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SUPEROXIDE-PRODUCING THERMOSTABLE ASSOCIATE BETWEEN NADPH CONTAINING LIPOPROTEIN AND NADPH OXIDASE FROM RATS BONE MARROW CELLS, NUCLEUS AND MITOCHONDRIA MEMBRANES: ISOLATION, PURIFICATION AND PROPERTIES

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From white rats bone marrow (BM) mitochondria, nucleus and cells membranes the isoforms of superoxide (O_2^-)-producing thermostable associate between NADPH containing lipoprotein (NLP) and total fraction of isoform of NADPH oxidase (Nox) – (NLP-Nox), were isolated and purificated for the first time. By the expense of own NADPH the separated NLP from associates of BM NLP-Nox, only reducing (antioxidant) effect was indicated. The isoforms of these NLP were stimulate the O_2^--producing activity of the immune cells (leukocytes and erythrocytes) membranes Nox also, by forming the hybrid associates between these Nox (NLP-hNox).. After incubation in boiling water during 10-15 min bone marrow associates practically are not decreased its superoxide producing activities. Thus, the isoforms of O_2^--producing thermostable NLP-Nox associates are a new structural-functional components of the bone marrow cells, nucleus and mitochondria membranes and produced O_2^-.by immediately mechanism in liquid and gas phases.

Bone marrow – superoxide producing associate – NADPH oxidase

Առաջին անգամ սպիտակ առնետի ոսկրածուծի (ՈԾ) միտոքոնդրիումների, կորիզների ու բջիջների թաղանթներից անջատվել և մաքրվել է սուպերօքսիդ (O_2^-)-գեներացնող ջերմակայուն ասոցիատ՝ կազմված ՆԱԴՖՀ-պարունակող լիպոպրոտեինից (ՆԼՊ) և ՆԱԴՖՀ օքսիդազի իզոձևերի գումարային ֆրակցիայից (Nox)՝ ՆԼՊ-Nox: Շնորհիվ սեփական ՆԱԴՖՀ-ի՝ ասոցիատներից տարանջատված ՆԼՊ-ի իզոձևերը ցուցաբերում են միայն վերականգնիչ (հակաօքսիդանտային) ազդեցություն: Այս ՆԼՊ-ի իզոձևերը խթանում են իմունային բջիջների (լեյկոցիտներ, էրիթրոցիտներ) թաղանթների Nox-երի O_2^--գեներացման ակտիվությունը՝ կազմելով այդ Nox-երի հետ հիբրիդային ասոցիատներ (ՆԼՊ-hNox): Ոսկրածուծի ասոցիատների O_2^--գեներացնելու ակտիվությունը չի նվազում դրանք եռացող ջրում 10-15 րոպե ինկուբացնելուց հետո: Այսպիսով, ոսկրածուծի բջիջների, կորիզների և միտոքոնդրիումների թաղանթների O_2^--գեներացնող ջերմակայուն ՆԼՊ-Nox ասոցիատի իզոձևերը հանդիսանում են այդ թաղանթների նոր կառուցվածքային-ֆունկցիոնալ բաղադրամասեր են և գեներացնում են O_2^--եր անմիջական մեխանիզմով հեղուկ և գազ փազերում:

Ոսկրածուծ – սուպերօքիդ գեներացնող ասոցիատ – ՆԱԴՖՀ օքսիդազ

Впервые из мембран клеток, ядер и митохондрий костного мозга (КМ) белых крыс выделены и очищены изоформы термостабильного ассоциата между НАДФН содержащим липопротеином (НЛП) и суммарной фракции терминальных изоформ НАДФН оксидазы

(Nox) – НЛП-Nox. За счет собственного НАДФН, отделенный НЛП из этих ассоциатов НЛП-Nox оказывает только восстановительный (антиоксидантный) эффект. Изоформы этих НЛП стимулируют O_2^--продуцирующую активность Nox мембран иммунных клеток (лейкоцитов и эритроцитов), создавая с ними гибридный ассоциат (НЛП-гNox). Ассоциаты КМ не дезактивируются после их инкубации в кипяченой воде в течение 10-15 мин. Таким образом, изоформы O_2^--продуцирующих термостабильных ассоциатов КМ являются новыми структурно-функциональными компонентами мембран клеток, митохондрий и ядер КМ и продуцируют O_2^- непосредственным механизмом в жидкой и газовой фазах.

