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FEATURES OF PRODUCTION, METABOLISM AND SIGNIFICANCE OF REACTIVE OXYGEN SPECIES AND PRODUCTS OF THEIR TRANSFORMATION

Bobrova M. S.

INTRODUCTION

A covalent chemical bond can be broken heterolytically (X:Y = $X:^-$ + Y^+, with the formation of ions and further ionic electrophilic and nucleophilic substitution and addition reactions) and homolytically (X:Y = X• + •Y, with the formation of radicals having an unpaired electron). Combinations of both types of transformations with the transfer of protons and electrons are possible, which gives charged and neutral particles with an unpaired electron (*•O•$_2$, •O•, •O_2^{-1}, •OH, •Cl, •NO, •NO_2, R_3C• (especially if it is triphenylmethane), RO•, ROO•, •V^{+2}, •Cr^{+3}, •Mn^{+2}, •Fe^{+3}, •Fe^{+5}, •Co^{+2}, •Ni^{+3}, •Cu^{+2}, •Mo^{+5} and others). Free radicals are highly reactive and therefore short lived. Proton transport determines acid-base reactions, electron transport determines redox reactions. Almost all of these reactions in living organisms can occur together.

The formation of an oxygen atmosphere contributed to the genetically fixed use of oxygen by living systems. In this case, along with electrophilic and nucleophilic reactions, four-electron reduction of oxygen, radical reactions with the participation of reactive oxygen species (ROS) formed by one-electron reduction become important. The prooxidant damaging effect of reactive oxygen species is expressed in the initiation and development of free radical peroxidation of biopolymers (FRPO), more often non-enzymatic; it caused the appearance of antioxidant protection (AOP), which, in addition to enzymes, includes low molecular weight essential food components for animals. The physiological prooxidant-antioxidant system (PAS) has a protective (effector) and regulatory function, but its imbalance leads to damage to the body's own tissues. Changes in PAS occur in normal and pathological conditions. Peroxidation is a variant of the radical.

1. Biochemical aspects of generation of reactive oxygen species

Active oxygen species initiate free radical peroxidation, which is supported by molecular (triplet) Oxygen – a stable biradical ($1s^2$, $2s^2$, $2p^4$, with two electrons paired in free cells $2p^1$ + $2p^1$), in which two unpaired electrons with identically directed spins are in molecular orbitals (↑•O_2•↓). Therefore, it cannot, under normal conditions, react with most organic molecules that have

antiparallel electron spins, even at an O_2 concentration in the atmosphere of 21%. In humans, 90% of all absorbed O_2 goes to mitochondrial cytochrome oxidase ($4H+ + 4e^- + O_2 = 2H_2O$); part of O_2 is used by other oxidases – heme (cytochrome P-450), flavin (xanthine oxidase, monoamine oxidase), copper-containing (ceruloplasmin). In aerobic cells, ROS are always formed, and 0.5–5% of the total absorbed O_2 goes to SRPO; if there were no AOP, then not 0.00000001 M H_2O_2, but 0.014 M would be formed in human tissues per day[1].

ROS are formed by one-electron reductions of an oxygen molecule: $O_2 + \lambda\eta \rightarrow {}^*O_2 + e^- \rightarrow {\bullet}O_2^- + e- + 2H^+ \rightarrow H_2O_2 + e^- \rightarrow {\bullet}OH + e^- + 2H^+ \rightarrow 2H_2O$ ($O_2 + 4e^- \rightarrow 2O^{-2}$). The main ROS are superoxidanion radical, hydroxyl radical, hydrogen peroxide, singlet oxygen, molecular oxygen, peroxyl radical (alkyl dioxide), oxyl radical (alkoxide), nitrogen monoxide, peroxynitrile, hypochlorite. Exogenous ROS are represented by ozone (O_3) and atomic oxygen ($\bullet O\bullet$)[2].

Singlet oxygen ($^*O_2$, $\bullet O\text{-}O\bullet$), in contrast to triplet oxygen, has ($\uparrow\bullet O_2\bullet\downarrow$) oppositely directed spins of unpaired electrons on degenerate pi orbitals. Formed when a light quantum is absorbed by triplet oxygen ($\downarrow\bullet O_2.\bullet\downarrow + \lambda h = \uparrow\bullet O_2\bullet\downarrow$ or $^*O_2$); in a cell, it can be formed upon absorption of light, in peroxidase reactions, microsomal NADPH-dependent oxidation, dismutation of the superoxide anion radical, in the Haber-Weiss reaction. Singlet oxygen initiates FRPO – cholesterol, polyunsaturated fatty acids, causes DNA breaks, inhibits enzymes. Singlet oxygen is inactivated by water, cholesterol, histidine, β-carotene, methionine, ascorbic acid, tocopherol.

Superoxidanion radical or superoxide ($\bullet O_2^-$, $\bullet O\text{:}O\text{:}^-$) is formed by one-electron transfer ($\bullet O_2^- + e- = \bullet O_2\text{:}^-$) from flavin-containing oxidases (monoamine oxidase); with the functioning of NADH dehydrogenase, possibly cytochrome oxidase, and in the ubiquinone-cytochrome c site in mitochondria (24 nm/min*mg of protein); NADPH-cytochrome-P-450-reductase microsomes (2-10 nm/min*mg of protein); in the formation of methemoglobin ($Hem\text{-}Fe^{+2} + O_2 = Hem\text{-}Fe^{+3} + \bullet O_2^-$ or metmyoglobin, which is oxidized by $Fe^{+3} + H_2O_2 = ferrimyoglobin\text{-}Fe^{+4}\text{-}O$, this compound takes away from the OH group tyrosine hydrogen, leaving a tyrosine radical in the protein); in the cytosol it is generated by xanthine oxidase. Non-enzymatically, the superoxide anion radical is formed by the interaction of metal ions of variable valence (iron, copper, manganese) with reducing agents (ascorbic acid, thiols,

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NADPH, hydrogen peroxide), although such a meeting is unlikely in the body under normal conditions. Ubiquinol can first donate one hydrogen to oxygen, forming a semiquinine radical (para-rarely ortho-R-$C_6H_3(OH)O$• and hydroperoxide radical (•OOH), then, donating the second, give ubiquinone (R-$C_6H_3O_2$). Detected in blood serum lipoprotein factor (suprol) with NADPH, which, together with iron ion and oxygen, produces superoxide anion radical [490].It is possible to add a proton to superoxide ($•O_2^{-} + H^+ = •O-O:H$). Superoxidanion radical induces and continues the FRPO chain, modifies membranes, carries out DNA single-strand breaks, protein fragmentation, is a source of other ROS, has a vasoconstrictor effect, activates ATP synthesis in mitochondria, is apparently necessary at the stage of translation of protein synthesis[3]. It is eliminated by superoxide dismutase and, to a lesser extent, ceruloplasmin and tocopherol. The superoxide anion radical exhibits oxidizing and reducing properties (accepts or donates an electron), and is a source for the formation of other ROS[4].

Hydrogen peroxide (H_2O_2 or H:O:O:H, H-O-O-H, is not a radical, has O_2^{-2} or O^{-1}) is formed ($RH_2 + •O_2• = H_2O_2$, $•O_2H + H• = H_2O_2$) during the functioning of flavin-, copper-, heme-containing oxidases (monamine oxidase, NAD-ubiquinone reductase, ubiquinone cytochrome-c-reductase in mitochondria, urate oxidase in peroxisomes, xanthine oxidase in the cytosol), its largest producer is superoxide dismutase, especially when catalase is inhibited. Hydrogen peroxide induces SRPO and oxidizes the sulfhydryl groups of enzymes, carries out double strand breaks in DNA. It is a weak acid ($H_2O_2 = H^+ + HO_2^-$), its salts (Na_2O_2) are known. Like all ROS intermediate in oxidation state (except for O_2^0 and O^{-2}), it is both an oxidizing agent (reducing from O^{-1} to O^{-2}) and a reducing agent (oxidizing from O^{-1} to O^0). Hydrogen peroxide is eliminated by catalase and peroxisome peroxidases[5]. Its messenger role is assumed. Stationary concentration of H_2O_2 in cells is 0.1-0.001 μM. H_2O_2 is a relatively stable molecule that can migrate by diffusion to other compartments of the cell, to other cells. Organic peroxides are formed by

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cytochrome P-450, cyclooxygenase, lipoxygenase and inhibit these enzymes (autoinhibition)[6].

