of GSH-Px in protecting against oxidative stress is fourteen-fold higher than that of catalase. Probability of cell damage after removal of GSH-Px and catalase increases by nearly 200 times. In described system, GSH-Px and catalase are more efficient than superoxide dismutase in fibroblasts protection, thus hydrogen peroxide and its derivatives are responsible for xenobiotic toxicity [62]. Hydrogen peroxide is more dangerous to the cell than superoxide anion [61]. This assumption is also confirmed by studies performed by Ceballos-Picot et al. [63]. The enhanced activity of Cu/ZnSOD at the unchanged GSH-Px activity increases concentrations of lipid hydroperoxide in brains of transgenic mice. The authors present an opinion that disturbances in the relation between antioxidative enzymes increased concentration of H_2O_2 and intensified prooxidative processes [63].

In *in vitro* studies, removal of GSH-Px from fibroblasts (precipitation with antibodies) even in normal culture (free of stressogenic agents) inhibits cellular proliferation,

leading to inactivation of the basic function of the cell. Removal of SOD under the same conditions enhanced the rate of cell division. The authors conclude that protection of cell from ROS depends primarily on the activity of GSH-Px. There is a linear relationship between concentrations of ROS and their scavenging by GSH-Px. The increased activity of SOD does not evoke a similar relationship, but the loss of the GSH-Px activity intensifies toxic processes in the cell [61].

Toussaint et al. [64] in *in vitro* studies indicated that a 44% inhibition of the GSH-Px activity in t-butyl hydroperoxide-exposed cells causes death of 50% of cells in culture. Transfection of human lines of breast cancer cells MCF-7 by cDNA cGSH-Px significantly enhances cell resistance to oxidative stress induced by H_2O_2 and doxorubicin [65]. The authors suggest that even in this case, removal of hydrogen peroxide is a critical process in the resistance of cells to oxidative stress induced by xenobiotics. GSH-Px acts in both hydrophobic and hydrophilic areas whereas other antioxidative enzymes act only in the hydrophilic area. This is an additional aspect that supports a unique role of GSH-Px in the cell [61].

In the early 1990s, thioredoxin reductases, a new family of selenoproteins of antioxidative function, was described [66]. Thioredoxin reductases catalyze thioredoxin reduction using varied substrates, protein disulfide, low molecular disulfide compounds, lipid peroxides or hydrogen peroxide [67]. This enzyme is also responsible for the reduction of ribonucleotides to deoxyribonucleotides and the maintenance of an adequate intracellular redox potential [68]. Two kinds of proteins of this group have been described to date, thioredoxin reductase-1 (TrxR1) and thioredoxin reductase-2 (TrxR2) [69]. TrxR1 was first isolated from lung cancer cells. This enzyme is composed of two identical subunits located in the cell cytosol. TrxR2 occurs in the mitochondria. A biological role of this enzyme has not yet been explained. It is likely that TrxR2 is one of the agents protected by the mitochondria from ROS [70].

Expression of Trx and TrxR has been observed in the skin keratinocytes, placenta, liver, leucocytes, and mammal secretory cells. Expression of these proteins is stimulated by a number of agents both exo- and endogenous (cytokines, lipopolisaccharides, mitogens, UV radiation, hydrogen peroxide). This suggests a significant involvement of these proteins in the protection of cells against oxidative stress [71].

It is also thought that Trx is an electron donor for reactions catalyzed by eGSH-Px. Glutathione peroxidases are oxidoreductases that exhibit high specificity to an electron donor substrate. Over many years, it has been believed that glutathione is the only electron donor for these enzymes. In view of the fact that plasma glutathione concentration is very low ($< 0.5\ \mu M$), it is supposed that under physiologic conditions plasma eGSH-Px activity depends on another electron donor, may be on Trx [59].

SELENIUM AND SELENOPROTEINS IN REGULATION OF GENE EXPRESSION

It has been evidenced that TrxR and Trx influence inactivation of nuclear factor κB and expression of antioxidative enzyme genes induced by exposure to tobacco smoke [72].

Enzymes containing selenium (Se) in an active site indirectly protect DNA and other components of the cell from damage caused by ROS. Organic Se compounds directly protect integrity of the cell genome [73,74]. High concentrations of selenomethionine (SeMet) in the cell activate processes of DNA repair through regulated expression of protein p53. Protein p53 activate almost 100 genes of proteins directly involved in the DNA repair [73]. It also plays an essential role in the control of cell division, oxygen metabolism, detoxification, and induction of apoptosis.

Selenium exhibits potential immunomodulatory properties. As a component of selenoproteins it is essential for neutrophils, macrophages, NK cells, T lymphocytes, and other defensive cells and processes [75]. On the other hand, it has impact on the state of balance between the production of superoxide anion and H_2O_2 in the mitochondria, which may contribute to death of the immune system cells. These effects of Se are evidenced by *in vitro* studies. Most likely it is one of the mechanisms by which Se diminishes immune response and protects from overproduction of hydrogen peroxide [76]. *In vivo* studies showed that low Se doses increase cytotoxic activity of NK cells, whereas Se

in higher doses exerts immunosuppressive effect on activity of NK cells [77,78].

