Polish Journal of Environmental Studies Vol. 13, No. 1 (2004), 5-13

Review

The Antioxidative Barrier in the Organism

E. Kulikowska-Karpińska*, J. Moniuszko-Jakoniuk

Department of Toxicology, Medical Academy of Białystok, Mickiewicza 2c, 15-222 Białystok, Poland

Received: 10 April 2003
Accepted: 2 June 2003

Abstract

Cell metabolism in organisms which use oxygen as a source of energy is closely associated with the generation and action of free oxygen radicals and their derivatives. Extra- and intracellular substances that are antioxidative in nature prevent overproduction of radicals and protect against propagation of peroxidative reactions. The list of compounds which can be treated as antioxidants becomes elongated. Many classifications of these compounds are used, of which the most common is the division according to their nature into enzymatic and non-enzymatic, according to their environment or the way they react with FOR. Enzymatic antioxidants include: superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase. Non-enzymatic antioxidants are: vitamin E, vitamin C, glutathione, carotenes and retinols, and some transition metals (Zn, Cu and Se). The balance between the actions of these two groups of compounds determines normal functioning of the organism. Impairment of the balance between pro- and antioxidative processes in the organism is called anitoxidative stress and may be induced by intensified reactions involving FOR and by depressed activity/concentration of antioxidants. It seems, however, that irrespective of the cause, oxidative stress is likely to result in many diseases.

Keywords: reactive oxygen species, oxidative stress, enzymatic and non-enzymatic antioxidants

Introduction

Chemically, antioxidants are known as compounds able to inhibit the processes of oxidation. They can be divided into two categories:

- antioxidants which inhibit the phase of initiation by removing "reactive oxygen species" (ROS) from the environment;
- antioxidants which inhibit the phase of propagation, e.g. by degrading hydroxyperoxides to interactive products.

*Corresponding author

Antioxidant-type compounds break the sequence of oxidative reactions by reacting with ROS on a single-stage pathway to generate non-reactive products or on a two-stage pathway, where at first a weak radical-type compound is formed from the antioxidant. Then, the weak radical is joined to another (or the same) compound to form a non-reactive complex, for instance:

ROS + dihydroquinone → p. hydroxyfenyl radical → quinone

Physiological antioxidants should undergo regeneration in the organism, e.g. the vitamin E - vitamin C

system. However, their action does not result in the total inhibition of peroxidation [16].

The presence of ROS in the cells which utilise oxygen as the source of energy leads to the formation of a number of mechanisms that are directed against them. These mechanisms have been divided into four categories [57]:

- compartmentization → separation of ROS generation sites in the cell, chelation of transition metals, specific distribution of antioxidative-type substances;
- detoxication → ROS removal through enzymatic and non-enzymatic pathways,
- repair,
- utilization.

These mechanisms referred to as the antioxidative protection or barrier are responsible for continuous monitoring of the organism demand for antioxidative substances. Control over the prooxidative processes in physiological and pathological conditions occurs through release, activation, synthesis and regulation of renal resorption and excretion of antioxidative compounds. It is assumed that the maintenance of normal homeostasis of pro- and antioxidative processes is based on the following feedback:

detection ↔ signal ↔ compensatory reaction (release of antioxidants from tissue deposits, de novo synthesis)

The third segment of this mechanism is relatively well known, the first and the second are vague.

The list of compounds which can be classified as antioxidants is being constantly enriched. They can be classified according to their nature, e.g. enzymatic and non-enzymatic [76], environmental or how they react with ROS [37, 92].

However, taking into account their function in the antioxidative processes, they can be divided into the first-line antioxidants and the so-called helper antioxidants.

Characteristics of Chosen Compounds Involved in the Antioxidative Barrier of the Organism

Enzymes

All cells of eukaryotic organisms contain strong antioxidative enzymes, including four major ones - superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase.

Superoxide Dismutase

Superoxide dismutase (superoxide : superoxide oxidoreductase SOD, EC.1.15.1.1) catalyses dismutase reactions of the superoxide anion radical and leads to hydrogen peroxide generation [25, 46, 51, 62]:

$$O_2^- + O_2 + 2H \xrightarrow{SOD} H_2O_2 + O_2$$

In the presence of superoxide dismutase, the superoxide anion radical is unstable and therefore its dismutation is spontaneous [42].

Three types of superoxide dismutase are known [31], differing in cofactors, sensitivity to inhibitors and location.

Cytosolic superoxide dismutase (Cu,ZnSOD-1) is built of two identical subunits. Each contains 151 amino acid residues and one ion of zinc and copper. Zinc is responsible for subunit stability, while copper for activity (neutralises the superoxide anion radical) [47, 89].

Copper ions can be found at two stages of oxidation, as cuprous (Cu^+) and cupric ions (Cu^{2+}). The superoxide anion radical can oxidize cuprous ions and reduce cupric ions:

$$SOD\text{-}Cu^+ + O_2^- + 2H^+ \rightarrow SOD\text{-}Cu^{2+} + H_2O_2$$

$$SOD\text{-}Cu^{2+} + O_2^- \rightarrow SOD\text{-}Cu^+ + O_2$$

The plasmatic isoenzyme differs from the cytosolic isoenzyme and constitutes a small part of total activity of superoxide dismutase (Cu,Zn SOD). Its role, however, has not yet been elucidated.

Manganese-containing superoxide dismutase (Mn-SOD; SOD-2) is present in the mitochondrial matrix. This isoenzyme is a protein composed of four subunits and is not inactivated by hydrogen peroxide. Ferrum-containing superoxide dismutase (FeSOD), whose activity in cells depends on the degree of exposure to ROS [89], can be found in a large number of aerobes.

Extracellular superoxide dismutase (EC-SOD) is a protein which also contains copper and zinc ions. EC-SOD molecules are tetramers with saccharic residues [68]. Like SOD-1, EC-SOD is inhibited by cyanide and azide ions, dietyloditio carbaminian and hydrogen peroxide. It is also very resistant to the effects of alkaline solution, urea, guanidine hydrochloride and high temperatures.

