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THE EFFECT OF OXIDATIVE MODIFICATIONS OF AMINO ACID RESIDUES IN THE ACTIVE CENTER OF TYROSINASE ON THE PROCESS OF GRAYING

WPŁYW OKSYDACYJNYCH MODYFIKACJI RESZT AMINOKWASOWYCH W CENTRUM AKTYWNYM TYROZYNAZY NA PROCES SIWIENIA

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Summary

Reactive oxygen species (ROS) are molecules that form during physiological processes of cellular metabolism. Production of ROS in the organism is strictly controlled by the system of numerous anti-oxidative mechanisms. Insufficiency of defense systems and an increase in the level of free radicals leads to disturbances of the oxidative-antioxidative balance, and consequently, to generation of oxidative stress.

Many observations indicate that the process which underlies graying is oxidative stress induced by hydrogen peroxide. Milimole concentrations of hydrogen peroxide (H_2O_2) are accumulated within gray hairs. Its presence inhibits transformation of tyrosine to melanin through the inactivation of the enzyme tyrosinase. The cause of an increase in H_2O_2 level is most probably a decrease in activity of catalase (CAT) responsible for its detoxication. This is a significant step forward, since the studies have indicated that another enzyme, called pseudocatalase, may prevent generation of gray hair. Scientists are working on obtaining effective inhibitors of the process of graying that could in the future serve as medicines preventing premature graying. Finding the mechanisms responsible for this process creates possibilities for developing new objectives for pharmacological therapy.

The aim of this work is to present current reports concerning the problem of graying and a potential participation of oxidative stress in the course of this process.

Streszczenie

Reaktywne formy tlenu (RFT) są molekułami powstającymi podczas fizjologicznych procesów metabolizmu komórkowego. Produkcja RFT w organizmie podlega ścisłej kontroli przez system licznych mechanizmów antyoksydacyjnych. Niewydolność systemów obrony oraz wzrost poziomu wolnych rodników prowadzi do zaburzeń równowagi oksydacyjno-antyoksydacyjnej, a w konsekwencji do generacji stresu oksydacyjnego.

Wiele obserwacji wykazuje, że procesem leżącym u podłoża siwienia jest stres oksydacyjny indukowany przez nadtlenek wodoru. W obrębie siwych włosów gromadzone są milimolowe stężenia nadtlenku wodoru (H_2O_2). Jego obecność hamuje przemianę tyrozyny w melaninę poprzez inaktywację enzymu tyrozynazy. Przyczyną wzrostu poziomu H_2O_2 jest najprawdopodobniej spadek aktywności katalazy (CAT) odpowiedzialnej za jego detoksykację. Jest to znaczący krok naprzód, ponieważ badania wykazały, że inny enzym, zwany pseudokatalazą, może zapobiec tworzeniu się siwych włosów. Naukowcy pracują nad uzyskaniem skutecznych inhibitorów procesu siwienia, mogących w przyszłości służyć jako leki zapobiegające przedwczesnemu siwieniu. Poznanie mechanizmów odpowiedzialnych za ten proces stwarza możliwości na opracowanie nowych celów dla terapii farmakologicznej.

Celem prezentowanej pracy jest przedstawienie aktualnych doniesień na temat problemu siwienia i potencjalnego udziału stresu oksydacyjnego w przebiegu tego procesu.

Keywords: catalase, hydrogen peroxide, melanogenesis, oxidative stress, tyrosinase.
Słowa kluczowe: katalaza, nadtlenek wodoru, melanogeneza, stres oksydacyjny, tyrozynaza.

INTRODUCTION.

Reactive oxygen species (ROS) are molecules that are formed during physiological processes of cellular metabolism. Released in small amounts, they perform the role of mediators and regulators of metabolism. The main source of ROS in cells are respiratory processes, where water is produces as a result of the complete reduction of an oxygen molecule. Partial reduction of the oxygen molecule leads to generating by-products of this process [1].

The presence of antioxidative enzymes, such as: glutathione peroxidase (GPx), glutathione reductase (GRx), superoxide dismutase (SOD), catalase (CAT) and low-molecular antioxidants: vitamin C, E, β-carotene, glutathione etc., allows for removal of the excessive ROS, repair of damage that occurred as a result of their action and maintaining the proper oxidoreductive potential inside the cells. Insufficiency of defensive systems and an increase in free radicals level leads to disturbances of prooxidative-antioxidative balance and, in consequence, to generation of oxidative stress. An increased or prolonged state of oxidative stress induces damage of important macromolecules in the cell. Nucleic acids, proteins and lipid components are the most exposed to oxidative damages [2,3].