Involvement of selenium in metabolic pathways associated with cell protection from oxidative stress causes that changes in activity of selenoproteins, resulting from, e.g., altered Se concentration or Se binding to inactive biological forms, may prove to be a potential factor affecting metabolism of the cell, leading finally to impairment of its function [79]. Expression of selenoproteins is regulated by the concentration of the microelement, however, the hierarchy of selenoprotein expression in cells is clearly defined [80,81]. Numerous reports have demonstrated that Se concentration does not have any impact on the transcription rate of selenoprotein genes [80,82]. Differences observed in selenoprotein expression in Se deficiency apparently result from changes in mRNA translation or its diminished stability. It is thought that factors other than Se concentration also play a significant role in expression of selenoproteins. The fact that oxidative stress caused by xenobiotics induces expression of TrxR and GSH-Px is now well documented [79,83].

The decomposition of ROS by GSH-Px and TrxR suggests that selenoproteins are involved in regulation of intracellular signal transduction.

Fibronectin is an extracellular component essential for intercellular communication. Exposure of cells to selenite decreases the number of surface receptors of fibronectin. This is an immediate action, therefore it cannot result from the activities of selenoproteins. Se stimulates activation of MAPK family protein kinases involved in pathways of cellular response to stimulation by inflammation factors and such exogenous stressogenic agents as NO, peroxides, superoxide anion or UV radiation [74,78]. These processes may be simultaneously inhibited by selenates. GSH-Px is also an inhibiting factor: hydroperoxide decay caused by GSH-Px inhibits activity of kinase p38 activated by selenite [79].

Selenoproteins directly influence cell growth: TrxR limits DNA synthesis; Trx/TrxR system activates rybonucleotide reductase, a key enzyme in DNA synthesis, responsible for production of deoxyribose. TrxR also regulates gene expression through activation of numerous DNA-binding transcription factors, e.g., NF-κB, glucocortycoide receptors, and modulates protein activator AP-1 [79].

However, it is known that not only Se incorporated into protein structure, but also other links influence metabolism of cells.

It was demonstrated in 1992 that Se^{+4} may induce cell necrosis by DNA single strand break [84]. It was observed at the same time that methylated Se compounds induce apoptosis [85]. Thioredoxin directly inhibits some of MAPK kinase family, e.g., ASK1. It is also documented that Se inhibits other pathways of signal transduction, protein kinase A, Ca^{+2}- dependent and independent kinases C (CPKC), diacylglicerol kinase and thymidine kinase [85].

Numerous literature data also indicate cytotoxic effect of Se-containing compounds. This phenomenon was observed in both *in vivo* studies and cellular cultures. Se toxicity results from increased oxidation of thiol groups and concomitant generation of $^{\bullet}O_2^-$. This process is limited to compounds that generate selenite anion during metabolic transformations or during reduction of disulfides [86].

LOW MOLECULAR ANTIOXIDANTS

Vitamin E (α-tocopherol), classified in the group of fat soluble vitamins, plays a crucial role in the protection of cellular membranes against lipid peroxidation. Vitamin E is a physiologic component of cellular membranes, which is faster than unsaturated fats in reacting with peroxyl radical, and thus prevents membranes from damage caused by ROS [5]. This reaction results in generation of a relatively stable radical, tocopheroxy, and organic hydroperoxide radicals [23].

Tocopheroxy radical can react with another α-tocopherol radical, which leads to the production of a stable form of dimer or to its total oxidation to tocopherol quinone [5]. Regeneration of α-tocopherol takes place in the presence of vitamin C responsible for supplying electrons for hydroperoxide reductase [42], which reduces α-tocopheroxy radical to α-tocopherol. Owing to this process, vitamin C contributes to protection of cellular membranes [87,88].

A bulk of literature data confirms the presence of interactions between vitamin C and α-tocopherol. Studies

performed on skin fibroblasts cultured in medium supplemented with vitamin C and α-tocopherol showed a considerable decrease in the level of ascorbate in α-tocopherol-containing medium. Changes in the level of ascorbate in the medium and the increased viability of cells confirm its involvement in the restoration of α-tocopherol. This phenomenon may be partly associated with direct reduction of polar groups, present in α-tocopheroxy radical, by vitamin C as well as with direct scavenging of free radicals, present in cultured medium, by ascorbate [88].

In vitro experiments also showed that in case of vitamin C deficiency, α-tocopherol exhibits prooxidative effect [89]. Vitamin C can also regenerate reactive forms of other low molecular antioxidants, e.g., glutathione and β-carotene [19].