Костный мозг – супероксид-продуцирующий ассоциат – НАДФН оксидаза

It is known that bone marrow-derived mesenchymal stem cells are the most frequently used stem cells in cell therapy and tissue engineering. Stem cells are a type of cells that have the potential to differentiate into various cells, including neurons. They exert a therapeutic effect by safely and effectively differentiating into neurons or replacing damaged cells, secreting neurotrophic factors, and inhibiting the inflammatory response. Simultaneously, the bone marrow (BM) is the major reservoir for endothelial progenitor cells (EPCs) [1,2]. In pathophysiological states such as aging, atherosclerosis failure, hypertension and diabetes, excess amounts of reactive oxygen species (ROS) create an inflammatory and oxidative microenvironment, which induces cell damage and apoptosis of stem and progenitor cells. NADPH oxidase (Nox) in BM and endothelial progenitor cells is potential therapeutic targets for promoting neovascularization in ischemic cardiovascular diseases [3, 4].

The stable O_2^--producing associates of NLP-Nox from mammals erythrocytes and leukocytes membranes, as well from Stevia medicinal plant cells membranes already were prepared for the first time [5, 6]. Are these associates presence in the cell and cell membranes of white rats BM? It is necessary to find out.

The aim of the work is to elaborate the method for isolation, purification and determination of the properties of O_2^--producing associates of NLP-Nox from cells, nucleus and mitochondria membranes of white rats BM, separation of NLP and total fraction of Nox1+Nox2 from these associates, to determine the mechanism of the production of O_2^- by these native and hybrid associates.

Materials and methods. *Isolation and purification of the white rats BM cells, nucleus and mitochondria membranes.*

After ether narcosis of sexually mature Wistar line white rats (220-250 g) the BM (up to 8-8,5 g) was collected. After homogenization of BM in 0,25 M sucrose (up to 120 ml), the nucleus was precipitated by centrifugation at 2500 × g, for 10 min, the mitochondria – 14.000 × g, 10 min and cells membranes – 5800 × g, for 15 min, at pH 5,6. After washing of nucleus, mitochondria and membranes precipitates with sucrose solution (1:50 v/v), them was collected by centrifugation in same conditions. The purificated nucleus, mitochondria and cells were homogenized in the water (up to 60 ml). After freezing and defrosting of these homogenates and dilution with water (4 times) the membranes of the cells, nucleus and mitochondria of the BM were precipitated at 5800 × g, for 10 min at pH 5,6. Then the precipitates of these purified membranes were homogenized in the water (up to 40 ml).

Isolation and purification of O_2^--producing associate NLP-Nox, as well total fraction of the isoforms of Nox1+Nox2 from cells, nucleus and mitochondria membranes of BM.

From purificated membranes of BM the isoforms of the total fraction of Nox1+Nox2 and associates of NLP-Nox isolated and purificated, using the process of formation of the nonstable complex between ferri-Hb (Hb) and Nox, as well, with NLP-Nox, also. The stimulation of releasing of the isoforms of Nox and NLP-Nox associates from these membranes to soluble phase were take place [7].

In particular, to the water mixtures of BM cells, nucleus and mitochondria membranes the 50 μM ferri-hemoglobin (Hb) from donors blood were added at the pH 9,5 (using deluted solution of KOH). After incubation of these mixtures at 37°C during 1,5 hours and centrufugation at 5800 × g for 10 min, the pH of the supernatantes were decreased to 4,8, by addition of HCI dilute solution. After centrifugation of these mixtures in above mentioned conditions, the isoforms of Nox1+Nox2 (fraction - I) as a unstable complex with Hb upon ion-exchanging chromatography on DE-52 cellulose at pH 9,5 from supernatants were isolated. After homogenization of above mentioned precipitates (fractions of NLP-Nox) in water, its pH again increased to 9,5 and after its centrifugation and delution with water up to 3-4 times and its ion-exchanging chromatography on DE-52 cellulose columns also at pH 9,5 were carried out. In these conditions NLP-Nox associates was not absorbed on DE-52 column and elutes without any delay. The total fraction of isoforms of Nox1+Nox2 (fraction-II) with Hb were absorbed on this column (as a nonstable complex between Nox and Hb). The Hb is eluated by 0,005 M potassium phosphate buffer (PPB), and total fraction of the isoforms Nox1+Nox2 was eluted by 0,2 M PPB.