Hydroxyl radical (•OH, •O:H) is formed during the radiolysis of water $H_2O = H• + •OH$ in the Fenton reaction between the ferrous ion (free or in the complex) and hydrogen peroxide $Fe^{+2} + H_2O_2 = Fe^{+3} + OH^- + •OH$, in the Haber-Weiss reaction between hydrogen peroxide and the superoxide anion radical $H_2O_2 + •O_2^- = OH^- + •OH + *O_2$; the latter reaction is possible in the spheres of xanthine oxidase and dihydroorotate dehydrogenase, in the reactions of nitric oxide with hydrogen peroxide or with the superoxide anion radical[7]. This is one of the main initiators of FRPO in tissues, although it is short-lived, it diffuses over a distance of no more than two of its diameters. The hydroxyl radical takes an electron from the anion, from the hydrocarbon residue, forming a carbon radical ($R• + •OH = ROH$, or $RH + •OH = R• + H_2O$), attaches via a double bond to polyunsaturated fatty acids or nitrogenous bases of nucleic acids. Its lifetime is milliseconds, but the rate constant of its reactions approaches the diffusion limit, that is, 10^9-10^{10}/M*sec. It is inactivated by α-tocopherol, bioflavonoids, β-carotene, reduced glutathione, ascorbic acid, and ethanol. •OH inhibits creatine phosphokinase[8].

There are other ROS that are derivatives of those described: ROOH, ROOR, ROO•, $•O_2^- + H^+ = HO_2•$, $•O_2^- + H• = HO_2^-$, $HO_2^- + H^+ \leftrightarrow H_2O_2 \leftrightarrow HO_2• + H•$, endoperoxides[9].

Mitochondrial oxidation includes a chain of hydrogen and electron transfer from 40 protein components, heme, iron-sulfur clusters (FeS), copper, phenolic, pyridine and flavin cofactors: NADH (as well as NADH dehydrogenase with FeS) → FMN (takes H and from $FADH_2$, NAD-KoQ reductase, FeS) → Ubiquinone (coenzyme Q or $KoQH_2$, as well as cytochrome c-reductase, FeS) → Cytochrome B (B1 and Bh heme-$Fe^{+2/+3}$) → Cytochrome C (C1 and C) → Cytochrome oxidase (cytochromes a3 and a1, 2 $Cu^{+2/+1}$ ions) → O_2. A sequential four-electron transfer occurs: 4H+ + 4e- = $2H_2O$, this is produced by cytochrome oxidase, which consumes more than

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95% of the O_2 absorbed by the body. In case of violations of this electron transport chain and, especially, cytochrome O_2 oxidase, mitochondrial membranes, hypoxia (all types of which cause tissue hypoxia), oxygen is not utilized and goes to SRPO, and electrons to O_2 can come from ubisemiquinone and the cytochrome region. Leakage from the mitochondrial superoxide oxidation chain originates from cytochrome C1. In the mitochondria of animal and plant cells, NADH oxidase is found, which carries out the reaction: $NADH + O_2 = NAD + H^+ + \bullet O_2^-$. It is the first intracellular source of ROS[1, 10].

The microsomal oxidation chain includes: NADPH → flavoprotein (NADPH-cytochrome P-450 reductase, FAD, FMN) → cytochrome b5 → cytochrome P-450 → O_2; less often (10-30%): $NADH_2$ → flavoprotein (NADH-cytochrome b5-reductase) → cytochrome b5 (or non-heme protein with FeS – adrenoxin and putidaredoxin in bacteria and mitochondria) → cytochrome P-450 → O2. The process is going on: $RH + O_2 + NADPH + H^+ = ROH + H_2O + NADP^+$. More than 150 isoforms of cytochrome P-450 combine into forms 1, 2, 2A, 3, 3A, 4, the inductor substrates for which are: for form 1 – aromatic polycycles, arylamines, giving epoxides; for 3 and 3A – aflatoxins, diols, steroids, anthracene; for 4 – arachidonate, clofibrate; for 2 and 2A, dioxin, phenobarbitals, carbon tetrachloride, these forms oxidize ethanol and participate in the synthesis of testosterone; ROS are generated from the useless cycling of cytochrome P-450 1, 2, and 4 forms. The synthesis of cytochrome P-450 is activated by polycyclic hydrocarbons, barbiturates, dioxins, the enzyme is autoinactivated by ROS and lipid peroxides, which are also generated by it. This is the second intracellular source of ROS[1.11].

Thus, for the normal and stimulated functioning of the mitochondrial and microsomal oxidation chains, elevated concentrations of reduced pyridine nucleotides ($NADH + H^+$ and $NADPH + H^+$) are required, from which an electron passes into transport chains and can prematurely leave them for O_2 with the formation ROS.

Superoxide is formed during the functioning of dioxygenases (DO). Dioxygenases include tryptophan DO (contains heme), indolylamine dioxidase (requires superoxidanion), the following have non-heme iron: pyrocatechase, metapyrocatechase, cysteine- or cysteamine DO (-SH → $-SO_3H$), polyunsaturated fatty acids DO, iron-α-ketoglutarate DO, thymine DO, prolyl

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hydroxylase, lysyloxygenase, ε-butyrobetaine DO, paraoxyphenylpyruvate DO, homogentisate oxygenase[12].

Weak ROS (superoxide and peroxide) are dangerous because the Haber–Weiss reaction produces strong ROS (hydroxyl radical and singlet oxygen). Simultaneously, superoxide and peroxide are formed by xanthine oxidase and dihydroorotate dehydrogenase (dihydroorotate oxygen oxidoreductase, EC 1.3.3.1, gives H_2O_2, has FAD or FMN). Xanthine oxidase (EC 1.2.3.2; dimer, M = 283 kD, contains 2 or 3 subunits, each of which contains FAD, Fe_2-S_2 iron-sulfur cluster, pterin, many cysteines for disulfide bridges between subunits, molybdenum, and Mo + 5 ($Mo^{+4/+6}$) has two bonds with FAD, two with pterin, one with cysteine sulfur, possibly one with the -S-SH group) is formed from xanthine dehydrogenase by limited proteolysis by a calcium-dependent protease or by oxidation of disulfide bridges, mainly at hypoxia. Oxidation of iron in the FeS center gives superoxide; FAD dehydrates the substrate to give active semiquinone. FAD dehydrates even water (participating in photosynthesis), forming a hydroxyl radical and $FADH_2$; the latter reduces superoxide to H_2O_2. The FAD electron can reduce iron, and two hydroxyl radicals combine to form H_2O_2[13]. Mo^{+5} donates an electron to H_2O_2 with splitting into •OH and OH^-, Mo^{+6} binds OH^- and hydroxylates the substrate, donating •OH and forming Mo^{+5}. Since 2 FeS accounts for 1 FAD and 1 Mo, superoxide is generated in excess. Xanthine dehydrogenase does not produce superoxide, that is, the FeS centers do not work, but Mo^{+5} donates an electron to xanthine nitrogen, which takes H^+ from water, and Mo^{+6} takes an electron from OH, forming •OH to hydroxylate the substrate. Interferon induces the expression of genes encoding enzyme subunits, molybdates activate xanthine oxidase apoenzymes from the Golgi apparatus; reduced glutathione or ascorbate activate (regenerate) the enzyme at concentrations of 0,15-0,4 mm, more than 0,6 mm inhibition occurs; allosteric (the active center has phenylaldanine, tyrosine, serine, histidine) inhibitors are corticosteroids and dioxins, which reduce the production of superoxide. In addition to xanthine, hypoxanthine, 30 aldehydes, pterin, adenine, histidine, cysteine, the enzyme oxidizes NO to nitrite; the enzyme is localized in the cytoplasm of liver cells (hepatocytes, Kupffer cells, endotheliocytes), small intestinal

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mucosa, blood serum, milk, kidneys[14]. An increase in the activity of xanthine oxidase causes the activation of superoxide dismutase and catalase, glutathione peroxidase and glutathione reductase, an increase in the concentrations of diene conjugates and malondialdehyde, and a decrease in the content of reduced glutathione. The enzyme has carcinogenic and apoptotic activity, which is explained by the effect of superoxide on DNA[15]

In terms of activity in the initiation and continuation of FRPO, endogenous ROS form the following series: hydroxyl radical > singlet oxygen > hydrogen peroxide > superoxide anion radical.

Known active forms of other elements HOCl, HOBr, HOJ, •NO, $•NO_2$, CO, ions with an unpaired electron $•Fe^{+3}$, $•Cu^{+2}$, $•Mn^{+2}$, $•Mo^{+5}$.

