REVIEW

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Trace elements as constituents of antioxidative proteins

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Abstract: The human body is under constant attack by free radicals (reactive oxygen species – ROS). Free radicals are highly reactive molecules generated by biochemical redox reactions which occur as a part of regular cell metabolism, and originate under the influence of some external causes, such as ultraviolet light, cigarette smoke, environment pollution, gamma radiation or some pharmacological agents. ROS trigger macromolecular damage inside the cell (lipids, proteins, nucleic acids). Over the centuries of evolution, organisms have created defence mechanisms against reactive oxygen species. The role of those mechanisms, among other things, is to stop free radical chain reactions, block any reaction of oxygen free radicals with compounds vital for the organism, and cleanse the body of the effects of free radical reactions with body molecules. The defence system of the organisms consists of enzymes whose task is to decompose radical compounds, binding proteins which transport and store metal ions, and enzymes which have the role of repairing the damage caused by free radicals. Trace elements support the antioxidative system within the organism. Their activity consists in blocking chain reactions of free radicals, as well as controlling the reaction of free radicals with the components of the organism. As co-enzyme ingredients and structural elements of macromolecules, they also act as metabolism regulators.

Key words: trace elements, antioxidative protein, reactive oxygen species, copper, manganese, selenium, zinc

Abbreviations: AD – Alzheimer's disease, CAT – catalase, Cu – copper, CuZnSOD – copper-zinc superoxide dismutas, GPX – glutathion peroxidase, GSH – reduced glutathione (gamma-glutamyl-cysteine-glycine), GSSG – oxidated glutathione, GSSG-R – glutathione reductase, H_2O_2 – hydrogen peroxide, lipid-OOH – lipid peroxide, lipid-OH – reduced lipide, Mn – manganese, MnSOD – manganese superoxide dismutase, MT – metallothionein, NADP – nicotinamide adenine dinucleotide phosphate, NADPH – nicotinamide adenine dinucleotide phosphate (reduced form), O_2 – oxygen, $O_2^{\cdot -}$ – superoxide, ROS – reactive oxygen species, Se – selenium, SH^- – thiolic groups, SLE – systemic lupus erythematosus, SM – sclerosis multiplex, SOD – superoxide dismutase, VTED – Venous thrombo-embolic disease, Zn – zinc, Zn-MT – zinc bound metallothionein

INTRODUCTION

The human body is under constant attack by free radicals. Free radicals are highly reactive molecules generated through biochemical *redox* reactions which occur as part of normal cell metabolism, and are a result of some external factors, such as ultraviolet light, cigarette smoke, environment pollution or gamma radiation. Some toxic compounds may also trigger the production of free radicals, among others, antineoplastic drugs, anesthetics, analgesics [1].

Free radicals are just one of the active elements in the pathomechanism of various diseases. Elevated level of free oxygen radicals is observed, among others, in such pathological conditions as Alzheimer's disease (AD), Parkinson's disease, venous thrombo-embolic disease (VTED), diabetes, sclerosis, neoplastic diseasees, peptic ulcer, glaucoma, hyperthyroidism, rheumatoid arthritis, multiple sclerosis (MS), systemic lupus erythematosus (SLE), and many others [1, 2-4].

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ANTIOXIDATIVE SYSTEM OF THE BODY

Over the centuries of evolution, organisms have developed specific mechanisms for defence against reactive oxygen forms. The role of those mechanisms, among others, is to stop chain reactions of free radicals, block the reaction of free radicals with compounds vital for the organism, and cleanse the body of the effects that the reactions of free oxygen radicals with body molecules have on the organism.

Some of the components of the defence system of the organism are enzymes which have the role of decomposing radical compounds [Figure 1]. These enzymes are, e. g.: catalase (converts hydrogen peroxide into water and oxygen), superoxide dismutases – SOD (catalyses the reduction of superoxide anions to hydrogen peroxide), peroxidases (catalyses the reduction of hydrogen peroxide to water), glutathione peroxidase (catalyses the reduction of hydrogen peroxide to water in the presence of glutathione), glutathione reductase (an enzyme which regenerates parts of glutathione, one of the small molecular antioxidants of the cell) [5].

