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Review Article

# Cell Antioxidant Systems

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**Abstract**

Oxidative reactions and the substances formed as a result of their course are important in the vital activity of the cells of the whole organism and the brain, in particular. It has been established that oxygen radicals function as a messenger, responsible for neuronal activity; they regulate cerebral blood flow, apoptosis, and other processes necessary for the functioning of the brain. It has been shown that nerve impulse conduction is also associated with the formation of free-radical forms of phospholipids. The constant formation of prooxidants in the body is balanced by their inactivation by the antioxidant system.

**Keywords:** antioxidant systems; oxidative reactions

## Background

Oxidative reactions and the substances formed as a result of their course are important in the vital activity of the cells of the whole organism and the brain, in particular. It has been established that oxygen radicals function as a messenger, responsible for neuronal activity; they regulate cerebral blood flow, apoptosis, and other processes necessary for the functioning of the brain. It has been shown that nerve impulse conduction is also associated with the formation of free-radical forms of phospholipids [1-4].

To date, a general idea has been formed about the prooxidant-antioxidant system of the body. An imbalance between the prooxidant and antioxidant links of this system leads to hyperproduction of oxygen radicals and damage to cells and tissues [6]. The general mechanisms underlying damage to body tissues with the participation of oxidative reactions are well studied and have a universal character. However, in the brain, as well as in other organs and systems, they have specific features, which is important to take into account when modeling the experimental pathology of the central nervous system [7-12].

Cellular antioxidant defense mechanisms

The constant formation of prooxidants in the body is balanced by their inactivation by the antioxidant system. Cells have a number of properties that allow them to resist the damaging effects of ROS and APs effectively [12-19].

To provide protection against oxidative stress, cells have a well-developed antioxidant system that contains low- and high-molecular compounds that can inhibit the initiation of ROS formation, "intercept" free radicals (ROS scavengers), or neutralize the formation of lipid free radicals and lipid peroxides [20].

Antioxidant protection (AOD) is represented by enzymatic and non-enzymatic mechanisms (Figure 1).