The total amount of SOD in the human organism is considered to be 3.5-4.0 g. Its highest activity can be observed in the liver, the lowest in fatty tissue [61].

In the liver, half of the total activity of SOD refers to the action of Cu,ZnSOD and half to MnSOD, while in the cerebrospinal fluid, the total activity of the enzyme is represented by Cu,ZnSOD. It is generally accepted that 85-90% of the total SOD activity in the organism involves Cu,Zn SOD.

The superoxide anion radical is produced only in the presence of oxygen [8]. SOD biosynthesis is under strict control; however, up to the present the process has been described only for bacterial cells [58]. Yet, it has been known that the product of molecular oxygen reduction is SOD synthesis inductor, while ferrum ions have a regulatory role [83]. Analysis of the mechanism of SOD conducted catalysis indicates that this enzyme is an incomplete antioxidant which prevents the action of one oxygen radical, its biological action being associated with a catalase effect. Tanford et al.[80], have shown that O_2^- inhibits the catalase effect, while H_2O_2 the action of dismutase.

Catalase

Catalase (hydrogen peroxide oxidoreductase; CAT, E.C. 1.11.1.6) localized in peroxisomes is a ferroporphy-

rin protein, 225 kDa of molecular mass, containing four ferrum atoms per mole of protein. Its molecule consists of four monomers. It catalyses dismutation of hydrogen peroxide. Catalase exhibits affinity only for free molecules of hydrogen peroxide. Due to this action two molecules of H_2O_2 are transferred to two molecules of H_2O and one molecule of oxygen [25]:

$$H_2O_2 + H_2O_2 \xrightarrow{\text{catalase}} 2\,H_2O + O_2$$

The reaction rate depends on the presence of hydrogen donors. Catalases exhibit two activities - a catalase effect at high concentration of H_2O_2 and a peroxidase effect at low concentrations of hydrogen peroxide. Its characteristic feature is the formation of the so-called complex I, when one particle of H_2O_2 is bound to the active centre of the enzyme [58]. Each catalase subunit binds one NADPH molecule for stabilisation [52,53,70].

Catalase most frequently exhibits peroxidase activity in the in vivo conditions. In mammals, it occurs mainly in the liver, erythrocytes and kidneys. It is believed that in the blood and hepatic cells catalase removes hydrogen peroxide produced due to the action of oxygen dehydrogenases.

In physiological conditions, catalase controls the concentration of H_2O_2, prevents its accumulation in cells and protects against oxidative insults which can lead to pathologies [38,52,70]. At low concentrations of H_2O_2, catalase acts jointly with glutathione peroxidase.

Glutathione Peroxidase

Glutathione peroxidase (glutathione:hydrogen peroxide oxidoreductase: GSH-Px, E.C. 1.11.1.9) is a protein made up of four subunits, each containing one atom of selene covalently bound to cystein. Selene is necessary for the catalytic action of the enzyme, which catalyses the reaction between glutathione and hydrogen peroxide to generate the oxidised form of glutathione, i.e. disulfide (GSSG) [4,15].

$$H_2O_2 + 2\,GSH \xrightarrow{\text{glutathion peroxidase}} 2\,H_2O + GSSG + 2\,H^+$$

The enzyme is highly specific to glutathione and less to peroxides; it catalyses the reduction both of hydrogen peroxide and organic peroxides, including lipid peroxides, steroid hormones and cholesterol. Free thiol groups are necessary for its normal functioning, their number depending on the physiological state of the enzyme (3-7/mol of protein) [33].

Glutathione peroxidase occurs mostly in the cytosolic fraction (70% of total activity), but also in the mitochondrial matrix (20%) and in the nuclear fraction (10%) [56].

Peroxidase aims the H_2O_2 attack at glutathione (protecting the thiol groups of enzymes), and thus prevents the involvement of hydrogen peroxide in the Frenton reaction.

The highest activity of glutathione peroxidase was found in the liver (detoxification), in the blood and lungs (high oxygen concentration); it was less intense in the heart and kidneys, the lowest in the brain and eye lens [89].

First of all, glutathione peroxidase protects cellular membranes against peroxidative insult, controls the lipid peroxidase rate and ensures the normal course of cellular metabolism. It participates in the regulation of a number of processes, e.g. the pentose cycle, and contributes to the maintenance of an adequate level of prostacyclines in the blood through a direct effect on the endogenous inhibitor of their synthesis. It also takes part in the transformation of prostaglandins and prostacyclines, and in the generation of the former [39]. It has been demonstrated that at a reduced activity of GSH-Px, ROS produced in phagosomes are able to cause self-destruction of the phagocytising cells [17].

Apart from selene-dependent glutathione peroxidase, another type, without selene, has been found. It is glutathione s-transferase which, like GSH-Px, catalyses degradation of organic peroxides, but does not degrade hydrogen peroxide.

The isoenzyme Se GSH-Px, which is exclusively specific to lipid peroxides, has been detected in the cellular membrane [64].

All peroxidases function as free unbound peroxides. In physiological conditions at a low H_2O_2 concentration, glutathione peroxidase exhibits stronger affinity for H_2O_2 compared to catalase. The action of glutathione peroxidase is directly related to glutathione reductase.

Glutathione Reductase

Glutathione reductase (NADPH:glutathione oxidoreductase; E.C. 1.6.4.2.) is an enzyme which reconstructs the form of glutathione at the cost of NADPH oxidation [4].

$$GSSG + 2\,H^+ + 2\,NADPH \xrightarrow{\text{glutathion reductase}} 2\,GSH + 2\,NADH^+$$

NADPH undergoes regeneration with the involvement of enzymes, including glucose-6-phosphate dehydrogenase (E.C.1.1.1.49) and isocitrate dehydrogenase (E.C. 1.1.1.42), which catalyse oxidation, with the resultant NADP as a co-substrate.

When glutathione reductase is unable to cope with the excess of glutathione disulfide produced in the cell, cells can actively "throw it out," at the cost of ATP energy [2].