Hypochlorite is formed by myeloperoxidase (it also forms hypobromite, hypoiodite) according to the reaction: $H_2O_2 + HCl = HClO + H_2O$. Hypochlorite (and other oxygen derivatives of +1 halogens) can decompose, giving atomic oxygen: HClO = HCl + O; which (•O•) is an active oxidizing agent. Hypochlorite, reacting with amino groups of proteins, forms chloramines R-NH-Cl ($R\text{-}NH_2 + HClO = R\text{-}NH\text{-}Cl + H_2O$), and according to the reaction of A.N. Osipov – hydroxyl radical ($Fe^{+2} + HClO = •OH + Cl^- + Fe^{+3}$) with a yield 20 times greater than in the Fenton reaction. HClO activates endotheliocyte gelatinase and collagenase, which contributes to vasculitis; hypochlorite can give singlet oxygen ($H_2O_2 + HClO = *O_2 + HCl + H_2O$). Actively form ROS systems of copper ions and ascorbic acid, iron and ascorbic acid, copper and homocysteine, iron and homocysteine[16].

Nitric oxide (•NO) is a free radical that activates guanylate cyclase in the calcium messenger system. Nitric oxide is formed from arginine by nitric oxide synthase (EC 1.14.13.39, homodimer in the cytoplasm, M=130–160 kD; reaction: arginine + $•O_2•$ + NADPH + H^+ = ornithine + •NO + $NADP^+$). There are constitutive NO-synthase type 1 of neurons, bronchial and gastric epithelium, skeletal muscles, activated by the calcium-calmodulin complex, dimerization and phosphorylation; expressed constantly and during inflammation. Inducible NO-synthase type 2 of macrophages, cardiomyocytes,

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vascular smooth muscles, hepatocytes, irreversibly binds to the calcium-calmodulin complex for activation, is not constantly expressed, is induced by tumor necrotic factor α, interleukin 1β and 2, interferon ε, bacterial lipopolysaccharide, is activated by cAMP, factors growth of the epidermis and fibroblasts, plasmin, is inhibited by glucocorticoids, insulin-like growth factor, platelet growth factor b, transforming growth factor β, thrombin. Type 3 NO synthase of endotheliocytes is represented by cytosolic and membrane-bound forms, expression is constant, but increases with shear acceleration of blood flow, and decreases with the action of tumor necrosis factor α, undergoes phosphorylation and myristylation, reversibly binds to calmodulin, and is activated by calcium. From the N-terminus of the enzyme there is cytochrome P-450 (thiol-bound heme – iron porphyrin lX), binding sites for arginine, 5,6,7,8-tetrahydrobiopterin and calmodulin; then cytochrome P-450 reductase is located, including FMN, FAD, NADPH and the C-terminus. Superoxide is formed on cytochrome P-450, which oxidizes $-NH_2$ in the guanidine group to -N=O; the next superoxide forms citrulline and •NO with the participation of tetrahydrobiopterin and H_2O_2. In addition to this pathway, it is possible to generate •NO from nitrites with the formation of methemoglobin ($Heme\text{-}Fe^{+2} + HNO_2 + H^+ = Heme\text{-}Fe^{+3} + \bullet NO + H_2O$), while nitrites are easily formed from nitrates. In turn, nitric oxide can be converted into nitrites and then into nitrates, and can also bind to iron and copper ions of enzymes, especially oxidases, blocking oxidative chains and ATP production. Nitric oxide binds to the iron ion of heme guanylate cyclase, activating it, which leads to a vasodilator effect in endotheliocytes, activates cyclooxygenase. •NO inhibits NADPH oxidase, 5-lipoxygenase and ribonucleotide reductase; •NO is not only a tertiary messenger, but also a factor of apoptosis, carcinogenesis; •NO leads to uncontrolled vasodilation, enhances the toxic effect of excess glutamate and calcium. Oxidation of •NO by a superoxidanion radical or hydroxyl radical gives peroxynitrite (ONOO-, which turns into peroxynitrous acid ONOOH, giving $\bullet NO_2$ and •OH radical), a vasoconstrictor that activates neutrophil adhesion and platelet aggregation, is toxic to the brain, and contributes to cardiovascular pathologies, decomposing with the formation of •OH and $\bullet NO_2$: $\bullet NO + \bullet O_2^{-1} = ONOO^-$; $ONOO^- + H^+ = ONOOH = \bullet NO_2 + \bullet OH$. Peroxynitrite damages DNA even more actively than superoxide[1]. It is significant that •NO reacts with superoxide 3 times faster than SOD. Superoxide, •NO, peroxynitrite are heme iron ligands (nitrosyl iron complexes are formed), which is the basis for self-inactivation of cytochrome P-450, these active forms suppress the expression of all forms of cytochrome P-450. Nitric oxide binds to metal ions in enzymes, forms nitrosamines through N_2O_3, inhibits SRPO ($ROO\bullet + \bullet NO = ROONO$), nitrates tyrosine in protein and SH-groups of thiols, deaminates nitrogen-containing substances, forms ONOO-,

modifies GGG from the 5-end -sequence in the telomere and promotes the formation of 8oG. Infections, inflammation, toxins generate ROS and •NO, the latter inhibits DNA repair enzymes and disrupts the structure of DNA itself, binds Zn^{+2} proteins; this leads to mutations in suppressor genes (p53), shortening of telomeres, and modification of genome expression signals[17].

Carbon compounds C +2 in the form of CO are formed during the oxidative breakdown of heme in animals by heme oxygenase and quercetin in Aspergillus by quercetinase. Copper-containing quercetinase breaks the pyran ring of quercetin to form a carboxyl group and CO. Heme oxygenase containing cytochrome P-450 oxidatively breaks the methen bridge between the rings of pyrroles A and B with the release of CO. Carbon monoxide can also be formed during NADPH-dependent PUFA oxidation. The messenger role of SO in neuronal systems is assumed.

Exogenous ROS include ozone (O_3, formally O=O=O, oxidation states –2, +4, –2, respectively), which is formed ($3O_2 + \lambda\eta = 2O_3$) in the atmosphere under the action of electric discharges or ultraviolet (UV) and decomposition gives active atomic oxygen ($O3 = •O_2• + •O•$) or ozonides. It is a toxic substance of the 1st hazard class: lung tissue suffers from ozone as well as from the free radical of nitrogen dioxide ($•NO_2$), which is prevented by tocopherol. O_3 enhances the permeability of tissue barriers, activates neuropeptides, induces the formation of IL-8 in the lungs, and reduces the expression of integrins by alveolar macrophages[1,18]. Sometimes the formation of these exogenous ROS is associated with immunoglobulins in the body.

2. Non-enzymative free radical peroxidation of biopolymers

Free radical oxidation can occur with the formation of peroxides, which is characteristic of very reduced hydrophobic compounds such as lipids (LPO), or without the formation of peroxides, which is more characteristic of more oxidized proteins, nucleic acids and carbohydrates (free radical oxidation – FRO)[1,19].

Cytotoxic effects of ROS are realized by peroxidation (impairment of the structure and function of the lipid membrane, in particular the transport, receptor, enzyme, electrical insulator), damage to structural proteins and proteoglycans, inactivation of enzymes through the oxidation of SH groups,

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damage to the DNA structure, increased sensitivity to hydrolases, activation of metalloproteinases, suppression of inhibitors of neutral proteinases, formation of lipid chemoattractants and secondary biotoxins (lipid peroxides, chloramines, oxidized lipoproteins), weakening of antioxidant protection. All biopolymers are subjected to SRPO to varying degrees; the most vulnerable are membrane lipids, which are easily repaired, the most dangerous may be the effects of DNA peroxidation, apparently, the results of peroxide degradation of proteins are significant. Therefore, the increase in ROS production (respectively, the level of FRPO) is greater than the power of antioxidant systems and is defined as oxidative stress. The ratio of ROS generation activity, FRPO and AOP levels is defined as antioxidant status or prooxidant-antioxidant homeostasis, balance[20].

Free radical peroxidation (FRPO) involves ROS initiation: RH + •OH = R• + H_2O, continuation: R• + O_2 = ROO•; ROO• + RH = ROOH + R• and chain branching: ROOH + Fe+2 = •Fe^{+3} + RO• + OH-, RO• + RH + ROH + R•, chain quenching: R• + R• = R-R or R• (•OH) + InH = RH (H_2O) + •In; where •In is the inactive radical of the inhibitor. Quenching of active radicals occurs under the influence of antiradical substances that form low-active (shielded) radicals[21].