The studies from recent years show a possible participation of oxidative stress in the course of graying process. DNA damage and apoptosis of melanocytes in the hair follicle is caused by reactive oxygen species. It was proved that milimole concentrations of hydrogen peroxide (H_2O_2) are accumulated within gray hairs. The cause of an increase in H_2O_2 level is most probably a decrease in the activity of catalase responsible for its catabolism and preventing from generation of other free radicals and reactive oxygen derivatives. The aim of this work is to present the current reports concerning the problem of graying and a potential participation of oxidative stress in the course of this process [4].

HYDROGEN PEROXIDE – SUBSTRATE FOR A REACTION CATALYZED BY CATALASE.

Hydrogen peroxide is a natural product of the cellular metabolism. It is produced in cells both during many enzymatic reactions and ego. It is produced in cells both during many

enzymatic and non-enzymatic reactions. Hydrogen peroxide easily diffuses through biological membranes and it may appear in different segments of the cell. Due to its oxidizing properties, H_2O_2 is regarded as a toxic compound. It can damage proteins, lipids, sugars and nucleic acids. From the biological point of view, the most important reactions with H_2O_2 as a mediator is oxidation of thiol residues of proteins and oxidation of transition metal ions in the Fenton reaction. The Fenton reaction results in formation of one of the most reactive radicals- hydroxyl radical initiating oxidation of the polypeptide chain. Oxidative damage of proteins leads to a change or loss of their biological function. Oxidized proteins easily form aggregates which in turn may inhibit enzymatic systems responsible for their degradation. These processes create favorable conditions for accumulation of changed proteins in cells [5].

Removing the excess H_2O_2 takes place with the use of catalase or glutathione peroxidase. Due to a higher affinity of GPx to H_2O_2,it is responsible for detoxication of this compound, especially when it occurs in a low concentration in the cell. In the case of a high concentration of H_2O_2 this role is overtaken by catalase [5].

Catalase is a homotetramer built of four identical subunits. Each of the subunits have a hem group in its active center, with a centrally located iron atom, usually bound to one molecule of NADPH. Catalase take part in the reaction of disproportioning H_2O_2 to oxygen and water. It may also show peroxidase activity towards some compounds. In mammals, catalase is located mainly in peroxisomes, although small amounts of this enzyme were also detected in mitochondria and endoplasmic reticulum. Peroxisomes are vesicles surrounded with a single membrane containing proteins that are unique for it, including transporters for substrates of peroxisomal enzymes. Inside peroxisomes, apart from catalase, there are also enzymes of ß-oxidation of fatty acids, enzymes of lipid biosynthesis and aminotransferases [5].

The activity of CAT depends on many factors, such as the temperature, pH, the presence of activators or inhibitors. Reduction of CAT activity is observed in the case of many diseases of inflammatory origin, where the participation of the oxidative stress is suggested. It was observed that in the initial stage of disease process with inflammatory character the level of CAT increases rapidly. This phenomenon is most likely resulting from releasing CAT from damaged cells and closely connected with the defensive reaction of the organism. Nevertheless, in the case of chronic inflammation states, especially those where a long-term oxidative stress is observed, CAT activity is decreased. CAT deficit is a result of mutation inherited in an autosomal recessive pattern. Deficiency or lack of this enzyme is

often connected with the occurrence of various ailments and may lead to developing α-thalassemia [5].

MECHANISM OF INHIBITING THE ENZYME TYROSINASE BY HYDROGEN PEROXIDE.

The color of hair is dependent on the presence of melanin – a pigment that occurs in the living organisms and has the ability to absorb and disperse UV radiation. Melanins effectively reduce the formation of free radicals and take part in their neutralization. Thanks to this they protect cells against oxidative damage of nucleic acid, proteins and lipids. Their synthesis proceeds in melanocytes during the process of melanogenesis. Regulation of this process is extremely complex and dependent of a number of physical and biochemical factors [6].