Non-Enzymatic low-Molecular Antioxidants

Reactions of low-molecular antioxidants with ROS and organic free radicals are less specific than enzymatic reactions. Therefore, these compounds are more universal defendants of the organism, capable of fulfilling different functions. Many of them exhibit both preventive (inhibit oxidation by reacting with oxidative factors) and interventive action (inhibit oxidation by reacting with indirect products of oxidation, i.e. free radicals).

Low-molecular antioxidants have been divided into two classes: hydrophobic (protecting the cell inside) and hydrophilic (protecting the cell lignid part). The former includes tocopherols, carotenoids, bilirubin and reduced coenzyme Q (ubiquinone) [9,35,67,69]. Female sexual hormones (estron and estradion derivatives and their metabolic products) also exhibit antioxidative properties [55]. The major hydrophilic antioxidants are: glutathione, ascorbic acid, cysteine, uric acid, flavonoids, creatinine, phytic acid, and melanines [54].

Tocopherol - Vitamin E

Vitamin E is a mixture of tocopherols and tocotrienols. Tocopherols (α, β, γ and δ) have a phytil chain, while tocotrienols (α, β, γ and δ) are characterized by a side chain with three double bonds. Differences between α, β, γ, and δ tocopherols and tocotrienoles lie in different distributions of methyli groups in the chromane ring. α-tocopherol is the most common compound, constituting 88% of the whole pool of vitamin E in the blood plasma. Its daily requirement for an adult is 8-10mg [14].

Vitamin E is the main antioxidant of the lipid phase [21]. Its absorption from the alimentary tract ranges from 21 to 74% [14,48]. In the cell, vitamin E occurs mainly in the mitochondrial membranes and endoplasmic reticulum. Its slight amounts can be found in peroxisomes and in the cytoplasm. In the membranes, vitamin E is irregularly distributed, forming clusters in the lipid part [47]. The vitamin E content in the membranes of various cells is varied: mononuclear leukocytes contain 10-fold more vitamin E than erythrocytes and blood platelets. In the mitochondrial membranes, the tocopherol molecule to phospholipid molecule ratio is 1 to 2,100 [40].

In the blood, vitamin E is transported with lipoprotein fractions and at the same time protects their components against oxidation [82]. Inside the cell, transport occurs via the so-called low-molecular binding proteins. In the cytosol of hepatocytes, these are 30 and 14.2 kDa proteins, the cytosol of cardiomyocytes showing the presence of one (14.2 kDa) transporter [30].

Vitamin E occurs in large amounts in the liver, heart, adrenal glands and ovaries. Particular importance has been ascribed to the presence of tocopherol in the plasma and erythrocytic membranes. Tocopherol prevents oxidative damage to cellular structures and tissues, and blocks the formation of nitrosamines [63]. It contributes to the maintenance of an adequate structure of the membranes, reduces neutrophil chemotaxis and improves the efficiency of the immune system [11]. It also reduces platelet aggregation [88], which protects the organism against microthrombi. Vitamin E is also involved in many other processes, not necessarily as an antioxidant, e.g. in the suppression of inflammatory states and DNA synthesis [30].

Vitamin E is a very efficient antioxidant, which can inhibit oxidative processes in the initiation phase, i.e. can react directly with $O_2^{\cdot-}$, $^1O_2$, $\cdot OH$ [21] and NO [26], as well as in the phase of propagation by reacting with organic peroxyl radicals.

The process of vitamin E regeneration from the radicals being its derivatives can occur through enzymatic and non-enzymatic pathways. It is difficult to determine which is the primary mechanism. The enzymatic pathway of tocopherol radical reduction occurs with the contribution of glycerolophosphate dehydrogenase, acetyl-CoA dehydrogenase and succinate -coenzyme Q reductase [59].

Non-enzymatic regeneration is likely to involve ascorbic acid, glutathione and reduced coenzyme Q. The reduced coenzyme Q is able to regenerate tocopherol bound in the membranes [16,18]. The process of vitamin E regeneration appears as follows:

$$ROO^{\bullet} + \text{vit. E-OH} \rightarrow ROOH + \text{vit. E-O}^{\bullet}$$

$$\text{vit. E - O}^{\bullet} + AH \rightarrow \text{vit. EOH} + A^{\bullet}$$

Ascorbic Acid - Vitamin C

L-ascorbic acid is an endiol form of δ-lactone of 3-keto-gulonic acid. The acid molecule is composed of endiol group [-C(OH)=C(OH)-], responsible for its acid and reductive properties [6, 22]. It has strong reductive properties and therefore is referred to as the major antioxidative factor in the aqueous solution. Ascorbic acid is well absorbed from the alimentary tract. Its daily requirement for an adult is 1-3 mg. The highest concentration of vitamin C in the organism is observed in the thymus, adrenal glands, salivary glands, liver and blood [13, 66].

Vitamin C is an unstable substance, sensitive to heating, particularly in the presence of oxygen and heavy metals (iron, copper). Its degradation is accelerated in the alkaline or neutral solution. Ascorbic acid is affected by certain drugs (acetylsalicylic acid, sulphonamides and barbituric acid derivatives), antiseptic agents (sodium benzoate) used for food conservation, as well as drying and ultraviolet radiation. Oxidation of ascorbic acid is also facilitated by enzymes included in the group of oxidases (ascorbinase, polyphenolooxidase and peroxidase) which are found in some plant products [43, 86].

In the organism, L-ascorbic acid is oxidised to L-dehydroascorbic acid through an indirect radical-type compound, namely L-monodehydroascorbic acid. The reaction is as follows [27]:

L-ascorbic acid ↔ free ascorbylic radical ↔
↔ L-dehydroascorbic acid

In aqueous solutions, L-ascorbic acid is unstable (the lactone ring opens spontaneously to form diketogulanic acid); hydrolysis of diketogulonic acid leads to the loss of activity [27]. Dihydroascorbic acid exhibits hydrophobic properties and thus can penetrate cellular membrane lipids. Dehydroascorbinian effectively protects the plasma LDL fraction against copper-induced peroxidation [71].