First of all, divinylmethane structures of polyunsaturated fatty acids of membrane phospholipids are oxidized (-CH=CH-CH_2-CH=CH-): -CH=CH-CH_2-CH=CH- + •OH = -CH=CH-CH•-CH=CH- + H_2O; -CH=CH-CH•-CH=CH- = -CH=CH-CH=CH-CH•-, that is, diene conjugates are formed; other primary products are formed –CH=CH-CH=CH-CH•– + *O_2 = –CH=CH-CH=CH-CH(O-O•)-; -CH=CH-CH=CH-CH(O-O•)- + H = -CH=CH–CH=CH-CH(OOH)–; further with a chain break, 4-oxyenals are formed: –CH=CH-CH=CH-CH(OOH)- = HO-CH=CH-CH=CH-CH=O. These processes occur according to the laws of the kinetics of liquid and solid-phase oxidation of hydrocarbons. At the same time, ROS first react with a carbon-hydrogen bond, which gives rise to free carbon radicals, then the active (radical) center is transferred from the alkyl to the allyl residue (here, ethane and pentane hydrocarbons are formed from the terminal fragments of PUFAs) and molecular oxygen is added. These processes are accompanied by chemiluminescence (chemiluminescent probes enhance it, so luminol enhances the luminescence from OH and HOCl phagocytes and the Fenton

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[21] Dat J.F., Vandenabeele S., Vranova E. et al. Dual action of the active oxygen species during plant stress responses. *Cell. Mol. Life Sci.* 2000. Vol. 57. P. 779–795.

reaction, and lucigenin from the superoxidanion radical of phagocytes and the xanthine-xanthine oxidase system). Aloxyl and peroxyl radicals are intermediate products of lipid peroxidation (LPO), registered by chemiluminescence and EPR spectroscopy. At the same time, the double bond moves and hydrogen is added. As a result, acyl hydroperoxides and diene conjugated structures are formed (absorption maximum at 231–234 nm). These are primary lipid peroxidation products registered by UV spectrophotometry, polarography, and amperometry[22].

The reduction of primary products gives hydroxy acids and alcohols, epoxides, and the oxidation gives 4-hydroxynonenal, triene conjugates and oxo compounds (at least 18 aldehydes and ketones that react with 2-thiobarbituric acid – TBA, oxiranes), among which malonic acid is most common (40%). dialdehyde (MDA, $O{=}HC{-}CH_2{-}CH{=}O$). These are secondary lipid peroxidation products registered by UV spectrometry, HPLC, TBA test for oxo groups[19].

The interaction of MDA with the amino groups of proteins, lipids, amino polysaccharides, nucleic acids, degradation products of chlorophylls, and heme gives fluorescent products of the Schiff base type. These aldimines are also formed non-enzymatically by the Maillard reaction. Cross-links ($>C{=}O + H_2N{-} \rightarrow >C{=}N{-} + H_2O$), as well as Schiff fluorescent bases (of the type 1-amino-3-imino groups R-N=CH-CH=CH-NH-R). For the latter, the excitation wavelength lies in the range of 360-400 nm, the emission wavelength is 420-470 nm. Schiff bases bind to partially oxidized membranes, carotenoids, proteins, forming granules of insoluble lipofuscin, an aging pigment; its accumulation in cells is determined by the intensity of lipid peroxidation and the amount of MDA. Lipofuscin is recognized by microscopy with specific staining of preparations. Schiff bases and lipofuscin are end products of peroxidation. The final products include gaseous hydrocarbons (pentane)[23].

Singlet oxygen during the oxidation of linoleic acid gives hydroxides in positions 9-, 10-, 12-, 13-, 15-, 16-, and hydroxyl radical – in 9-, 12-, 13-, 16-positions. These changes in PUFAs lead to membrane hydrophilization and disruption of the properties of the hydrophobic barrier, an increase in their fluidity and permeability, and a decrease in viscosity, while MDA inhibits

[22] Gautam V., Kaur R., Kohli S.K., Verma V., Kaur P., Singh R., Saini P., Arora S., Thukral A.K., Karpets Yu.V., Kolupaev Yu.E., Bhardwaj R. ROS compartmentalization in plant cells under abiotic stress condition. Reactive Oxygen Species and Antioxidant Systems in Plants: Role and Regulation under Abiotic Stress / Eds. Khan M.I.R., Khan N.A. Springer, Singapore, 2017. P. 89–114.

[23] Scandalios J.G. Oxidative stress: molecular perception and transduction of signals triggering antioxidant gene defenses. *Braz. J. Med. Biol. Res*. 2005. V. 38. P. 995–1014. DOI: 10.1590/s0100-879x2005000700003.

protein replication and synthesis, and forms intermolecular crosslinks at amino groups. MDA itself is in two tautomeric forms depending on pH – unstable aldehyde and hydrated into a tetrahydroxy derivative. By binding to amino groups, MDA can crosslink DNA strands, glucoseaminoglycans, proteins, and cause potassium leakage from cells. Hepatic aldehyde dehydrogenases can oxidize MDA to acids, which are decarboxylated to active acetyl; the latter is used in the tricarboxylic acid cycle (energy metabolism) and for the synthesis of fatty acids. Experimental administration of MDA at doses of 0.1–10 mg/kg increases the incidence of hepatomas[1, 19].

As a result of lipid peroxidation, their hydrophobicity decreases and hydrophilicity increases, pores are formed, lipid peroxidation of membranes leads to an increase in its permeability, which is especially important for substances with active transport, to disruption of the receptor structure[24]. Membrane functions are disturbed – localization of electrical potential, enzymes, receptors. Destruction of the inner membrane of mitochondria, membranes of the endoplasmic reticulum disrupts oxidative phosphorylation and microsomal detoxification. Peroxidation of lysosomal membranes leads to autolysis and cell death. LPO of membranes acidifies the environment, which inhibits the activity of phospholipases A, but activates phospholipase C, which gives lysophospholipids. Tocopherol is the most effective inhibitor of membrane lipid peroxidation; together with phospholipases, it participates in membrane repair. Peroxidation of membrane structures disrupts the functioning of receptors, the conductivity of membrane channels, as it promotes the formation of ionophore pores, leads to inhibition of membrane-bound enzymes (ATPase, creatine phosphokinase); peroxidation of the erythrocyte membrane leads to hemolysis. The intracellular concentration of potassium, sodium, calcium, magnesium, chlorine, hydrogen ions, the lipid composition of membranes, their fluidity change, up to membrane rupture and osmotic shock, which causes free radical necrobiosis. The result of FRPO of membrane lipids, initiated by short-lived ROS, is massive cell death – necrosis[25].

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[24] Piotrovskii, M.S., Shevyreva, T.A., Zhestkova, I.M., Trofimova, M.S. Activation of plasmalemmal NADPH oxidase in etiolated maize seedlings exposed to chilling temperatures. *Russian Journal of Plant Physiology: a Comprehensive Russian Journal on Modern Phytophysiology.* Vol. 58. No 2. P. 290–298. http://dx.doi.org/10.1134/S1021443711020154.

[25] Казначєєва М.С., Цебржинський О.І. Дослідження розподілу активності цитохромоксидази в тканинах цибулі ріпчастої різних за рівнем стійкості до хвороб сортів. *Світ медицини та біології.* Полтава. 2011. Вип. 3. С. 10–12. https://womab.com.ua/upload/7.3/SMB-2011-03-010.pdf.

FRPO of cholesterol ROS produces 25-hydroxycholesterol, which hormone-like inhibits at the gene level the expression on hepatocyte membranes of transport receptors for the B-100 low-density lipoprotein protein, which is one of the mechanisms of atherogenesis. Oxidation of cholesterol with hypochlorite gives about 20 products (diols, triols, epoxides, diones).

The most reduced compounds are most susceptible to FRPO – lipids with unsaturated fatty acid residues, complex and cyclic lipids to a lesser extent. A distinction is made between NADPH-dependent LPO, which stimulates the functioning of predominantly microsomal oxidases with ROS leakage as a substrate (the process is stimulated to a lesser extent by NADH) and non-enzymatic LPO stimulated by ascorbic acid; however, in both cases, iron salts are often added in in vitro experiments.

The chain process of FPRO is less pronounced for nucleic acids, proteins, and even weaker for polysaccharides, since these are more oxidized compounds. Therefore, in these cases, the term FRPO damage to biopolymers is used. FRPO and low molecular weight metabolites are exposed. Molecular and singlet oxygen are non-polar compounds and easily penetrate the lipid membrane to the inner compartments of the cell. It should be noted that the oxidation of ROS substrates, •NO reactions, and the condensation of oxo groups (FRPO products) with amino groups of biopolymers are nonenzymatic processes. The course of LPO processes requires sufficient substrates, sufficient molecular oxygen, and the presence of inductors that initiate the formation of radical ROS. The degree of damaging effect of FRPO depends on the partial pressure of O_2, that is, hyperoxia aggravates the condition.