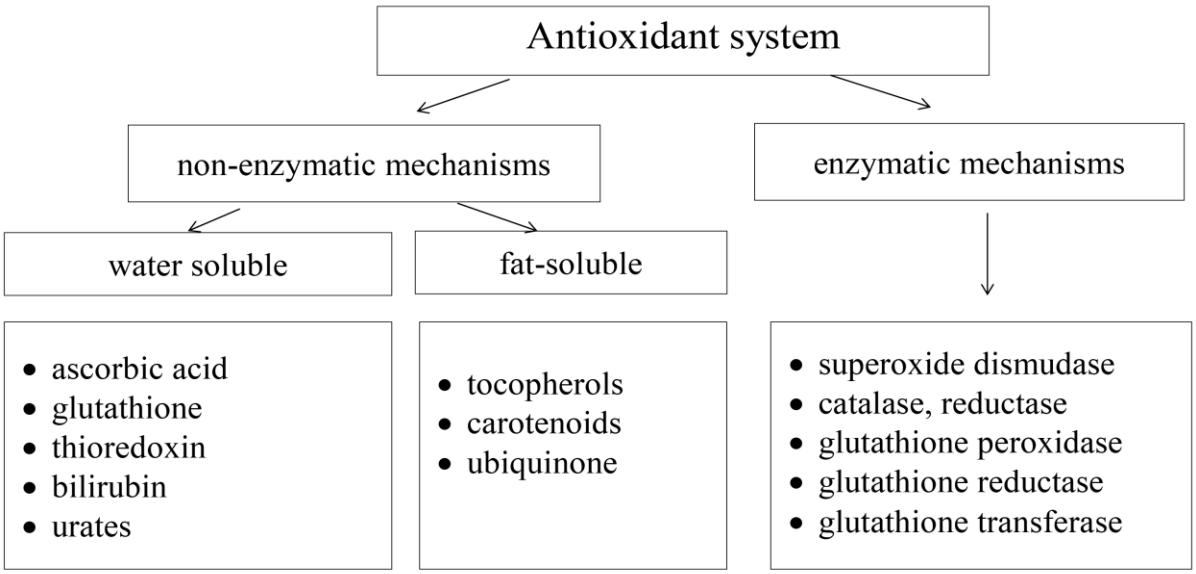


**Figure 1:** Antioxidant system.

**Non-enzymatic mechanisms of the antioxidant system**

Important elements of AOP are low-molecular non-enzymatic antioxidants – "scavengers" of ROS and AFA. Non-enzymatic antioxidants are water-soluble (ascorbic acid, glutathione, thioredoxin, bilirubin, urates act in the aqueous phase (cytoplasm, mitochondria, nucleus)) and fat-soluble (tocopherols, carotenoids, ubiquinone act in the lipid phase). The antioxidants realize their action both intra- and extracellularly [17].

**Ascorbate** (AN) is a "scavenger" for hydroxyl radicals, alkoxy radicals (RO•), peroxyl radicals (RO2•), hypochloric acid (HOCI), N2O3, peroxynitrite (ONOO) and others [20]. It takes part in the reduction

of tocopheroloxyl radical (TocO•) to α-tocopherol with the appearance of an ascorbate radical (semidehydroascorbate):

TocO•+ AH → TocOH + A•

The ascorbate radical (A•), in turn, is reduced to ascorbate (AN) with the help of reduced glutathione and the enzyme glutaredoxin:

A• + 2GSH →AH- + CSSG + H+ , as well as

NADH-dependent semidihydroascorbine reductase:

A• + NADH → AH- + HAD+, and

NADPH-dependent thioredoxin-reductase:

A• + NADP H → AH- + NADP+

**Reduced glutathione**. Tripeptide glutathione (GSH) –

γ-L-glutamyl-L-cysteine-glycine is the most common sulfhydryl compound in cells, which has its own antioxidant activity, directly inactivates the superoxide radical, hydroxyl radical and peroxynitrite, acts as a cofactor of antioxidant enzymes containing thiol group, hydrogen donor, metabolite and substrate of enzymes of the glutathione system. Glutathione is constantly synthesized in the liver and released into the blood, enters all cells of the body, except for red blood cells.

It takes part in the synthesis of proteins and nucleic acids, protects against ROS by restoring disulfide bonds, affects the activity of enzymes and other proteins, supports membrane functions, takes part in the metabolism of eicosanoids, is a reserve of cysteine, takes part in the metabolism of xenobiotics, increases cell resistance to pathogenic influences, affects proliferation. The exceptional importance of reduced glutathione is to protect

cells from damage. So, glutathione is involved in the reduction reaction of the ascorbate radical (A•) to ascorbate

(AH-), catalyzed by the enzyme glutaredoxin [4, 19].

In the cell, glutathione is used to reduce the sulfene groups in the protein molecule (Pt-SOH), which are formed during the oxidation

of the SH groups of cysteine residues.

In this case, the interaction of the reduced glutathione with the sulfene group first leads to the appearance of a mixed disulfide formed by the protein and glutathione:

Pt-SOH + GSH→ PtS-SG + H2O

and then to the reduction of the thiol group of the protein

PtS-SG + GSH→ Pt-SH + GSSG

The importance of the reduction reaction of thiol groups of proteins with glutathione is explained by the fact that the formation of sulfene ionic groups in the molecules of a number of proteins that regulate the phenotypic properties of cells changes the properties of these proteins. As a result, the function of cells as a whole can change [11].

**Ergothioneine** (natural betaine) inactivates hypochlorous acid (hypochlorite), hydroxyl radical and peroxynitrite [19].

**α-lipoic acid** is a trap for hydrogen peroxide and hypochlorite, and dihydrolipoic acid – is for superoxide radical [6].

Vitamin E (α-tocopherol), carotenoids (lycopene, β-carotene, oxycarotenoids, and xanthophylls) and ubiquinone Q act in the lipid (hydrophobic) phase.

**Vitamin E (α-tocopherol)** blocks the free radical oxidation of cell membrane lipids by interacting with the lipid peroxyl radical.