Proteins that bind, transport and store metallic ions (transferrin, ferritin, haptoglobin, hemopexin, ceruloplasmin, metallothionein, β-globulin, albumin) can also be considered as defensive mechanisms. It is known that free metallic ions, i.e. those not bound to protein molecules or other chelating compounds, catalyse the formation of free radicals in the Fenton reaction [1].

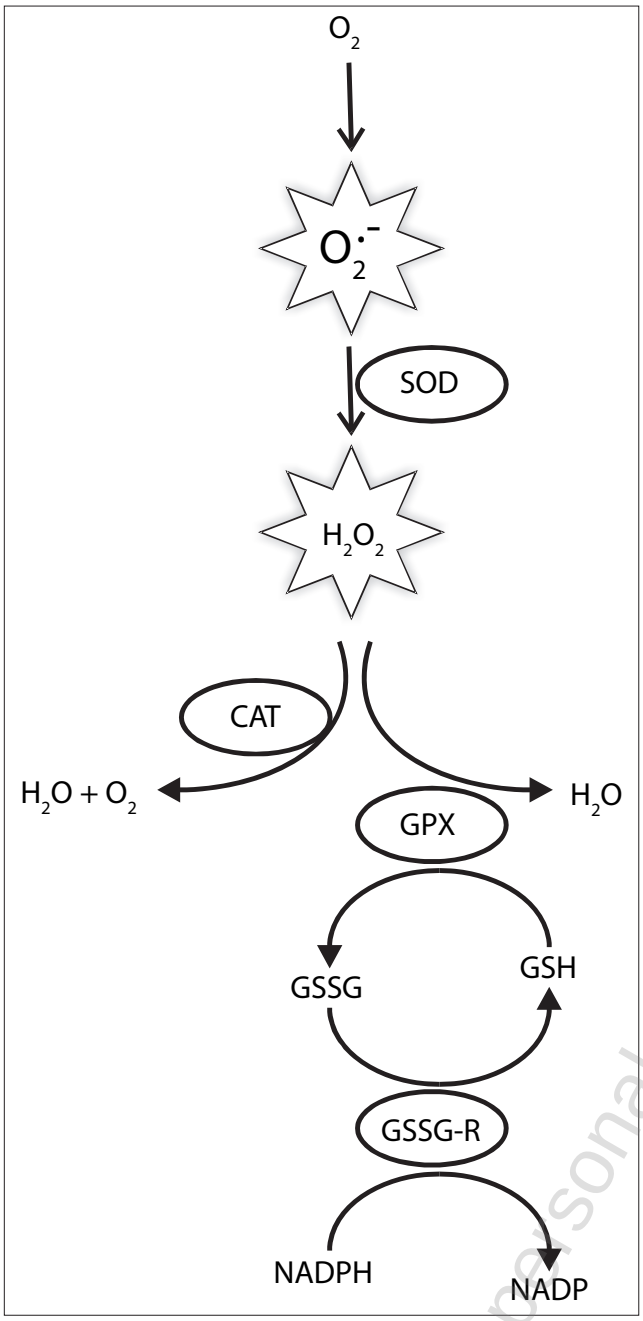

Figure 1 Antioxidant enzymes.
CAT – catalase; GPX – glutathione peroxidase; GSH – reduced glutathione (gamma-glutamyl-cysteine-glycine); GSSG – oxidated glutathione; GSSG-R – glutathione reductase; H_2O_2 – hydrogen peroxide; NADP – nicotinamide adenine dinucleotide phosphate; NADPH - nicotinamide adenine dinucleotide phosphate (reduced form); O_2 – oxygen; $O_2^{\cdot-}$ – superoxide; SOD – superoxide dismutase.

The enzymes that repair damage caused by free radicals, e. g. the destruction of purine and pirimidine bases constituting nucleic acids or even nucleic acid strand breaks, are also a part of the organism's defence system.

Such small molecular free radical scavengers (small molecular antioxidants) as vitamins and trace elements are also of crucial importance for the defence system.