Undergoing oxidative modification, proteins become more sensitive to proteolysis. In proteins, hydrogen peroxide oxidizes sulfhydryl groups to disulfide bridges, especially in the active sites of enzymes (Na-K-ATPase), hydroxyl oxidation of aromatic amino acid residues, oxidation of hydrophobic amino acid radicals with the formation of oxo groups, cross-linking of protein strands by forming tyrosine dimers, cross-linking strands of protein with nucleic acids according to the type of Schiff bases, modification of the peptide group with its break. The latter also produces the superoxide anion radical, causing protein fragmentation through the oxidation of the peptide group. LPO products of oxo compounds can bind like Schiff bases with protein amino groups of radicals of basic amino acids – lysine and arginine. Proteins containing metals (collagenase) are more damaged, complex proteins are deacylated by ROS. ROS act on histones, antiproteases, disrupting their structure and function; oxidation of low density lipoproteins contributes to their antigenicity. ROS promote deacylation of proteins, disruption of membrane receptors. Peroxidation of lipids and hydrophobic radicals of

protein amino acids disrupts the immobilization of membrane-bound proteins (cytochrome P-450, creatine phosphokinase)[26].

Of particular importance is the FRPO of low-density lipoprotein proteins (LDL, β-lipoproteins). LDL during atherogenesis accumulate in the blood plasma as cholesterol transporters in cell membranes. Hyper-β-lipoproteinemia activates the respiratory burst of neutrophils (element of inflammation), ROS oxidize surface proteins with the formation of antigenic products.

In addition to •NO, guanylate cyclase is activated also by $\bullet O_2^-$ and •OH, and ribonucleotide reductase itself has a radical center on the tyrosine phenolic residue. In the reaction: myoglobin Fe^{+3} + H_2O_2 = ferrimyoglobin-Fe^{+4}-O, the resulting compound takes hydrogen from the OH group of tyrosine, giving a tyrosine radical in the protein[27].

FRPO damage to nucleic acids has been proven, and many pro-oxidant substances, oxidizing agents, are known, causing breaks in the ester bond of phosphate and pentose residues, oxidation of pentoses and nitrogenous bases. In this case, single- (superoxide, hydroxyl radical) and double-stranded (hydrogen peroxide) DNA breaks occur, MDA is formed from deoxyribose, thymine peroxide, 8-hydroxy-2-deoxyguanosine (8oG), as well as about 50 more products are detected, which disrupts the structure and function of DNA. If guanine is complementary to cytosine, then 8oG is complementary to adenine, which replaces cytosine in the opposite DNA strand, i.e., forms a mutant site. 8oG has fairly stable bonds in DNA, almost does not undergo depurination by DNA glycolases, but the antimutagenic enzyme 8-oxo-2-deoxyguanosine-5-triphosphate pyrophosphorylase is known. The hydrophobic region of DNA is formed by DNA cytosine methylase, which uses methionine. 5-methylcytosine (5mC) is abundant in DNA telomeres, viral eukaryotic genome sequences, embryonic and tumor cells, being an epigenetic factor[28].

Among the products of ROS action ($*O_2$ and •OH, but not H_2O_2) on DNA, 8oG, 8-hydroxyadenine, 5-hydroxycytosine, 5-hydroxymethyluracil, 2,6-diamino-4-hydroxy-5-formamidopyrimidine (Fapy), 5-hydroxyuracil, cis-5,6-dihydroxythymine, 5,6-dihydrothymine, 5-hydroxymethyluracil, 5-hydroxy-5-methylhydantoin. The action of ROS (Fe^{+2} system with H_2O_2) on a DNA fragment with 5mC gives 5-hydroxy-5-methylcytosine, and the formation of 8oG contributes to the demethylation of 5mC and the replacement of C by A.

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[26] Mittler, R. ROS Are Good. *Trends in Plant Science.* 2017. Vol. 22. No. 1. P. 11–19. https://doi.org/10.1016/j.tplants.2016.08.002.

[27] Мерзляк М.Н. Активированный кислород и жизнедеятельность растений. *Соросовский образовательный журнал*. 1999. № 9. С. 20–26.

[28] Morales M, Munné-Bosch S. Malondialdehyde: Facts and Artifacts. *Plant physiology.* 2019 Vol. 180(3). P. 1246–1250. DOI: https://doi.org/10.1104/pp.19.00405

Hypochlorite forms 5-chlorocytosine, 5oU, 5oC, but not 8oG, which is formed from the action of peroxynitrite. Paraquat, salts of lead, cobalt, nickel, mercury, iron, copper contribute to the formation of 8oG, and this is largely due to inhibition of the activity or synthesis of 8oG-DNA glycosylase. It is a lyase-class repair enzyme that cuts out 8oG and Fapy, maintains a low level of 8oG in DNA, is associated with calmodulin, and is encoded by the HOGG1 gene. Enzyme activity is regulated by the SoxRS, fnr, mexa, and arcA genes, but not by OxyR (not induced by H_2O_2). Butionine sulfoximine (GSH-transferase inhibitor) inhibits the enzyme and promotes the accumulation of 8oG. 8oG binds complementary to C and A, promotes transversions. 8oG is found in bacteria (Salmonella, collies), yeast, eukaryotes, and with age (rats 3 days, 5 months, 30 months) its content in the DNA of the liver, lungs, kidneys, spleen, small intestine, brain increases. The iron nitrile acetate complex is a renal carcinogen that increases the proportion of 8oG in DNA. 8oG was detected under the action of hepatocarcinogens, in leukocytes, in small cell lung cancer, in brain DNA in Parkinson's disease, ischemia and reperfusion of the brain after 20 minutes (while 8oA, 5oC, Fapy are still formed). Retinoic acid derivatives, N,N-dimethyl-paraphenylenediamine, inhibit induced hepatocarcinogenesis and accumulation of 8oG[19].

Carbohydrates, like lipids, nucleic acids, and proteins, form MDA under the influence of ROS. Peroxidation of carbohydrates enhances the formation of cross-links of polysaccharide filaments between themselves and with proteins. Monosaccharide monomers can form radical intermediates, and their chains can cross-link with MDA, especially outside the cell. The radicals of proteins and carbohydrates do not give a chain reaction of SRPO. Polysaccharides protect bacteria from ROS.

ROS can act on low molecular weight metabolites (NAD, FAD, porphyrins, succinic acid) and activate guanylate cyclase. Organic peroxide radicals, tyrosine residues in ribonucleotide reductase, nitrosyl complexes, iron (+3), copper (+2), molybdenum (+5), and manganese (+2) ions, which have unpaired electrons, give EPR signals[29].

3. Enzymative free radical peroxidation of biopolymers

ROS are formed intracellularly by leakage from the sphere of enzymatic (xanthine oxidase) or non-enzymatic reactions of predominantly mitochondrial and microsomal oxidation, but they are involved in enzymatic lipid peroxidation. Enzymatic peroxidation is subject to arachidonic acid, to a lesser extent linoleic and linolenic acid, from which eicosapentaenoic acid can be formed; these polyunsaturated fatty acids are essential nutritional factors.

[29] Bhattacharjee S. Reactive oxygen species and oxidative burst: roles in stress, senescence and signal transduction in plants. *Curr. Sci.* 2005. Vol. 89. P. 1113–1121.

These acids are cleaved by phospholipase A2 (activated by the calcium messenger system through the PLAP protein, with glucocorticoids binding to the nuclear protein NF-kB, which regulates gene expression; inhibited by the protein lipocortin, the synthesis of which is induced by glucocorticoids) from membrane phospholipids. Enzymatic peroxidation occurs with the help of isoforms of lipoxygenase, cyclooxygenase, and cytochrome P-450, all these enzymes are inhibited by their products, lipid peroxides, and can give •OH radical upon activation. Peroxide products of enzymatic compartmentalized LPO also give diene conjugates and MDA. Products of lipoxygenase and cyclooxygenase (eicosanoids) are formed in all cells (very little in erythrocytes), including alteration of cell membranes, and act on neighboring cells, including an acute reaction to damage; eicosanoids are rapidly cleaved in the bloodstream[1]. The numerical index at the abbreviated name of eicosanoids indicates the number of double bonds.

The most common PUFAs have more than 16 carbon atoms (from 2 to 24 C atoms in total) in the skeleton and 1-4 double bonds, mainly in the cis configuration (hydrogen atoms are on one side of the rigid double bond, and hydrocarbon radicals are on the other). Unlike a single bond, free rotation around a double bond is impossible; therefore, PUFA in membrane phospholipids contributes to their rigidity. Usually PUFAs are unbranched chains ending with a carboxyl group (–COOH) on one side and a methyl group ($–CH_3$) on the other. Counting from the end (omega, ω), that is, from methyl, the numbers of C atoms with double bonds are put. Oleic is a ω-9 monounsaturated fatty acid. Linoleic ($C_{17}H_{31}COOH$) is an ω-6 (and 9) cis-, cis-dine-unsaturated fatty acid. ω-3 PUFAs important for human nutrition include α-linolenic ($C_{17}H_{29}COOH$, three double bonds at 3, 6, 9 C atoms), which is a precursor for eicosapentaenoic, docosapentaenoic, docosahexaenoic ω-3 PUFAs, these acids do not produce inflammatory mediators. During the technological processing of oils, the formation of lipid peroxides and dienes is possible, the transfer of double bonds to omega-9 to 7, 8, 10, 11 C atoms, the transition of the cis-configuration to trans, the hydrogenation of vegetable oils gives margarine with saturated fatty acids and unsafe trans-PUFAs , long-term storage of them contributes to oxidation (rancidity of fats)[1].