The ascorbylic radical which is generated in this proces can be regenerated on a non-enzymatic pathway with the involvement of uric acid [75].

The basic function of ascorbic acid is its contribution to reactions of hydroxylation, e.g. hydroxylases which take part in the synthesis of collagen as a cofactor require the presence of ascorbate [86].

In the organism, ascorbic acid plays a role of antioxidant which protects body fluids against ROS. It can react with peroxide and thus prevents DNA damage [6, 10, 28, 72].

Together with cytochromes a and c, pirimidine and flavine nucleotides and glutathione, ascorbic acid forms redox systems which affect the behaviour of normal oxidation-reduction potentials in cells [10, 28].

Vitamin C is essential for vitamin E regeneration. Through the reduction in α-tocopherol radical, it can play a role of an "adjunct" antioxidant on the surface of cellular membranes, thus regenerating α-tocopherol, which can again function as an antioxidant [40, 54].

It is assumed that ascorbic acid exhibits peroxidative properties, since at low concentrations (50 mM) and in the presence of transition metal ions - through the reduction of Fe^{3+} to Fe^{2+} or Cu^{2+} to Cu^{1+} - it facilitates establishment of an optimal Fe^{3+}/Fe^{2+} or Cu^{2+}/Cu^{1+} ratio, which promotes peroxidation of NADPH-dependent lipids. At higher concentrations, ascorbate excessively reduces Fe^{3+} to Fe^{2+} and inhibits NADPH-dependent lipid peroxidation [19].

Vitamin C is believed to decrease the risk of certain civilisation-related diseases, such as hypertension [66, 83], atherosclerosis [34, 77, 81, 86], cataract [87] and neoplastic diseases [13, 22].

Glutathione

Glutathione (δ-glutamylocysteinyloglycin) is a tripeptide, a compound soluble in water which exhibits reductive properties conditioned by the thiol group. It is synthesized by many types of cells from glutaminian, cystein and glycin [2]. However, the major site of its synthesis is the liver, from where it is released to the blood and bile [7].

Glutathione activity on the surface of hepatocytes is relatively low, and therefore its release by these cells predominates over the uptake. However, high activity of the enzyme on the surface of renal, pancreatic and intestinal cells allows its efficient uptake from the blood [7].

In the cell, glutathione occurs at high concentrations in the cytoplasm, mitochondria and nucleus (5-10 mM), its concentration being substantially lower in the endoplasmic reticulum (2 mM) [56].

In the mitochondria, glutathione is the major compound which protects the cell against physiological antioxidative stress.

In physiological conditions, over 99% of glutathione occurs in cells in a reduced form (GSH), and only a small per cent in an oxidized form (disulfide, GSSG) [7].

Glutathione takes part in the detoxification of xenobiotics and heavy metals (copper, silver, zinc, chrom), and forms stable complexes with them. It also participates in transformations of many endogenous compounds, including lipid peroxidation products.

Its major physiological task is scavenging reactive electrophylic compounds and the playing role of intracellular "redox buffer" [7].

The functions of glutathione in the organism are associated with the thiol group (-SH) present in its molecules, which is part of the cystein residue. Glutathione acts as a substrate or co-substrate with a number of enzymes which exhibit antioxidative properties in mammals, e.g. with glutathione S-transferase or glutathione peroxidase. At the same time it can react with $^1O_2$, $HO^{\bullet}$, $O_2^{\bullet}$ itself [76].

Its thiol group reacts with hydroxide radicals (the fastest) and with radicals of the organic aqueous phase (more slowly). Glutathione reactions with the radicals of organic substances (including free radicals of other molecules) may lead to their "repair", but also result in free glutathione radical formation [7].

The major function of glutathione is to maintain thiol groups of proteins at a reduced level, which in many cases is necessary for their functional activity. Glutathione can affect the oxidation-reduction condition of certain proteins through the non-enzymatic exchange of SH/SS groups [56].

It appears that glutathione combines both enzymatic and non-enzymatic protection of cellular structures against oxidation.

Carotenes and Retinols

The main form of vitamin A is a β-ionic derivative showing the structure similar to transretinol. Its provitamins, α and β carotenes, are the most important for the human organism.

In the plasma, retinols are carried by the transporting proteins and are lypoproteins, while β-carotene is transferred mainly by the LDL lypoprotein fraction [87]. The largest amounts of retinol and carotene are stored in the liver. These compounds can modify cell differentiation and the immune system [29].

It has been also found that carotenes substantially reduce oxidation of cellular membrane lipids and lypoprotein fractions of the plasma [45]. They are also involved in the prevention of neoplastic disease [22], myocardial infarct and atherosclerosis [23].

β-carotene is considered to be a multifunctional antioxidant, soluble in lipids. Vitamin E and β-carotene act synergistically for the inhibition of microsomal peroxidation in lipids. β-carotene can also act as a scavanger of single oxygen, break the continuity of peroxidative reactions and inhibit the actions of lypooxygenases [23].

The mechanism of β-carotene antioxidative effect occurs in three stages [76]:

1. extinction of factors which trigger processes of peroxidation.

Triggering factors (flavines and porfines) can detach atom of hydrogen or electron from other molecules and form radicals able to react with carotene.

2. Scavenging of single oxygen at a rate of 10^{-9} -10^{-10} M^{-1} s^{-1}

$$^1O_2 + \text{carotene} \rightarrow O_2 + \text{carotene}$$

3. Scavenging of peroxyl radicals.

Zinc (Zn), Copper (Cu), Selene (Se)

It was long believed that the role of zinc, copper and selene in the maintenance of balance between pro- and antioxidative processes in the cell depends on their presence in the molecules of the antioxidative enzymes.

Bettger and O'Dell [11] have revealed that zinc takes part in the maintenance of normal structure and function of cellular membranes. Later reports [6] have described its antioxidative effect irrespective of the enzymes it forms. The mechanism of antioxidative action of zinc still remains unknown.

It is assumed that Zn exhibits its antioxidative effect only at high concentrations. The likely antioxidative role of extracellular Zn is to protect sulfhydryl groups against oxidation and inhibition of ROS generation (with the involvement of transition metals) [6].