After joining of above mentioned Nox fractions of I and II and treatment with 15 ml ethanol and 0,9 ml chloroform mixture (for 10 ml solution of Nox or associates) the precipitates of Hb traces was separated by centrifugation [8]. In the following stage the gel-filtration of these associates on the column of G-100 Sephadex at pH 9,5 were carried out (on this column these associates were not decay).

Separation of NLP from NLP-Nox associate of BM cells, nucleus and mitochondria membranes.

Separation of NLP from NLP-Nox associates of bone marrow cells, nucleus and mitochondria membranes by transmition of its water solutions through Sephadex DEAE A-50 at pH 9,5 was carried out. The Nox was absorbed on this column, and NLP was eluted without any delay. The traces of Nox was denaturized by deluted hydrogen peroxide [9], and its traces decay with catalase. The traces of catalase in these NLP was removed by ion-exchanging chromatography on DE-52 column at pH 9,5. As opposed to catalase, on this column the NCL doesnt absorbed.

Isolation and purification of erythrocytes and leukocytes membranes from donor blood.

Plasma of the 2nd group of donor blood (20 ml each one, from donor blood bank) was isolated from erythrocytes by using 3% Dextran-70 ("Loba Finchemie", Germany), dissolved in saline [10]. After washing of the erythrocytes precipitates with 0.9% NaCl and its hemolysis in water (1:4 v/v), erythrocytes membranes (EM) was precipitated by centrifugation (5800 × g, 10 min). Then, EM was washed by 0.04M potassium phosphate buffer, pH 7.4 (PPB) and precipitated under above mentioned conditions until colorless supernatant.

Donors' blood leukocytes were precipitated by centrifugation in similar conditions. After washing the leukocyte precipitate in saline it was homogenized with water and after freezing and thawing leukocytes membranes (LM) was precipitated by pH 5.6, also. After washing by 0.04 M PPB, the LM precipitate was collected by centrifugation (13000 × g, for 10 min). Afterwards, the obtained EM and LM precipitates were mixed with water (1:5 v/v). The prepared blood serum was again centrifuged by 14000 × g, for 15 min.

Isolation and purification of the total fraction of Nox1+Nox2 isoforms from EM and LM.

In aqueous mixtures EM and LM the pH was adjusted to 9.5 by the addition of 0.1 M KOH as well as 50 μM Hb from human erythrocytes and was incubated at 37° for 1,5 hours. After centrifugation at 5800 × g 10 minutes the supernatants underwent ion-exchange chromatography on DE-52 cellulose columns from which after elution of Hb fraction by 0.005M PPB total fractions of Nox1+Nox2 from EM and LM were eluted by 0.2 M PPB. After gel-filtration of total fraction of Nox1+Nox2 isoforms in the column with Sephadex G-100, the Hb traces, included in the complex with Nox, were removed by treatment with the ethanol/chloroform fractionation [8].

Electrophoresis of the obtained associates NLP-Nox or NLP was realized in 10% polyacrylamide gel (PAAG) for acidic and basic proteins. The content of isoforms of the associates of NLP-Nox, NCL, total fractions of Nox1+Nox2, as well as EM and LM were determined by its weighing after desalting and vacuum lyophilization.

Determination of percentage of increase of the stationary concentration of produced O_2^-.

Stationary concentration of produced O_2^- by NLP-Nox complexes in the presence of sodium carbonate (pH 10,2) buffer were determined both by adrenaline [11] and nitrotetrazolium blue (NTB) [12] methods. In the latter case the 2 μM phenazine methosulfate (PMS), as initiator, was used. The produced O_2^- in homogeneous and gas phases were oxidized the adrenaline (2.10^{-4} M) to adrenochrome (at 500 nm) and reduces NTB (4.10^{-4}M) to formazan (at 560 nm). The percentage of the increase of maximal optical absorption of adrenochrome and formazan were determined by the produced O_2^- upon isoforms of the NLP-Nox in the absence (100%) and presence of these associates. The stimulation of O_2^- producing process by Nox1+Nox2 isoforms in the homogeneous phase and EM and LM in the heterogeneous phase in the presence and absence of NLP was determined after incubation of the above mentioned Nox (0.2mg/ml from EM or LM), as well as EM and LM (2mg/ml) with the obtained NLP (2mg/ml) at 37ºC for 30 min. For the suppression of these processes, the 2.10^{-8} M Cu Zn-COD from bovine liver was used.