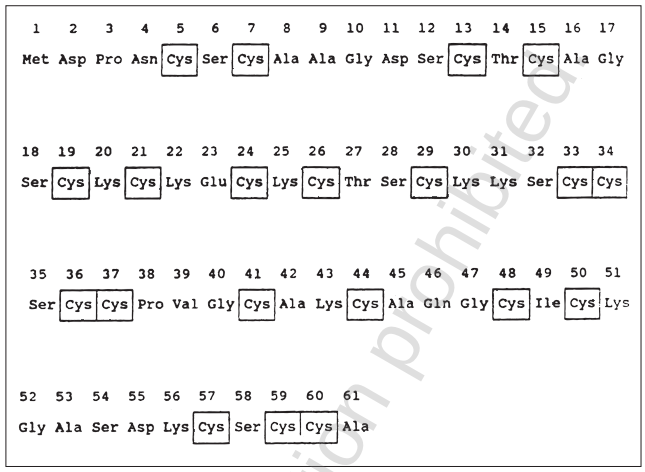

Figure 2 Structure of metallothionein.
Metallothioneins (MT) are low molecular weight (6~7 kDa), cysteine-rich metal binding proteins found in a wide variety of organisms, including bacteria and fungi, as well as plant and animal species. The high number of cysteines (20 residues in mammals) allows a high capacity for binding metal ions through sulfhydryl groups, forming metal-thiolate complexes. As a consequence, neither disulfide nor free sulfhydryl groups are present in the MT structure.

TRACE ELEMENTS IN THE ANTIOXIDATIVE SYSTEM

The volume of trace elements (microelements) in the organism does not exceed 0.01% (mass percent). Microelements act as enzyme co-factors or are structural elements of macromolecules [6].

Selenium (Se) is part of an enzyme called glutathione peroxidase which has an important antioxidative function. Four molecular forms of this enzyme are distinguished:
– classic glutathione peroxidase (located mainly in the liver and erythrocytes);
– plasmatic glutathione peroxidase;
– glutathione peroxidase (found in the alimentary tract);
– phospholipid peroxide glutathione peroxidase.

The first three molecular forms of the enzyme display a tetrameric structure (each monomer includes a selenium atom contained in selenocystein), the fourth is not a monomer [7]. The enzyme was discovered for the first time in bovine erythrocytes. It catalysed the reaction of the decomposition of toxic hydrogen peroxide (H_2O_2) with the participation of a reduced glutathione [8]:

$$2\,GSH + H_2O_2 \longrightarrow GSSG + 2\,H_2O$$

(GSH – reduced glutathione; GSSG – oxidated glutathione)

Glutathione peroxidase is present in cytosol and in small amounts in the mitochondria. Its function is to protect the cell structures from the devastating consequences of activities of free radicals. It seems that the first three molecular forms of the enzyme mainly operate within the aqueous spaces of the cell, whereas the fourth – phospholipid peroxide glutathione peroxidase – operates within the lipid cells. The enzyme decelerates the process of lipid peroxidation and reduces the already present lipid peroxides:

$$\text{lipid-OOH} + 2\,GSH \longrightarrow \text{lipid-OH} + GSSG + HzO$$

(lipid-OOH – lipid peroxide, lipid-OH – reduced lipid,
GSH – reduced glutathione GSSG – oxidated glutathione).

Glutathione peroxidase acts in cooperation with other elements of the organism, constituting the antioxidative system, by means of peroxide dismutases and catalases.

The components of superoxide dismutases are copper, zinc and manganese (in microbial dismutases it is also iron) [9]. In eucariotic organisms there are two kinds of dismutases: a copper-zinc enzyme (CuZnSOD) – present in the cytosol and biological solutions, and a manganese enzyme (MnSOD) – present in the mitochondria. Both catalyse the reduction of the toxic superoxide anion radical [10]:

$$SOD(Cu^{1+}Zn) + O_2^- + 2H^+ \longrightarrow SOD(Cu^{2+}Zn) + H_2O_2$$

During the reaction process changes occur in the degree of copper oxidation (in copper-zinc dismutase) or manganese oxidation (in manganese dismutase). Zinc, which is a part of copper-zinc dismutase, only has a constituting function. Hydrogen peroxide (H_2O_2) is decomposed by catalases – hemoprotein enzymes (with iron incorporated into a haemic component [11]:

$$2\,H_2O_2 \longrightarrow O_2 + 2\,H_2O,$$

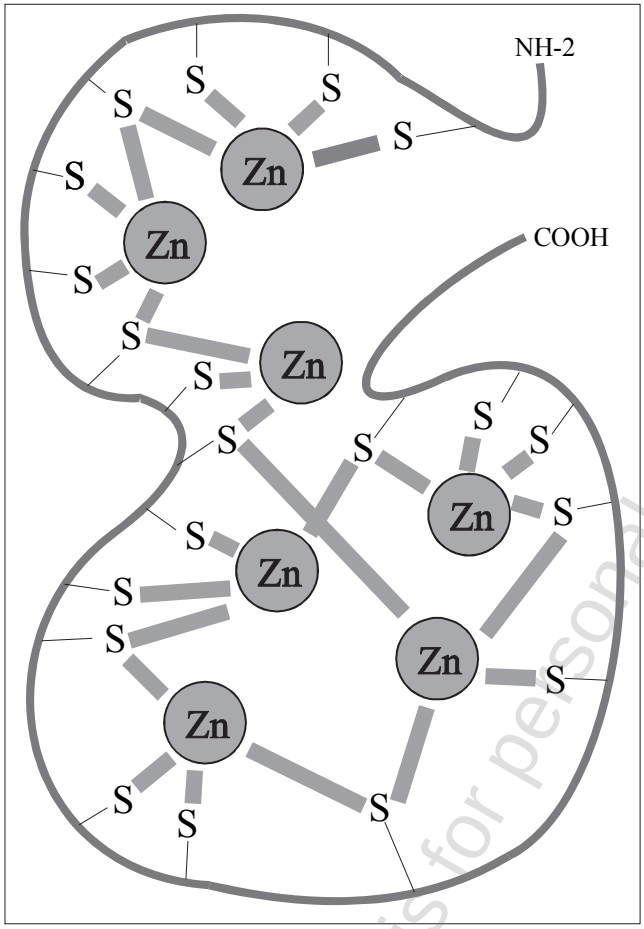

Figure 3 Metallothionein binding 7 Zn.
The main functions of MT are homeostatic control, metabolism, and detoxification of a number of essential (Cu, Zn) and toxic (Cd, Hg) trace metals. At acidic pHs, MT becomes depleted of metals and are called apothionein. MTs are unique low molecular weight proteins with a high cysteine content, and high affinity for heavy metal ions (typically Cd and Zn). They have ~60 amino residues of which 20 are cysteines which bind 7 equivalents of divalent transition metals.

Copper and zinc are part of metallothioneins [Figure 2] – small molecular proteins with a recognized antioxidative function. They have a single chain of 61 aminoacids (6-7 kDa) containing 20 residues of cystein appearing in repeating sequences: Cys-X-Cys, Cys-X-Y-Cys or Cys-Cys. Cystein is a regular structural unit of metallothioneins in all animal species. The electrophillic character of sulphur in the sulphydril groups of amino acid is responsible for its high affinity to metallic ions. Metallothioneins display the highest affinity for metals of the transitory groups (zinc, cadmium, copper, silver). One molecule of protein can bind 7 atoms of bivalent metals (e.g. zinc) [Figure 3] or a larger amount (12 atoms) of univalent metals (e.g. silver). The chief function of metallothioneins is to bind and distribute zinc and copper, and in the case of environmental pollution – to bind toxic metals [12, 13].

Microelements assist in the elimination of free oxygen radicals, not only by acting as enzyme co-factors, but also indirectly – by shutting off the production of free radicals as they compete with toxic metals (cadmium, nickel, iron, lead) [14-18].

Trace elements assist the antioxidative system within the body. Their activity consists in blocking free radical chain reactions as well as obstructing reactions of free radicals with components of the organism. As constituents of co-enzymes and structural elements of macromolecules, they also perform the function of metabolic coordinators. Every pathological condition is followed by changes in metabolism which calls for increased consumption of vitamins and microelements.

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