A positive correlation has been revealed between low zinc concentration and reduced α-tocopherol levels in plasma [89]. It has also been demonstrated that a reduction in zinc concentration leads to the increased production of H_2O_2 and elevated activity of NADPH-dependent reductase and cytochrome P-450, which in turn causes increased generation of active forms of oxygen [36]. There is a correlation between zinc concentration and cell damage by TNF (tumour necrosis factor). Zinc inhibits TNF-induced DNA fragmentation, cytolysis and cytotoxic ADP [1]. It prevents inflammatory processes of the endothelium induced by unsaturated fatty acids and their derivatives [41], and delimits atherosclerosis. It also has a regulatory role in the process of cytokin production [12].

Normal zinc concentration is indispensable in the functioning of approximately 200 enzymes; e.g. a decrease in zinc content leads to reduced activity of Zn-dependent dehydratase of delta-aminolevulinic acid, the enzyme which takes part in heme synthesis [50]. Studies which involved patients with sickle cell anaemia revealed zinc deficiency. Zinc, with no change in the Bohr effect, increases affinity for oxygen.

A 10-fold reduction in a daily zinc intake causes a decrease in SOD activity by approximately 20% [74], and at the same time attenuates the potential of the antioxidative barrier. On the other hand, the increased supply of zinc to the organism considerably diminishes copper absorption from the alimentary tract, which also determines the activity of superoxide dismutase [32].

Copper exhibits a dual effect. In the form of free cupric ions, as a transition metal, it is a strong peroxidative factor. On the other hand, it is part of SOD and Cp, the enzymes which prevent these processes.

Copper is the main factor responsible for peroxidative modifications of the LDL lypoprotein fraction, reduction in blood cholesterol and LDL levels [49].

A positive effect of copper on lipid metabolism is probably associated with the fact that at its adequate concentration glutathione level and activity of 3-hydroxy-3-metylglutaryl reductase of the Co-A in hepatocytes remain low. Its reduced concentration leads to increased activity of the enzyme [3] and to attenuated activity of the lecitin:cholesterol acetyltransferase and lypoprotein lypase. Changes in the activity of these enzymes due to alterations in copper concentrations are responsible for hypercholesterolaemia [49].

The normal Cu/Zn ratio in the organism is necessary to regulate the production of interleukins, the primary mediators of inflammatory processes.

The antioxidative action of selene is determined mainly by its role as an essential component of the active centre of glutathione peroxidase [73]. Recent years have brough new data concerning other antioxidative mechanisms with selene involvement. In the cell, the highest selene concentration can be observed in the cellular nucleus, cytoplasm, mitochondria amd microsomes [90]. Its transport between cells occurs via the plasma [65]. Selene stabilizes erythrocytic membranes [91] and normalizes the production of $^{\cdot}OH$ by neutrophils during the immune response [5].

Balance violation between pro- and antioxidative processes in the organism is referred to as oxidative stress. Balance disturbances can be induced by an increase in ROS-catalyzed reactions and a decrease in the activity or concentration of antioxidants. It seems, however, that irrespective of the cause, the oxidative stress has biomedical implications which lead to numerous pathologies. Yet it should be remembered that there are a large number of risk factors of various origin, e.g. environmental or genetic.

Increased generation of ROS and their metabolites or attenuated antioxidative efficiency have been demonstrated in the course of such diseases as atherosclerosis, stroke, myocardial infarct, carcinoma, cataract, AIDS, acute respiratory insufficiency syndrome, Parkinson's disease, rheumatoid arthritis, emphysema, neurological diseases and malaria [40]. Epidemiological reports suggest that the intake of increased amounts of antioxidants in a diet reduces the risk of circulatory disorders. A protective effect of vitamin E in this case consists of the delimitation of LDL fraction oxidation, platelet adhesion and aggregation, atherosclerotic changes, onset of angina pectoris and mortality associated with these diseases [22,86].

Numerous reports have described a positive role of ROS in cell metabolism, mainly its regulatory function. Reactive oxygen forms take part in many processes essential for cell life. It has been found that the action of the so called secondary messenger, a cyclic GMP, is regulated by O_2^-, $^{\cdot}OH$ and H_2O_2 [85]. ROS are bound to participate in such processes as cell differentiation and maturation [79]. The presence of the supraoxide anion radical determines normal metabolism of xenobiotics

(e.g. hydroxylations), synthesis of vitamin K-dependent coagulation factors (prothrombin, factor VII, factor IX), normal platelet aggregation and maintenance of adequate potential of the cellular membranes [75].

Free oxygen radicals and their derivatives play a significant role in the metabolism of cells which utilise oxygen as a source of energy. Extra- and intracellular substances, antioxidative in nature, constitute an important barrier of the organism against excessive generation of radicals and propagation of peroxidative reactions. Balance maintenance between the actions of these compounds determines normal functioning of the organism, while its violation may lead to a number of abnormalities.