Determination of NADPH in NLP-Nox complex or in NLP.

The presence of NADPH group in the NLP-Nox complex or NLP was determined by spectrofluorimetric method, by determine the fluorimetric intensity ("F") in relative units. The emission peak of NADPH group as part of NCL was recorded at 430nm with 370 nm excitation length. NADPH solutions of certain concentration were used as a control group.

By the known gel-filtration method molecular mass of associates on the Sephadex G-100 were determinated. The content of ascorbat-depending lipid peroxidation product – malone dialdehide (MDA) in the associates or NLP from BM cells, nucleus and mitochondria membranes were determined by the method of Wladimirov and Archakov [13].

During the work the cellulose DE-52 ("Whatman" England), Sephadex DEAE A-50 and G-100 ("Pharmacia" Sweden), Dextran-70 ("Loba Finchemie", Germany), as well as adrenaline, nitrotetrasolium blue and PMS ("Sigma") were used. A spectrophotometer "Cary 60 UV/VIS" (USA) and spectrofluorimeter "Perkin Elmer" (USA), as well as, centrifuges K-24 and K-70 ("Janetzki", Germany) were used.

The statistical treatment of the received results by Student-Fisher with the determination of the criterion of reliability "p" (M±m, n=6) were carried out.

Results and Discussion. From BM cells, mitochondria and nucleus membranes the O_2^--producing associates were isolated and purified for the first time. During electrophoresis these associates were precipitate on the entry of PAAG tubules. However, the color band for the acidic and base water soluble proteins doesn't detect. On the other hand, during gel-filtration of these associates in the Sephadex G-100 column at pH 9,5, the first eluted fractions with symmetrically elution diagram a non changed value of optical absorption spectra (A_{260}/A_{530}) for these associates on the average were: 7,7-7,9; 7,0-7,2 and 7,3-7,4, for the associates from cells, nucleus and mitochondria membranes of the BM, correspondingly. These indicates indirectly testify about purity of the associates from above indicated BM cells, nucleus and mitochondrial membranes. In visible region the optical absorption spectra of associates of NLP-Nox from cell membranes and subcell membranes of BM, the low absorption at 412 nm (the absorptiosn of heme group of the Nox), as well at 530 nm (tune up the optical spectra of NLP and Nox) were indicated. In UV region the maximal optical absorption at 260 nm for the above mentioned associates was presence. As a result, for the isolation and purification by above mentioned way, the optical spectral index (A_{260}/A_{530}) of these associates doesn't changed already. The presence of the traces of water soluble acidic and basic character proteins with these associates from BM membranes doesn't indicated. The opalescence of the water solutions at pH 9,5, as well as, the presence of the product of lipid peroxidation – malone dialdehide (MDA), to 12,4 - 12,6 μM/мг) in the composition of the associates from BM membranes were indicated.

The optical absorption spectra of the isoforms of associates from cells, nucleus and mitochondrial membranes of BM are presented in fig.1.

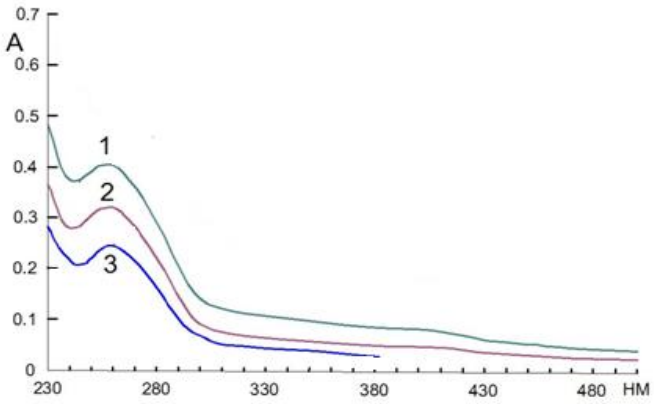

Fig.1. The optical absorption spectra of O_2^--producing associates from BM mitochondria (1), nucleus (2) and cell (3) membranes at pH 9,5 in visible and UV region. In the visible region the weak absorption at 412 nm and 530 nm, as a result of the traces of Nox were inditioned. In UV region the characteristic absorbance at 260 nm are present.