The role of **ubiquinone Q** (from the French word ubiquitaire – to be everywhere, "ubiquitous quinone"), as an antioxidant, is to reduce the tocopheroxyl radical (TocO•) to α-tocopherol. In this case, ubiquinone Q itself is converted into an ubisemiquinone radical, which is then reduced to ubiquinone Q either in the membrane electron transport chain or with the participation of vitamin C.

The antioxidant properties of carotenoids have not been studied enough. They are singlet oxygen traps and have an ability to reduce the tocopheroxyl radical (TocO) to α-tocopherol [5].

In addition to low molecular weight non-enzymatic antioxidants, scavengers ACF and APA, there are **enzymatic defense mechanisms**. Enzymatic antioxidants include membrane-bound and cytosolic enzymes (superoxide

dismutase, catalase, glutathione-dependent peroxidases and transferases), which provide the reduction of ubiquinone Q (DT-diaphora), ascorbate radical, oxidized glutathione (glutathione reductase). AOP enzymes contain the active center of metal ions with variable valence, which, depending on the conditions, act as both an oxidizing agent and a reducing agent [7].

Superoxide dismutase (SOD) catalyzes the dismutation reaction

of superoxide anion radicals:

$O2- + O2- = O2 + H2O2$

During the reaction, hydrogen peroxide is formed, which is able

to inactivate SOD, so SOD always “works” in tandem with catalase, which quickly and efficiently breaks down hydrogen peroxide into neutral compounds:

$2H2O2 = 2H2O + O2$

The property of SOD to reduce the formation of peroxynitrite is due to its ability to reduce the concentration of the superoxide radical

in the cell, which also limits its availability for the Fenton reaction. The superoxide radical stimulates the release of iron from ferritin and from the "iron-sulfur centers" of cellular proteins. There are other mechanisms that provide the protective effect of SOD as an antioxidant enzyme.

In most cells, **peroxidase** is localized in peroxisomes, and is found in significant amounts in mitochondria [7-19].

**System "glutathione-enzymes of the antioxidant system"**

The glutathione enzyme system includes three glutathione-dependent enzymes: glutathione peroxidase (GPO), glutathione reductase (GR), and glutathione transferase (GT) [5].

**Glutathione peroxidases** are selenium-containing enzymes. Currently, four types of glutathione peroxidases that differ in their structure and localization within cells have been identified.

Glutathione peroxidase catalyzes reactions in which the enzyme reduces hydrogen peroxide to water, as well as organic hydroperoxides (ROOH) to hydroxy derivatives, resulting in the conversion to the oxidized disulfide form GS-SG:

$H2O2 + 2GSH = GS-SG + H2O$

$ROOH + 2GSH = GS-SG + ROH + H2O$

Glutathione peroxidase neutralizes peroxynitrite:

$ONOO- + 2GSH \rightarrow NO2- + GSSG + 2H2O$

Glutathione reductase is a flavoprotein with a flavin adenine dinucleotide prosthetic group and consists of two identical subunits.

It catalyzes the reduction of glutathione from the oxidized form

of GS-SG, and all other enzymes of the glutathione system use it:

$2\ NADPH + GS-SG \rightarrow 2\ NADP + 2\ GSH$

The reduction of oxidized glutathione is carried out with the participation of NADP + reduction systems on the pentose phosphate pathway of glucose-6-phosphate oxidation ("Embden-Meyerhoff shunt"):

$NADP + + G-6-P \rightarrow NADP + H+ + 6 – phosphogluconate$

Glutathione transferase catalyzes the reaction:

$RX + GSH = HX + GS-SG$

Some forms of glutathione transferases, like glutathione peroxidases, are capable of reducing lipid hydroperoxides to hydroxy acids (LOH) [12].

Under normal conditions, more than 90% of glutathione is in the reduced form in the cell. Marked oxidative stress of the cell can lessen the content of reduced glutathione with an increase in the concentration of oxidized glutathione. Oxidized glutathione stimulates the formation of disulfide bonds between adjacent thiol groups in the protein molecule and changes their properties:

$GSSG + Pt-(SH)2 \leftrightarrow Pt(S-S) + 2GSH$, and also interacts with SH-groups of proteins and forms "mixed disulfides", which changes the properties of proteins, causing dysfunction of cells.