References

1. AGARWAL S., DRYSDALE B.E., SHIN H.S. Tumor necrosis factor-mediated cytotoxicity invokes ADP-ribosylation. J. Immunol. **140**, 4187, **1988**.
2. AKERBOOM S.P., SIES H. Transport of glutathione, glutathione disulfide, and glutathione conjugates across the hepatocyte plasma membrane. Methods Enzymol. **173**, 525, **1989**.
3. ALLEN K.G.D., KLEVAY L.M, Copper, An antioxidant nutrient for cardiovascular health. Review. Curr. Opin. Lipidol. **5**, 22, **1994**.
4. ARORA S.S., GORES G.J. The role of metals in ischemia/reperfusion injury of the liver. Semin. Liv. **16**, 31, **1996**.
5. ATROSHI F., WESTERMARCK T. Trace elements and glutathione-enzymes in inflamation. Trace Elements in Health and Disease, Trace'89, ed. G.T. Yuregi, O. Donma, L.Kayrin, Adana, Turkey. 575, **1991**.
6. BARJA G., LOPEZ-TORRES M.M., PEREZ-CAMPO R., ROJAS C., CADENAS S., PRAT J., PAMPLONA R. Dietary vitamin C decreases endogenous protein oxidative damage, malondialdehyde, and lipid peroxidation and maintains fatty acid unsaturation in the guinea pig liver. Free Radic. Biol. Med. **17**, 105, **1994**.
7. BARTOSZ G. Metabolizm glutationu. Post. Biochem. **39**, 32, **1993**.
8. BAST A, GORIS R.J.A. Oxidative stress. Biochemistry and human disease. Pharm. Weekbl.Sci. **6**, 199, **1989**.
9. BAYER R.E. An analysis of the coenzyme Q in free radical generation and as an antioxidant. Biochem. Cell. Biol. **70**, 390, **1992**.
10. BENDICH A., Physiological role of antioxidants in the immune system. J. Dairy. Sci. **76**, 2789, **1993**.
11. BETTGER W.J., O˙DELL B.L. A critical physiological role of zinc in the structure and function of biomembranes. Life Sci. **28**, 1425, **1981**.
12. BIAŁKOWSKA M., NOWICKA G., MAJCHRZAK J., KACZOROWSKI J., DANKO B. Hair zinc and copper concentration in surviviors of myocardial infarction. Ann. Nutr. Met. **31**, 327, **1987**.
13. BLOCK G., Epidemiologic evidence regarding vitamin C and cancer. Am. J. Clin. Nutr. **54**, 1310S, **1991**.
14. BLOMSTRAND R., FORSGREN L. Labrlled tocopherols in man. Int. J. Vitamin. Nutr. Res. **38,** 328, **1968**.
15. BRENT A.J., RUMACK B.H. Role of free radicals in toxic hepatic injury. I. Free radical biochemistry. Clin. Toxicol. **31**, 139, **1993**.
16. BONORDEN W.R., PARIZA M.W. Antioxidant nutrients and protection from free radicals. Nutritional Toxicology, ed. F.N. Kotsonis, M. Mackey i J. Hjelle, Raven Press Ltd, New York. 19, **1994**.
17. BRAY T.M., BETTGER W.J. The physiological role of zinc as an antioxidant. Free Radical Biol. Med. **8**, 281, **1990**.
18. BRIVIBA K., SIES S. Nonenzymatic antioxidant defense systems. Natural antioxidants in human health and disease, ed. B. Frei, Acad. Press, San Diego, Ca. **1994**.
19. BUETTNER G., JURKIEWICZ B.A. Catalytic metals, ascorbate and free radicals:combinations to avoid. Nutr.Res. **145**, 532, **1996**.
20. BUNK M.J., DNISTTRIAN A.M., SCHWARTZ M.K., RIVLIN R.S. Dietary zinc deficiency decreases plasma concentrations of vitamin E. Proc. Soc. Exp. Biol. Med. **190**, 379, **1989**.
21. BURTON G.W., INGOLD K.U. Vitamin E. Application of physiol organic chemistry to the exploration of its structure and funktion. Act. Chem. Res. **19**, 194, **1986**.
22. BYERS T., PERRY G. Dietary carotenes, vitamin C, and vitamin E as protective antioxidants in human cancers. Annu. Rev. Nutr. **12**, 139, **1992**.
23. CANFIELD L.M., FORAGE J.W., VALENZUELA J.G. Carotenoids as cellular antioxidants. Proc. Soc. Exp. Biol. Med. **200**, 260, **1992**.
24. CHANCE B. Effect of pH upon the reaction kinetics of the enzyme-substrate Compounds of catalase. J. Biol. Chem. **194**, 471, **1952**.
25. CIECHANOWSKI K. O wolnych rodnikach w medycynie. Pol. Tyg. Lek. **42**, 939, **1987**.
26. DE GROOT H., HEGI U., SIES H. Loss of α-tocopherol upon exposure to nitric oxide or the sydonimine SIN-1, FEBS Lett. **315**, 139, **1993**.
27. DEUTSCH J.C. Ascorbic acid oxidation by hydrogen peroxide. Anal. Biochem. **255**, 1, **1998**.
28. DEUTSCH J.C. Spontaneous hydrolysis and dehydration of dehydroascorbic acid in aqueous solution. Anal. Biochem. **260**, 223, **1998**.
29. DROGE W., ECK H.P., GMUNDER H., MIHM S. Requirement for prooxidant and antioxidant states in T cell mediated immune responses. Relevance for the pathogenetic mechanisms of AIDS? Klin. Wochenschr. **69**, 118, **1991**.
30. DUTTA-ROY A.K., GORDON M.J., CAMPBELL F.M., DUTHIE G.G, JAMES W.P.T. Vitamin E requirements, transport and metabolism: Role of α-tocopherol-binding proteins. J. Nutr. Biochem. **15**, 562, **1994**.
31. FREDERIKS W.M., BOSCH K.S. Lokalization of superoxide dismutase activity in rat tissues. Free Radic. Biol. Med. **22**, 241, **1997**.
32. FLEMING C.R., LIE J.T., McCALL J.T., O'BRIEN J.F., BAILIE E.E., THISTLE J.L. Selenium deficiency and fatal cardiomyopathy in patients on home parenteral nutrition. Gastroenterology. **83**, 688, **1982**.
33. FLOHE L., GUNZLER W.A. Glutathione peroxidase. Glutathione, ed. G. Thieme, Publishers, Stuttgart. 132, **1974**.
34. GAZIANO J.M., MANSON J.E., BURING J.E., HENNEKENS C.H. Dietary antioxidants and cardiovascular disease. Ann. N.Y. Acid. Sci. **669**, 249, **1992**.
35. GENOVA M.L., BONACORSI E., D'AURELIO M., FORMIGGINI G., NARDO B., CUCCOMARIO S., TURI P., PICH M.M., LENAZ G., BOVINA C. Protective effect of exogenous coenzym Q in rats subjected to partial hepatic ischemia and reperfusion. Biofactors. **9**, 345, **1999**.
36. GIRELLI D., OLIVIERIO O., STAZIAL A.M., AZZINI M., LUPO A., BERNICH P., MENINI C., GAMMARIO L., CORROCHER R. Low platelet glutathione peroxidase activity and seru selenium concentration in patients with chronic renal failure. Relations to dialysis treatment, diet and cardiovascular complications. Cli. Sci. **84**, 611, **1993**.