From J. Liebig, the assessment of fats mainly concerns calorie content, PUFAs are also part of these fats. Linoleic and linolenic acids are not synthesized in the human body, being indispensable essential components of food (vitamin F, daily requirement 2-6-10 g). PUFAs contribute to the transmission of nerve impulses, have a hypotensive and hypocholestrinemic effect, and are necessary for the normal development and functioning of the brain. PUFAs undergo, with some differences, β-oxidation, and less often α- and ω-oxidations.

Functions of PUFAs: 1) are part of (in the second position) membrane phospholipids and reserve fats; 2) only the cholesterol esterified by them is converted in the liver into bile acids – the form of its removal from the body; 3) are used to form arachidonic acid, which is necessary for the synthesis of eicosanoids. Losing two hydrogen atoms (2H), linoleic acid turns into gamma (γ)-linolenic acid, which adds acetyl to form homo-γ-linolenic acid, the latter loses 2H and gives arachidonic acid.

Synthesis of eicosanoids begins with excision of PUFAs, such as arachidonate, from membrane phospholipids by phospholipase A2. Then enzymatic peroxidation of eicosa-8,11,14-triene, eicosa-5,8,11,14-tetraenoic – arachidonic, eicosa-5,8,11,14,17-pentaenoic acids occurs to convert them into eicosanoids.

Lipoxygenase (LOG, EC 1.13.11.12) is an enzyme of plant and animal tissues, contains non-heme iron $(FeS)_n$, has a number of forms, including calcium-activated, is activated by the FLAP protein, LDL, is inhibited by quercetin (vitamin P)[30], •NO, tocopherol, excess PUFA, vitamin D derivatives, zylevton. LOX is involved in the destruction of mitochondrial membranes of reticulocytes; its substrates can be polyunsaturated fatty acids, cholesterol esters. There are a number of forms of LOG according to the direction of attack of oxygen on the number of the carbon atom of PUFA (5-LOG, 13-LOG, etc.). The 9-LOG, 12-LOG and 15-LOG forms give 12- or 15-HETE (hydroxyeicosotetraenoate), and the 5-LOG form gives 5-HPETE (hydro-peroxideicosotetraenoate), which, through the epoxy form and reaction with glutathione, starts the synthesis of lipoxins (L_x) and leukotrienes (LT). Melatonin through nuclear receptors affects the synthesis of 5-LOG. Lipoxins are synthesized in neutrophils, these are trihydroxyeicosanoids. Leukotrienes (slow-reacting substances of anaphylaxis) function primarily through the calcium messenger system, stimulate leukocyte (5-HETE, LTA_4, LTV_4) and platelet chemotaxis, vasospasm (LTS_4), bronchospasm (LTD_4), increase vascular permeability, promoting exudation and edema (LTE_4), which is essential in the pathogenesis of asthma and coronary heart disease, the role of others (LTF_4) is unclear. LTS_4, LTE_4, LTD_4 are hundreds of times stronger than histamine, reduce the smallest air-conducting systems of the bronchi, LTS_4 and LTD_4 increase the separation of mucus, which contributes to the removal of nematode larvae from the body. LTV_4 is the strongest neutrophil chemoattractor. LTC_4, LTD_4, LTE_4 are slow reacting substances of anaphylaxis. Tartrazine, excess aspirin increase the synthesis of leukotrienes

[30] Rhoads D. M., Umbach A. L., Subbaiah C. C., Siedow J. N. Mitochondrial reactive oxygen species. Contribution to oxidative stress and interorganellar signaling. *Plant Physiol*. 2006. Vol. 141. P. 357–366. DOI: 10.1104/pp.106.079129

over prostaglandins, which can contribute (especially in cyclooxygenase deficiency) to asthmatic cough, bronchospasm (aspirin bronchial asthma)[31].

Cyclooxygenase (COX, EC 1.14.99.1) contains an iron-heme structure and starts the synthesis of prostaglandins (PG), prostacyclins (PGI) and thromboxanes (Tx) through 11-HPETE with the participation of peroxidase, reductase, isomerase, functioning mainly through the adenylate cyclase messenger system and involved in inflammation and platelet aggregation. COX is inhibited by non-steroidal anti-inflammatory drugs (aspirin, indomethacin, nimesin), activated through the calcium messenger system with the participation of a number of proteins. Two isoforms of COX are known. COX-1 is encoded by a dominant gene that is expressed in the stomach and kidneys; it is a constitutive form that gives TxA2, PG2, PGE2, and its products may have a cytoprotective role. COX-2 is represented by an inducible form during inflammation, is induced by cytokines and inflammatory mediators, is blocked by meloxicam, nimesid, nimesulide; under the influence of interleukins (IL-1, possibly IL-6, TNF, inhibited by adenosine) is activated in synovial cells (with rheumatoid arthritis), chondrocytes, endothelial cells, macrophages. COX-2 products have pro-inflammatory properties.

First, PGG_2 is formed, which is oxidized to PGN_2, the latter gives TxA_2 by oxidation in megakaryocytes, platelets and endotheliocytes, and TxB_2 is formed from it. From PGN_2, prostacyclin PGI_2 is formed in the endothelium, from PGN_2, PGD_2 is formed, and then PGE_2, PGF_{2a}. Most PGs cause smooth muscle contraction. TxA_2 and TxB_2 cause rapid platelet aggregation, activate hypercoagulability, increase the activity of mucous glands, and are smooth muscle constrictors. PGD_2 is produced by mast cells and involves degranulation of mast cells. PGF_2 is a smooth muscle constrictor, PGE_2 dilates the bronchi, PGE_1 and PGE_2 are formed in eosinophils, in particular under the influence of parathyroidin-like peptide, tumor necrosis factor, interleukin-1, PGE_2 increases vascular permeability, dilates microvessels, stimulates gastric blood supply and secretion of mucin with a decrease in secretion HCl (exactly the opposite effect refers to the side effect of aspirin), activates renin secretion, renal blood flow, natriuresis and diuresis, inhibits vasopressin-dependent water resorption, therefore, with an excess of PGE_2, hypokalemic excretory alkalosis (Bartter's syndrome) develops. PGE_2 mediates the effects of endogenous pyrogens (IL-1); PGE_2 and PGI_2 inhibit insulin secretion, but act oppositely on adipose tissue lipolysis (PGE_2 is inhibitory). PGE_2 and PGF_{2a} stimulate uterine contractions and involution of the corpus luteum, PGF_2 is a vasoconstrictor.

[31] Nandi A., Liang-Jun Y., Jana C.K., Dascorresponding N. Role of Catalase in Oxidative Stress- and Age-Associated Degenerative Diseases. Oxid Med Cell Longev. Volume 2019, Article ID 9613090, 19 pages https://doi.org/10.1155/2019/9613090.

The balance of T_xA_2 and PGI_2 secreted by platelets and endothelium, respectively, determines the maintenance of the liquid state of the blood and the non-wetting of the intact vascular wall. After taking aspirin, the enzymatic synthesis of PGI_2 is restored faster than new platelets are produced, which gives anticoagulant and antithrombogenic effects. Synthesis of PHA_2 occurs in large quantities in marine coelenterates. Constitutional insufficiency of cyclooxygenase (Vidal's syndrome), an excess of currants and green apples in the diet, and aspirin intake in case of PUFA deficiency cause a predominance of LT over PG, which contributes to asthma and bronchospasm. The addition of omega-3-PUFA to the diet reduces the production of eicosanoids with double bonds in positions 2 and 4, but increases the yield of derivatives of ω-3-dihomo-γ-linolenic and ω-3-eicosapentaenoic acids. This reduces the risk of thrombosis and atherogenesis.

Cytochrome P-450 forms epoxyeicosotetraenoic acids, which are cleaved by epoxyhydratases and glutathione transferases[1,32].

4. Sites of ROS production in plant cells

The ROS is being produced under both normal and stressful conditions at various locations in the chloroplasts, mitochondria, peroxisomes, plasma membranes, ER and the cell wall. In presence of light, chloroplasts and peroxisomes are the major sources of ROS production, while the mitochondrion is the leading producer of ROS under dark conditions[33].