$GSSG + Pt-SH \rightarrow PtS-SG + GSH$

Deficiency of reduced glutathione deprives the cell of its antioxidant reserve, making it more susceptible to the damaging effects of ROS [14].

**The study of the prooxidant-antioxidant state of the brain**

**in the simulation of experimental cerebral pathology**

To determine the prooxidant-antioxidant state of the brain in its homogenates (20% dilution in PBS (pH 7.2)) determine the activity of lipid peroxidation processes (the content of products that react with thiobarbituric acid (TBKRS)), the concentration of reduced glutathione (GSH), thiol groups (TSH), and glutathione peroxidase activity [19].

To determine the content of TBCRS, 2.4 mL of a 0.07 N sulfuric solution and 0.3 mL of a 10% solution of phosphotungstic acid are sequentially added to the test sample of 10% brain homogenate (0.3 mL). The precipitate is washed twice, dissolved in 3.0 mL of bidistilled water, 1 mL of a 0.85% aqueous solution of TBA is added, dissolved in 25 mL of acetic acid with the addition of 5 mL of H2O. The color reaction takes place in hermetically sealed tubes at 96 °C for 60 minutes. After cooling them in water for 5 minutes, the optical density of the centrifuged supernatant is determined on a PV 1251C spectrophotometer (Solar, Belarus) at wavelengths of 532 nm and 580 nm. The concentration of TBCR is calculated by the formula: TBCR = (E532 – E580)/0.156 × K, where E is the extinction at the corresponding wavelengths, V1 is the volume of the TBA solution; V2 is the volume of the test sample; K is the dilution factor of the brain sample (147.7). The calculation of the concentration of TBBS is carried out using the absorption coefficient for the resulting product 532=1.56×105 M-1×cm-1 and expressed in nanomoles per gram of protein (gram of tissue) [2].

When measuring the concentration of GSH to 1 mL of 15% brain homogenate add 0.2 mL of 25% trichloroacetic acid, shake, and centrifuge at 5,000 rpm for five minutes. 1.2 mL of 0.5 M phosphate buffer (pH 7.8) and 50 µl of Ellman's reagent were added to the resulting supernatant (0.2 ml). The GSH concentration is calculated taking into account the molar extinction coefficient (ε412 =13600 M-1 cm-1) by determining the optical density of the samples under study at λ=412 nm on a PV 1251C spectrophotometer. The determination of the TSH concentration is carried out as follows: add 30 µL of 3% dodecyl sulfate sodium salt solution

to 60 µL of brain homogenate. Take 25 µL of the resulting mixture and combine with 1.2 mL of 0.5 M phosphate buffer (pH 7.8) and 50 µL of Ellman's reagent. After 10 minutes of incubation at room temperature determine the optical density on the PV 1251C spectrophotometer at λ=412 nm, taking into account the molar extinction coefficient. The molar extinction coefficient in determining the content of TSH is 13600 M-1 cm-1.

To measure the activity of glutathione peroxidase, 0.1 mL of 0.1 mL of brain homogenate and 20 mm tert-butyl hydroperoxide, incubated for 10 minutes at a temperature of 37 °C, the reaction is stopped with 0.02 mL of a solution of 25% trichloroacetic acid; to obtain a zero point, a similar procedure is carried out immediately after the introduction of tert-butyl hydroperoxide. The samples are centrifuged (5,000 rpm, 5 min), 30 µL of the resulting supernatant and 30 µL of the Ellman’s reagent are added to 1 mL of phosphate buffer (pH 7.8), and the optical density is measured at λ=412 nm and λ=700 nm [19].

Thus, oxidative stress, which is the result of an imbalance between prooxidants and antioxidants towards the former due to excessive production of free radicals and/or a decrease in the activity of antioxidant defense, underlies the pathogenesis of many diseases and its study in modeling experimental pathology serves as a fundamental basis for clinical research.

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