37. GRACE P.A. Ischaemia-reperfusion injury. Brit. J. Surg. **81**, 637, **1994**.
38. GROVER A.K., HUI J., SAMSON S.E. Catalase activity in coronary artery endothelium protects smooth muscle against peroxide damage. Eur. J. Pharmacol. **387**, 87, **2000**.
39. GUIDI G., SCHIAVON R., BIASIOLI A., PERONA G. The enzyme glutathione peroxidase in arachidonic acid metabolism of human platelets. J. Lab. Clin. Med. **104**, 574, **1984**.
40. HALLIWELL B., GUTTEIDGE J.M. The antioxidants of human extracellular fluids. Arch. Biochem. Biophis. **280**, 1, **1990**.
41. HENNING B., DIANA J.N., TOBOREK M., McCLAIN C.J. Influence of nutrients and cytokinines an endotheliall cell metabolism. J. Am. Coll. Nutr. **3**, 224, **1994**.
42. HYLAND K., VOISIN E., BANOUN H., AUCLAIR Ch. Suproxide dismutase assay using alkaline dimethylsulfoxide as superoxide anion-generating system. Anal.Biochem. **135**, 280, **1983**.
43. INYANG U.E., IKE C.I. Effect of blanching, dehydration method and temperature on the ascorbic acid, colour, sliminess and other constituents of okra fruit. Int. J.Food Sci. Nutr. **49**, 125, **1989**.
44. ISCHIROPOULOS H., AL-MEHDI A.B. Peroxynitrite-mediated oxidative protein modifications. FEBS Lett. **364**, 279, **1995**.
45. JIALAL I., NORKUS E.P., CRISTAL L., GRUNDY S. Beta-carotene inhibits the oxidative modification of low-density lipoprotein. Biochem. Biophys. Acta . **1086**, 134, **1991**.
46. JEWETT S., ROCKLIN A.M. Variation of one unit of activity with oxidation rate of organic substrate in indirect superoxide dismutase assays. Anal. Bioch. **212**, 555, **1993**.
47. KAGON V.E., BAKALOVA E.A., STOYTCHEV E.S. Fluorescent measurment of incorporation and hydrolysis of tocopherol and its esters in biomembranes. Oxygen radicals in biological systems. Part B.: Oxygen radicals and antioxidants, ed. L. Packer, A. Glazer,: Methods in enzymology. Acad. Press. San Diego. 355, **1990**.
48. KELLEHER J., LOSOWSKI M.S. The absorption of α-tocopherol in man. Brit. J. Nutr. **24**, 1033, **1970**.
49. KLEVAY L.M. Soy protein may affect plasma cholesterol through copper. Am. J. Clin. Nutr. **2**, 300, **1994**.
50. KULIKOWSKA E., MONIUSZKO-JAKONIUK J., MINIUK K. Rola cynku w procesach fizjologicznych i patologicznych organizmu. Pol. Tyg. Lek. **24-26**, 470, **1991**.
51. KWON O.J., LEE S.M., FLOYD R.A., PARK J.W. Thiol-dependent metal-catalized oxidation of copper, zinc superoxide dismutase. Biochem. Biophys. Acta. **1387**, 249, **1998**.
52. LARDINOIS O.M., MESTDAGH M.M., ROUXHET P.G. Reversible inhibition and irreversible inactivation of catalase in presence of hydrogen peroxide. Biochem.Biophys. Acta. **1295**, 222, **1996**.
53. LARDINOIS O.M. Reactions of bovine liver catalase with superoxide radicals and hydrogen peroxide. Free Radical Res. **22**, 251, **1995**.
54. LASKOWSKI H., MINCZKOWSKI A, WYSOCKI H. Mortality and clinical course of patients with acute myocardial infarction treated with streptokinase and antioxidants: mannitol and ascorbic acid. Int. J. Cardiol. **48**, 235, **1995**.
55. LEAL A.L., BEGONA-RUIZ-LARREA R., LACORT M. Cytoprotective actions of estrogens against tert-butyl hydroperoxide-induced toxicity in hepatocytes. Biochem. Pharmacol. **56**, 1463, **1998**.
56. LENARTOWICZ E., WUDARCZYK J., DĘBSKA G. Regulacja stopnia oksydoredukcji grup tiolowych w komórkach zwierzęcych. Post. Biochem. **42**,154, **1996**.
57. LEWIN G., POPOV I. The antioxidant system of the organism. theorethical basis and practical cosequences. Med. Hypoth. **42**, 269, **1994**.
58. LICZMAŃSKI A.E. Toksyczność tlenu II. Mechanizmy obronne. Postępy Bioch. **4**, 293, **1988**.
59. MAGUIRE J.J., KAGON V., ACKRELL B.A., PACKER L. Succinate-ubiquinone reductase linked recycling of alpha-tocopherol in reconstituted systems and mitochondria, requirement for reduced ubiquinone. Arch. Biochem. Biophys. **292**, 47, **1992**.
60. MARKLUND S.L. Distribution of Cu-Zn superoxide dismutase and Mn superoxide dismutase in human tissues and extracellular fluids. Acta Physiol. Scand. **492**, 19, **1980**.
61. MARKLUND S.L. Properties of extracellular superoxide dismutase from human lung. Biochem. J. **220**, 269, **1984**.
62. McCORD J.M., FRIDOVICH I. Superoxide dismutase. An enzymatic function for erytrocuprein (hemocuprein). J. Biol. Chem. **244**, 6049, **1969**.
63. MERGENS W.J., KAMM J.J., NEWMARK H.L. Alpha tocopherol: uses in preventing nitrosamine formation. Environmental aspects of N-nitroso compounds, ed. E.A. Walker, M. Castegnaro, L. Gricute, R.E. Lyle, IARC Scientific Publ., Lion. 190, **1978**.
64. MICHIELIS C., RAES M., TOUSSAINT O., REMACLE J. Importance of Se-glutathione peroxidase, catalase and Cu/Zn-SOD for cell survival against oxidative stress. Free radical. Biol. Med. **3**, 235, **1994**.
65. MOTSENBOCKER M.A., TOPPEL A.L. A selenocysteine containing selenium-transport protein in rat plasma. Biochem. Biophys. Acta. **704**, 253, **1982**.
66. NESS A.R., KHAW K.T., BINGHAM S., DAY N.E. Vitamin C status and blood pressure. J. Hypertens. **14**, 503, **1996**.
67. NEUZIL J., STOCKER R. Bilirubin attenuates radical-mediated damage to serum albumin. FEBS Lett. **331**, 281, **1993**.
68. OSHIMA H., GILIBERT I., BIANCHINI F. Induction of DNA strand breakage and base oxidation by nitroxyl anion through hydroxyl radical production. Free. Radical Biol. Med. **26**, 1305, **1999**.
69. PAIVA S.A., RUSSELL R.M. Beta-carotene and other carotenoides as antioxidants. J. Am. Coll. Nutr. **18**, 424, **1999**.
70. PUTMAN C.D., ARVAI A.S., BOURNE Y., TAINER J.A. Active and inhibited human catalase structures: ligand and NADPH binding and catalitic mechanism. J. Mol. Biol**. 296**, 295, **2000**.
71. RETSKY K.L., FREEMAN M.W., FREI B. Ascorbic acid oxidation productes protect human low density lipoprotein against atherogenic modification. J. Biol. Chem. **268**, 1304, **1993**.
72. RUTKOWSKI M., GRZEGORCZYK K. Witamina C w medycynie: „prawidłowe stężenie" w osoczu. Pol. Merk. Lek. **6**, 57, **1999**.
73. SCHOLZ R.W., SAINI A.K., REDDY P.C., REDDY C.C. Dietary vitamin E and selenium effects on resistance to oxidative stress in rat liver mitochondria. Biochem. Mol. Biol. Intern. **6**, 1215, **1994**.
74. SEMMAN S. Dietary versus cellular zinc. The antioxidant paradox. Free Radical Biol. Med. **14**, 95, **1993**.
75. SEVANIAN A., DAVIES J.A., HOCHSTEIN P. Serum urate as en antioxidant for ascorbic acid. Am. J. Clin. Nutr. **54**, 1129S, **1991**.
76. SIES H. Strategies of antioxidant defense. Europ. J. Bio-