The specific amount of these associates from BM membranes was inadequate changed (tab. 1).

Table 1. The specific content (mg/g membrane) of o_2^--producing associates from bm cells, nucleus and mitochondria membranes ($p<0,05$, n=6).

Associate from membrane of:	BM
cells	21,3±2,1
nucleus	25,2±3,0
mitochondria	32,0±3,4

As indicated on tab.1, the specific amount of released NLP-Nox associate in mitochondria membranes was higher in comparison with the indices of BM cells and nucleus membranes. The presence of these associates testify about its important role, as a new structural-functional component of BM cell, nucleus and mitochondrial membranes. As a result of the influence of the produced O_2^-, the incubation of these associates during 15-20 hours at room temperature in aerobic conditions at pH 9,5, the some aggregation and increase of opalescent phone of its solutions is take place. The connection with increase of peroxidation of own lipid residues (responsible for solubility of associates) by produced O_2^-, is probably. In the presence of Cu,Zn-SOD in catalytically amounts, the increase of the turbidity of associates solution doesn't indicated. In the presence of 0.1M PPB with saline, the opalescent degree of the solutions of these associates practically doesn't increase. The molecular mass of these BM associates were 280-300 kDa, also, as in the case of O_2^--producing lipoprotein – suprol from woman's placental blood serum. The relationship of optical absorptions A_{412}/A_{530} for total fraction of the isoforms of Nox1+Nox2 from BM mitochondria membranes is composed up to 22,8±1,7; from nucleus membranes – 15,1±0,5, and cells membranes – 12,3±0,4 ($p<0,05$, n=6).

Moreover, the relationship A_{412}/A_{530} for the isoforms of Nox1+Nox2 from erythrocytes membranes (EM) and leukocytes membranes (LM) is composed up to 10,2±1,1 and 11,4±0,6 ($p<0,05$, n=6), correspondingly. These indices testify, that the ligand surround of Fe(III) of hem group of the total fraction of Nox1+Nox2 from BM mitochondria membranes a bit differed from dates of Nox1+Nox2 from membranes of nucleus and cells of BM, as well EM and LM. It is possible, that these changes of the Nox were continued just as easily, that of these Nox, isolated from stem cells of BM membranes , which are participates in various way of aerobical metabolism [1-4]. On the other hand, the increase of the absorption at 412 nm is a factor of stability of hem group of Nox, thus the stability of hem group of the Nox from membranes of BM mitochondria is higher than that of the Nox from nucleus and cells membranes of BM, as well as, from EM and LM.

As early indicated, the decrease of the absorption at 412 nm of the isoforms of Nox from biomembranes under the influence of hydrogen peroxide, the corresponding deactivation of these enzyme was take place, at the same time, the high tollerance against hydrogen peroxide the extracellular Nox (eNox) was indicated [9]. By the other hand, the optical spectral indexes of total fraction of the isoforms of Nox1+Nox2 from rats BM cell membranes in oxidized and reduced state doesn't differed from EM and LM Nox. The characteristic for the isoforms of Nox maximal optical absorption at 560 nm (α - band), 530 nm (β-band) and 412 nm (Soret absorption) were presented. After reduction of total fraction of Nox1+Nox2 by sodium dithionite, the characteristic for the Nox of optical absorptions maximums at 558 nm, 525 nm and 418 nm were evidenced, as indicates in fig.2.

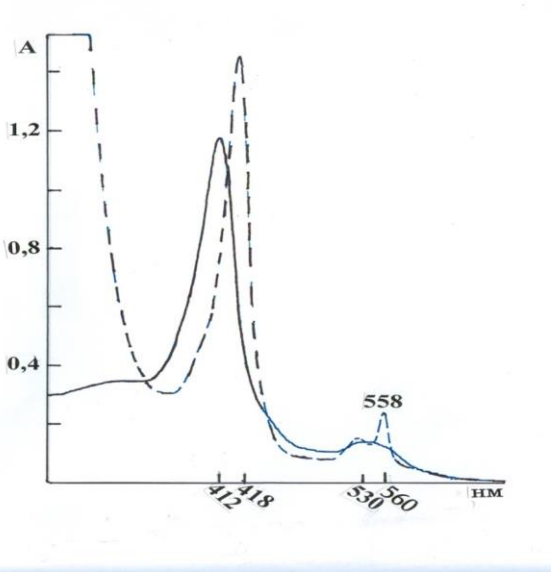

Fig.2. The optical absorption spectra of the total fraction of Nox1+Nox2 from EM and LM in oxidized (—) and reduced by sodium dithionite (----) stages.