The ***chloroplast*** comprises of an extremely ordered system of thylakoid membranes which houses the light capturing photosynthetic machinery as well as anatomical requirements for efficient light harvesting[34]. The photosystems, PSI and PSII which form the core of the light harvesting system in the thylakoids are the major sources of ROS production. The photosystems, PSI and PSII which form the core of the light harvesting system in the thylakoids are the major sources of ROS production. Abiotic stress factors like drought, salinity, temperature extremes, all of which cause water stress and limit CO_2 concentrations, coupled with excess light, leads to the formation of $O^{\bullet-}_2$ at the PS, via the Mehler reaction.

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[32] Sagi M., Fluhr R. Production of reactive oxygen species by plant NADPH oxidases. *Plant Physiol.* 2006. Vol. 141. P. 336–340. DOI: 10.1104/pp.106.078089.

[33] Choudhury, S., Panda, P., Sahoo, L., and Panda, S. K. Reactive oxygen species signaling in plants under abiotic stress. *Plant Signal. Behav.* 2013. Vol. 8. P. 23–68. doi: 10.4161/psb.23681.

[34] Pfannschmidt, T. Chloroplast redox signals: how photosynthesis controls its own genes. *Trends Plant Sci.* 2003. Vol. 8. P. 33–41. doi: 10.1016/S1360-1385(02)00005-5.

Mitochondria are also the site of generation of harmful ROS, like H_2O_2 and $O^{\bullet-}_2$, though in a smaller scale. Plant mitochondria differ from animal counterparts in having O_2 and carbohydrate-rich environment and also being involved in photorespiration. The mitochondrial ETC (mtETC) is the major culprit as it houses sufficiently energized electrons to reduce O_2 to form the ROS. The two major components of the mtETC responsible for producing ROS are Complex I and Complex III[35].

Peroxisomes are single-membrane-bound spherical microbodies and are the major sites of intracellular H_2O_2 production due to their integral oxidative metabolism. They also produce $O^{\bullet-}_2$, like chloroplasts and mitochondria during the course of various metabolic process. The $O^{\bullet-}_2$ is generated at two different locations. The Xanthine oxidase (E.C.1.17.3.2), located in the peroxisomal matrix, metabolizes both xanthine and hypoxanthine into uric acid and generate $O^{\bullet-}_2$ as a by-product. Second is the NADPH-dependent small ETC, composed of NADH and Cyt b localized in the peroxisomal membrane which utilizes O_2 as the electron acceptor and releases $O^{\bullet-}_2$ into the cytosol[36].

Apoplast, the diffusible space around the plant cell membrane is responsible for converting the incoming CO_2 into a soluble, diffusible form which enters the cytosol to undergo photosynthesis. At times of adverse environmental conditions, stress signals combined with abscisic acid (ABA) make the apoplast a prominent site for H_2O_2 production. The NADPH oxidases expressed by the AtRbohD and AtRbohF in the guard cells and the mesophyll cells of *Arabidopsis*, account for generating the apoplastic ROS which is required for ABA-induced stomatal closure. Besides these enzymes, there are additional ROS-generating enzymes which comprise of pH dependent peroxidases (POXs), cell wall-linked oxidases, germin-like oxalate oxidases and polyamine oxidases, all of which mainly produce H_2O_2[37].

Plasma membrane which surrounds the entire plant cell plays an important role in interacting with the ever changing environmental conditions and provides information necessary for the continual survival of the cell. The NADPH-dependent-oxidases which are localized in the plasma membrane are in the spotlight due to their gene expression and presence of different

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[35] Møller, I. M., Jensen, P. E., and Hansson, A. Oxidative modifications to cellular components in plants. *Annu. Rev. Plant Biol.* 2007. Vol. 8. P. 459–481. doi: 10.1146/annurev.arplant.58.032806.103946

[36] Palma, J. M., Corpas, F. J., and del Río, L. A. Proteome of plant peroxisomes: new perspectives on the role of these organelles in cell biology. *Proteomics.* 2009. Vol. 9. P. 2301–2312. doi: 10.1002/pmic.200700732

[37] Hu, X., Zhang, A., Zhang, J., and Jiang, M. Abscisic acid is a key inducer of hydrogen peroxide production in leaves of maize plants exposed to water stress. *Plant Cell Physiol.* 2006. Vol. 47. P. 1484–1495. doi: 10.1093/pcp/pcl014

homologs during different stress conditions. The NADPH oxidase produces $O^{\bullet-}_2$ by transferring electrons from cytosolic NADPH to O_2, which either spontaneously dismutates to H_2O_2 or is catalyzed by SOD. The fact that NADPH oxidase plays an important role in plant defense against pathogenic infection and abiotic stress conditions is well supported[2].

During stress, the ***cell wall***-localized lipoxygenase (LOX) causes hydroperoxidation of polyunsaturated fatty acids (PUFA) making it active source of ROS like $OH^{\bullet}$, $O^{\bullet-}_2$, H_2O_2, and $^1O_2$. The cell wall-localized diamine oxidases utilize diamines or polyamines to generate ROS in the cell wall. During pathogen attack, lignin precursors undergo extensive cross-linking, via H_2O_2-mediated pathways to reinforce the cell wall with lignin[38].

The NADPH-mediated electron transport involving $CytP_{450}$, localized in the ***endoplasmic reticulum*** generates $O^{\bullet-}_2$. Organic substrate, RH interacts with the $CytP_{450}$ followed by reduction by a flavoprotein to give rise to a free radical intermediate (Cyt P_{450} R^-). This intermediate promptly reacts with triplet oxygen ($^3O_2$) to form an oxygenated complex (Cyt P_{450}-ROO^-). The complex may occasionally decompose to Cyt P_{450}-Rh by generating $O^{\bullet-}_2$ as byproduct[39].

5. The role of ROS in plant cells

ROS in plant cells are by-products of normal metabolism due to the leakage of electron transport chains in chloroplasts, mitochondria, as well as in those compartments where there are enzymes of redox reactions. In a normally functioning cell, there is a certain balance between the activation and deactivation of Oxygen, so the number of its active forms remains at a safe level, however, damage to plant tissues under the influence of stress factors, as a rule, leads to the activation of Oxygen, while the balance between the formation and destruction of ROS is disturbed. It is generally accepted that the main target of ROS is cell membranes, the lipids of which undergo enzymatic and free radical peroxidation, which primarily damages polyunsaturated fatty acid molecules. The effect of ROS on protein molecules is manifested in the oxidation of their –SH groups, FeS-centers of enzymes, fragmentation of peptide chains, increased sensitivity of proteins to the action of proteases. The interaction of free radicals with DNA causes damage to nitrogenous bases, deoxyribose, single- and double-strand breaks, the occurrence of covalent crosslinks, and mutations. ROSs are inducers of apoptosis and carcinogenesis[20].

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[38] Higuchi, E. T. Look back over the studies of lignin biochemistry. *J. Wood Sci.* 2006. Vol. 52. P. 2–8. doi: 10.1007/s10086-005-0790-z

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ROS generation by a plant cell occurs in response to environmental stressors of abiotic origin – drought and salinity, low and high temperatures, heavy metal ions, herbicides, UV radiation, air pollution, hypoxia.

At the current stage, the British school of biochemistry, headed by Dr. Nicholas Smirnoff, is engaged in the development of the problem of ROS and AOZ of plant organisms, whose latest and most powerful comprehensive work is "Antioxidants and reactive oxygen species in plants".

The study of the role of ROS in the anti-infection protection of animals, the processes of oxidative explosion, the mechanisms of aging and apoptosis opened the prospects of finding analogs in the plant world. This direction is the subject of research by A.A. Aver'yanov, according to whose work a balance exists between the activation and deactivation of oxygen in a normally functioning plant cell, so the amount of its active forms remains at a safe level. However, structural and functional disorders of plant tissues, as a rule, lead to oxygen activation. The normally existing balance between the formation and utilization of ROS can be disturbed in various pathological conditions of plants. Since the activation of oxygen is one of the first responses of a plant cell, it is not excluded that it is ROS that plays an important role in suppressing the development of pathogens. Thus, during the implementation of the hypersensitivity reaction, phenols are released from the vacuoles and their enzymatic oxidation occurs. And since this process is accompanied by the generation of activated oxygen in toxic concentrations, it is possible that it is the cause of the death of both the infected host cells and the invading pathogen. In the laboratory of I.A.Tarchevsky, it was established that pathogenic microorganisms induce a cascade of protective reactions in the plant cell long before resistance or susceptibility is fully manifested. This is achieved by the functioning of signal systems, the main of which in the plant body are: calcium, lipoxygenase, NADPH oxidase (superoxide synthase), NO synthase, adenylate cyclase, phosphoinositol, and MAP kinase. ROSs play a key role in the functioning of the first four of them. In the superoxide synthase signaling system, when NADPH localized in the cytoplasmic membrane is oxidized by molecular oxygen, $\bullet O_2^-$ is formed, which is transformed into H_2O_2 as a result of a reaction catalyzed by SOD[7]. It is assumed that $\bullet O_2^-$ and H_2O_2 are secondary messengers in the superoxide synthase signaling system. H_2O_2 causes the activation of transcriptional regulation factors and, as a result, the expression of protective genes.$\bullet O_2^-$ and H_2O_2 do not always ensure an effective course of the hypersensitivity reaction, since $\bullet O_2^-$ has a too short lifetime, and H_2O_2 is toxic only in sufficiently large concentrations, therefore, in addition with these AOP functions in the plant cell NO, which is formed in the NO signaling system (L-arginine + NADPH + O_2 → citrulline + NO + $NADPH^+$) and enhances the effect of APO, causes transcriptional activation of protective genes and a