chem. **215**, 213, **1993**.
77. SIMON J.A. Vitamin C and cardiovascular disease: A reviev. J. Am. College Nutr. **11**, 107, **1992**.
78. SOHAL R.S. The free radical hypothesis of aging: An appraisal of the current status. Ageing Clin. Exp. Res. **4**, 3, **1993**.
79. TAN I.K., CHUA K.S., TOH A.K. Serum magnesium, copper and zinc concentrations in acute myocardial infarction. J. Clin. Lab. Anal. **6**, 324, **1992**.
80. TANFORD C., LOVRIEN R. Dissociation of catalase into subunits. J. Am. Chem. Soc. **84**, 1892, **1962**.
81. TOUSOULIS D., DAVIES G. Vitamin C increases nitric oxide availablity in coronary atherosclerosis. Ann. Intern. Med. **131**, 156, **1999**.
82. TRABER M.G., SCHIANO T.D., STEEPHEN A.C., KAYDEN H.J., SHIKE M. Efficacy of water-soluble vitamin E in the treatment of vitamin E malabsorption in short-bowel syndrome. Am. J. Clin. Nutr. **59**, 1270, **1994**.
83. TSAN M.F. Superoxide dismutase and pulmonary oxygen toxicity. P.S.E.B.M. **203**, 286, **1993**.
84. TSE W.Y., MAXWELL S.R., THOMASON H., BLANN A., THORPE G.H., WAITE M., HOLDER R. Antioxidant status in controlled and uncontrolled hypertension and its relationship to endothelial damage. J. Hum. Hypertens. **8**, 843, **1994**.
85. ULLRICH V., BRUNE B., HECKER G., SCHMIDT K.U., MULSCH A., BUSSE R. Physiological target of superoxide anion and hydrogen peroxide in reperfusion injury. Free Radical. Res. Comms. **3-6**, 265, **1989**.
86. UPSTON J.M., KARJALAINEN A., BYGRAVE F.L., STOCKER R. Efflux of hepatic ascorbate: a potential contributor to the maintenance of plasma vitamin C. Biochem.J. **342**, 49, **1999**.
87. WEFERS H., SIES H. Antioxidant defense: vitamin E and C and beta-carotene. Oxy-Radicals in molecular biology and pathology, ed. P.A. Cerutti, I. Fridovich, J.M.McCord, Alan R. Liss Inc. San Diego, New York. 481, **1988**.
88. WILSON K., LANG D., HAWES A.S., ZELANO J., ISOM W., KRIGER K.H. Platelet-activating factor antagonism attenuates platelet and neutrophil activation and reduces myocardial injury during coronary reperfusion. J. Surg. Res. **55**, 504, **1993**.
89. WISPE J.R., CLARK L.C., BURHANS M.S., KROPP K.E., KORFHAGEN T.R., WHITSETT J.A. Synthesis and processing of the precursor for human mangano-superoxide dismutase. Biochim. Biophis. Acta. **994**, 30, **1989**.
90. ZACHARA B.A. The unusual and unknown function of selenoprotein P. Appl. Biol. Commun.. **2**, 89, **1992**.
91. YANG F.Y., YANG M.L. Reaction of the Se with SH groups in spectrin is involved in the stabilization of erytrocyte membrane skeleton. Biochem. Int. **15**, 1085, **1989**.
92. Yu B.P., Cellular defenses against damage from reactive oxygen species. Physiol. Rev. **1**, 139, **1994**.