Melanocytes inhibiting hair derive from cutaneous melanocytes and they constitute a kind of reserve of stem cells for pigment cells present in the skin. Melanocytes are located in the hair bulbs and they are responsible for production of melanosomes – organelles containing and producing melanin. Melanosmomes supply the hair cortical layer. The amount of melanosomes is closely connected with the activity of melanocytes and is reflected in the hair color. Dark hairs are characterized by a rather large amount of melanocytes and a substantial amount of eumelanosomes with the dark eumelanin. Brown hairs have slightly smaller melanocytes than those in dark hair. In blond hair, in turn, poor melanization is observed. The presence of pheomelanosomes with red and yellow pheomelanin is observed in people who have hair with reddish color [7,8].

The process of melanogenesis begins from transformation of L-tyrosine to DOPAchinon. The substrate in biosynthesis of eu- and pheomelanin is tyrosine, which forms from L-phenylalanine as a result of multi-stage process of oxidation and polymerization. L-tyrosine undergoes hydroxylation to 3, 4-dihydroksy-L-phenylalanine (L-DOPA) in the melanosome. Regulation of the process of melanogenesis is extremely complex and involves the participation of physical and biochemical factors. The reaction of transformation is catalyzed by the enzyme tyrosinase (TYR) [9].

Tyrosinase is a glycoprotein composed of 511 amino acids. Within the structure of TYR one can distinguish the N – terminal domain, located inside the melanosome, the C – terminal domain located in the melanocyte cytoplasm and transmembrane one, connecting both domains. The N – terminal domain is responsible for the catalytic activity of the enzyme and for melanin synthesis in the melanosome. In the active center of this domain there are two copper atoms (Cu). Different TYR forms are distinguished, depending on the degree of Cu

oxidation and the type of bound ligands: oxytyrosinase, mettyrosinase and deoxytyrosinase. The C – terminal domain is responsible for the location and intracellular transport of tyrosinase. While examining the mechanism of reactions involving tyrosinase, it should be noted that it is the key enzyme taking part in the course and regulation of melanogenesis [9].

The latest scientific research proves that milimole concentrations of hydrogen peroxide are accumulated within gray hair. Its presence inhibits transformation of tyrosine to melanin through the inactivation of the enzyme tyrosinase. The cause of an increase in H_2O_2 level is most probably a decrease in the activity of catalase responsible for its detoxication. The influence of hydrogen peroxide leads to the oxidation of methionine residues in the TYR active center and production of methionine sulfoxides (Met-S=O). Oxidative modifications of amino acid residues of tyrosinase lead to the loss of catalytic function of this enzyme. The majority of cells are equipped with methionine sulfoxide reductase (MSR), allowing for restoration of amino acids to their reduced form. In this case, additionally, a decrease in MSR activity was indicated, thanks to which the introduced modifications become irreversible [4,1].

PSEUDOCATALASE – PROMISING MEDICINE INHIBITING THE PROCESS OF GRAYING.

The latest studies have proved that the loss of hair pigmentation may be corrected by the local application of pseudocatalase (PC-KUS). This compound is activated by UVB radiation and considerably reduces accumulation of hydrogen peroxide in hair follicles. This finding was made studying a culture of human cells of hair follicles. The compound PC-KUS, performing the function of catalase, effectively inhibits the progression of pigment loss thanks to H_2O_2 reduction in hair follicles and epidermis in people with vitiligo. A team of German scientists from the Ernst Moritz University in Greifswald recorded positive results of treatment with the use of this compound [10, 4].

The results of the study seem to be highly promising and in the future the compound PC-KUS may turn out to be an effective medicine against the symptoms of premature graying.

CONCLUSION.

Many observations indicate that the process which underlies greying is oxidative stress induced by hydrogen peroxide. Scientists are working on obtaining effective inhibitors of the

process of graying which could in the future serve as medicines preventing premature graying. Finding the mechanisms responsible for this process creates possibilities for developing new objectives for pharmacological therapy.

The results of studies published in recent years have indicated that pseudocatalase can effectively prevent the accumulation of excessive H_2O_2 within hair follicles and serve as a medicine preventing premature graying in the future. Moreover, it appears that it also can be effective in people suffering from vitiligo. Some experts suggest that additional supplementing with minerals, such as selen and zinc, which are used by the organism to produce catalase, methionine sulfoxide reductase and tyrosinase, may increase the effectiveness of therapy.

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