Ascorbate is one of the first antioxidants that scavenge free radicals (hydroxyl and peroxyl, O_2^-, nitrogen oxide and dioxide, perchloric acid, ozone, singlet oxygen) generated during cellular metabolism [7]. Vitamin C also plays a crucial role in maintaining the integrity of blood vessels, in functioning of thrombocytes, and in biosynthesis of cholesterol and catecholamines [0]. During ROS scavenging, ascorbate is first reduced to semiascorbate radical and then to dehydroascorbate. Semiascorbate radical is a relatively stable and low reactive compound [91]. In contrast, dehydroascorbate is unstable and rapidly proceeds hydrolysis to diketogulonate, which is then transformed into oxalic acid.

Dehydroascorbate can undergo *de novo* enzymatic reduction to ascorbate with involvement of NADPH-dependent thioredoxin reductase, and also non-enzymatic reduction with participation of GSH [5,53]. Vitamin C, depending on the concentration applied, can also show prooxidative effect [92,93]. *In vivo* studies revealed that vitamin C supplementation increased markers of DNA damage induced by oxygen radicals, e.g., 8-oxoguanine and 8-oxoadenine [92]. Prooxidative action of ascorbate also occurs in the presence of iron and copper ions. Reduction of these metals under the influence of vitamin C *in vitro* leads to generation of reactive hydroxyl radicals. Reduced metal ions can react with lipid hydroxide, leading to the production of alcoxyl radicals, which initiate lipid peroxidation [87].

REACTIVE OXYGEN SPECIES AND PROCESS OF APOPTOSIS

Numerous reports show that ROS produced by the mitochondria are involved in processes leading to cell necrosis. ROS contribute to oxidative stress responsible for cellular destruction observed during necrosis and generate the apoptosis via the respiratory chain [7].

Apoptosis is a physiologic process essential for survival of multicellular organisms, during which redundant cells are eliminated [38,94,95]. Apoptosis also plays an essential role in early stages of carcinogenesis and in autoimmune disorders [38]. Apoptosis induced by oxidative stress is also involved in important immune processes, e.g., activation of T cells since this process is associated with the increased rate of oxidative phosphorylation [96].

Apoptosis can be induced by activation of membrane receptors, protein kinases, inhibition of cellular cycle and activation of p53 by DNA-damaging factors as well as by oxidants changing mitochondrial transition pore (MPT).

The increase in mitochondrial transition pore associated with opening of permeability transition pore complex (PTPC) plays a key role in the induction of apoptosis [97]. This increase is regulated by numerous agents, e.g., bivalent cations (Ca^{+2}, Mg^{2+}), mitochondrial membrane potential (MMP), concentration of adenine nucleotides, ROS or NO production. Numerous findings suggest that changes in MPT are influenced by intracellular redox potential associated with redox potential of GSH and –SH proteins [98]. This process frequently precedes the development of morphological changes, such as chromatine condensation, phosphatidylserine inversion in external cellular membrane, and activation of endonucleases [98].

Proteins able to activate (Bax, Bag, Bcl-XS, Bock) and to inhibit the process of apoptosis (Bcl-2, Bci-XL, A1, Mcl-1) are also important in MPT regulation [95].

Protein Bcl-2 is a heterodimeric complex, located in the external mitochondrial membrane, able to inhibit apoptosis through regulation of cell redox potential, however, the mechanism by which this process is induced has not yet been conclusively explained [99]. Bcl-2 exhibits antioxidative effect as it prevents from ROS generation [7], increas-

es the level of GSH and its redistribution to different cell compartments, and prevents from depletion of the GSH pool and cell destruction due to lipid peroxidation [100].

There are several ways, in which ROS can disturb the level of proapoptic proteins Bcl-2. ROS can activate protein p53 [16] probably through induction of DNA damage, which decreases activity of Bcl-2 and increases activity of Bax. However, this pathway needs very high concentrations of ROS, which are required to induce DNA damage [90]. This process leads to changes characteristic of apoptotic process, e.g., chromatin condensation, nucleus fragmentation, and DNA damage [98].

Some antioxidants can inhibit activation of caspases, leading in later stages to apoptosis, e.g., overexpression of Mn-dependent superoxide dysmutase restores mitochondrial membrane potential and inhibits cell necrosis caused by arresting the respiratory chain, whereas the increased expression of Cu/Zn SOD delays apoptosis by removal of $^{\bullet}O_2^-$ and facilitates the release of cytochrome c [7]. GSH-Px is also an agent that directly inhibits the process of apoptosis. *In vitro* studies showed that GSH-Px probably diminishes the expression of Bax or prevents the activation of caspase 3 [101].

Reactive oxygen species in their popular understanding are compounds fully integrated with processes disadvantageous to the cell, leading to its damage. Now we have a lot of evidence that ROS are an integral element of signal transduction in the intercellular communication [102]. There is balance between ROS-generating pathways and their transformations. It has been known since 1954 that chronic disturbance of this balance may lead to the development of certain diseases, e.g., cardiovascular, pulmonary or neoplastic [18,103,104]. Recent studies have gathered convincing evidence that the balance between pro- and antioxidative processes determines normal metabolism of the cell, which is pronounced by activation of relevant genes or by expression of proteins in response to exo- and endogenous stimuli affecting the cell.

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