hypersensitivity reaction, effectively killing microbes. This is additionally confirmed by the presence of Ca-dependent NO-synthase localized in the cytosolic fraction in plants. The interaction of AFO with •NO as well as salicylate is synergistic, since the amount of NO increases tenfold in the hypersensitivity reaction. Accumulation of salicylic acid in the infected tissue inactivates catalase, increasing the amount of H_2O_2, which enhances the oxidative burst, and the latter, in turn, increases the synthesis of salicylic acid, which for this reason is called a "signal amplifier". NO reacts with superoxide, forming peroxynitrile (OHOO), an extremely toxic compound that inhibits pathogens. Next, a hydroxyl radical is formed from peroxynitrile, which further increases toxicity. An increase in NO concentration indirectly affects Ca^{2+}-dependent protein kinases, the protein factor of transcription regulation and the synthesis of protective proteins. It should also be added that the very first stages of the hypersensitivity reaction are activated by phospholipase C, which is part of the Ca^{2+} signaling system. Thus, upon contact with a pathogen, the concentration of Ca^{2+} in the cytoplasm increases, which activates soluble and membrane-bound Ca^{2+}-dependent protein kinases, which in turn participate in the phosphorylation of protein factors regulating the expression of protective genes. In the LOG signaling system, free unsaturated fatty acids released under the action of phospholipase A2 independently activate protein kinases or, with the help of LOG, add oxygen and turn into hydroperoxy derivatives. The latter in the course of lyase, peroxygenase, and adenylate cyclase reactions form compounds toxic to microorganisms (hexinals, nonadienals, hydroxy- and epoxy derivatives), as well as cyclic acids (phytodienic and jasmonic), which, with the participation of protein kinases, also cause the expression of protective genes. In this way, the activation of the signaling system in response to the action of pathogens leads to the activation of the expression of protective genes and an increase in plant resistance. In 1933, the American scientist Chester expressed an opinion about the existence of physiological acquired immunity in plants, during the formation of which ROS act as signaling intermediates that participate in the activation of enzyme genes that carry out protective reactions, in particular, to the synthesis of phytoalexins[20].

Thus, ROSs arising during the oxidative burst are not only the direct cause of the hypersensitivity reaction, but also the inducer of genes that include subsequent protective reactions in plant cells, such as the SAR state. There is an assumption that changes in the PAS balance affect the immune resistance of plants. These data, as well as the constantly growing number of publications on the participation of ROS in other important physiological processes (metabolism and synthesis of phytohormones, regulation of photosynthetic reactions and mitochondrial oxidation, apoptosis, aging), require a more

detailed, qualitatively new approach to studying the biological role of ROS and AO in vital activities of plants

CONCLUSIONS

The sources of ROS in the body are mitochondrial, microsomal, phagocytic electron transport chains of oxidation, monoamine oxidase, xanthine oxidase, interaction of metal ions of variable valence with oxygen and reducing agents. Under physiological conditions, ROS are formed mainly in the following systems[40]:

- in the respiratory chain, mitochondria are small amount (up to 100 pmol) due to the transfer of 5-10% electrons from physiological acceptors to molecular oxygen (in this case, mainly 2amo acceleration of $O_2^{\bullet -}$ is generated, the rate of formation of which is directly dependent on the degree of conjugation of the respiratory chain); enzymatic complexes of the respiratory chain of mitochondria, which generate $O_2^{\bullet -}$ (NADP-dependent dehydrogenase, NAD-dependent ubiquinone reductase), can be activated during physical exertion (muscle contraction), energy-dependent processes in the kidneys, transmembrane processes, etc.; believe that $O_2^{\bullet -}$ is the precursor of all other forms of ROS in vivo;

- in the process of NADPH oxidase activation; expression of this enzyme is characteristic of blood phagocytes, endotheliocytes, chondrocytes and astrocytes; NADPH-oxidase catalyzes 2 accelerates the reduction of O_2, taking the reducing equivalent from NADPH; activation of NADPH oxidase occurs under the influence of cytokines (INF-γ, TNF-β, IL-1β, some growth factors) and is accompanied by the formation of $O_2^{\bullet -}$ and H_2O_2;

- in the synthesis of prostaglandins both by the cyclooxygenase pathway in the process of converting PgG_2 into PgH_2 (the peroxidase function of PgH-synthase), and by the lipoxyganase pathway – in the process of converting hydroperex of arachidonic acid into oxyacid; this process is controlled by a number of peptide hormones (angiotensin), cytokines (TNF-β) and growth factors;

- in the system of myeloperoxidase–H_2O_2-halogens (Cl^-, Br^-, I^-), which is triggered due to the activation of phagocytosis and leads to the formation of $O_2^{\bullet -}$ OCl^- and HO;

- during spontaneous MAO oxidation of dopamine and adrenaline (spontaneous oxidation produces $O_2^{\bullet -}$, and catalyzed MAO produces H_2O_2;

- when glutamate receptors are activated; for example, activation of the NMDA subtype of glutamate receptors on the postsynaptic membrane leads to the opening of channels permeable to Ca^{2+} and K^+; the consequence of the

[40] Netyukhailo L.G., Kharchenko S.V. Active forms of oxygen (literature review). Young Scientist. 2014. Vol. 9 (12). P. 131-135.

activation of these receptors is the intracellular production of ROS ($O_2•^-$ and NO), and the result of the activation of Ca^{2+}-dependent NO-synthase is NO;

- when activating AMPA receptors, based on both Ca^{2+}-dependent and mitochondrial mechanisms;

- during the synthesis of NO.

In addition to the complete restoration of the O_2 molecule to water in the respiratory chain of mitochondria in the cells of aerobic microorganisms always occurs and is incomplete – one-three-electron reduction with the sequential formation of various ROS, which include the free radical anion, singlet oxygen, hydroxyl radical, hydrogen peroxide, molecular oxygen, peroxyl radical (alkyl dioxide), oxyl radical (alkoxide), nitrogen monoxide, peroxynitrile, hypochlorite, ozone (O_3) and atomic oxygen (•O•). ROS cause the formation of organic hydroperoxides ROOH – DNA, proteins, lipids, as well as small molecules.

SUMMARY

Reactive oxygen species (ROS) are formed by one-electron reductions of an oxygen molecule. The main ROS are superoxidanion radical, hydroxyl radical, hydrogen peroxide, singlet oxygen, molecular oxygen, peroxyl radical (alkyl dioxide), oxyl radical (alkoxide), nitrogen monoxide, peroxynitrile, hypochlorite. Exogenous ROS are represented by ozone (O_3) and atomic oxygen (•O•). In terms of activity in the initiation and continuation of free radical peroxidation of biopolymers, endogenous ROS form the following series: hydroxyl radical > singlet oxygen > hydrogen peroxide > superoxide anion radical. Known active forms of other elements HOCl, HOBr, HOJ, •NO, $•NO_2$, CO, ions with an unpaired electron $•Fe^{+3}$, $•Cu^{+2}$, $•Mn^{+2}$, $•Mo^{+5}$. The sources of ROS in the body are mitochondrial, microsomal, phagocytic electron transport chains of oxidation, monoamine oxidase, xanthine oxidase, interaction of metal ions of variable valence with oxygen and reducing agents. Free radical oxidation can occur with the formation of peroxides, which is characteristic of very reduced hydrophobic compounds such as lipids, or without the formation of peroxides, which is more characteristic of more oxidized proteins, nucleic acids and carbohydrates (free radical oxidation). ROS are formed intracellularly by leakage from the sphere of enzymatic (xanthine oxidase) or non-enzymatic reactions of predominantly mitochondrial and microsomal oxidation, but they are involved in enzymatic lipid peroxidation.

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Information about the author:
Bobrova Mariia Serhiivna,
Candidate of Biological Sciences,
Associate Professor at the Department of Natural Sciences and their Teaching Methods
Volodymyr Vynnychenko Central Ukrainian State University
1, Shevchenka str., Kropyvnytskyi, 25006, Ukraine