The elevation of the Soret absorption in the isoforms of total fraction of Nox1+Nox2 from BM cell and subcellular membranes testify about increase of the stability of hem group of these Nox, especially, at the total fraction of Nox1+Nox2 from BM mitochondria membranes. It is necessary to indicate, that in aerobic conditions above mentioned associates, even in aggregated stage, contineously was produced O_2^-, at the

comparatively long time, during 25-30 days, even at 4ºC (the loss of O_2^--producing activity composed to 14-17 %).These associates practically were preserved its activity in anaerobic conditions during year.

The intensity of fluorescence ("F") in relative units of NADPH in composition of NCL-Nox or NCL from cells, nucleus and mitochondria membranes were 0,56±0,02; 0,50±0,03 and 0,65±0,01($p<0,05$, $n=6$), correspondingly. The separated NLP from NLP-Nox associate from BM cells, nucleus and mitochondria membranes were indicated only reductive (antioxidative) effect, due to own NADPH. These NLP were inhibited the process of oxidation of adrenaline by superoxide radicals, generated during the decay of hydrogen peroxide with ammonia [14]. Although, in the presence of total fraction of Nox1+Nox2 (0,2 mg/ml) from EM and LM, these NLP from BM produces O_2^-, which cause the increase of the optical density of adrenochrome (at 500 nm) or formazan (at 560 nm), and Cu,Zn-SOD was suppressed these processes. At the same time, the increase of the oxidation of adrenaline to adrenochrome or reduction of NTB up to formazan by produced O_2^- upon Nox in the presence of NLP in homogeneous phase (in solution) up to 55-74%, in comparison with control (indices in the absence of NCL), the maximal increase of these processes by the influence of Nox and NLP from BM mitochondria membranes was carried out. The increase of percentage of the maximal optical density of forming adrenochrome or formazan (up to 43-52%) in heterogeneous phase in the presence of EM, LM, membranes of BM cells, nucleus and mitochondria (up to 0,2 mg/ml) and NLP (0,2 mg/ml) were observed. At the same time, in comparison with control data (without NLP), the increase of the oxidation of adrenaline to adrenochrome or reduction of NTB up to formazan by produced O_2^- upon Nox in presence of NLP in homogeneous phase (in solution) up to 55-74% was carried out. The maximal increase of these processes by the influence of Nox and NLP from BM mitochondria membranes sach as the increase of percentage of the maximal optical density of formed adrenochrome or formazan (up to 43-52%) in heterogeneous phase in presence of EM, LM, membranes of BM cells, nucleus and mitochondria (up to 0,2 mg/ml) and NLP (0,2 mg/ml) were observed.

At the same time, the process of stimulation of the superoxide production is essentially under the influence of Nox1+Nox2 and NCL from BM mitochondria membranes. It is possible, that these effects were conditioned with the increase of the Soret absorption (at 412 nm), as a factor of stabilization of hem group of these Nox, as well, with the increase of the content of NADPH in NLP of BM mitochondrial membranes. It is possible, that these effects were caused by the increase of the Soret absorption (at 412 nm), as a factor of stabilization of hem group of these Nox, as well as by the increases of the content of NADPH in NLP of BM mitochondrial membranes.

The mechanism of the production of O_2^- by associates of NLP-Nox from BM cells, nucleus and mitochondria membranes was conditioned by electron transfer from NADPH to Fe(III), as electronic bridge of the hem group of total fraction of Nox1+Nox2 to molecular oxygen, reduces it to O_2^-. At the same time, for the NLP from BM membranes the Fe(III) of hem group of the total fraction of Nox1+Nox2 from membranes of immune cells (leukocytes and erythrocytes [5]) in homogeneous phase, as well, in gas phase, can be used. It is possible, that by the associates of NCL-Nox, as a natural, vigorous, stable and new structural-functional components of biomembranes, was conditioned the increase of the production of O_2^- by the isoforms of Nox BM stem cells in the various aerobic metabolic ways [3,4]. Thus, the isoforms of the NLP from BM cells, nucleus and mitochondrial membranes were formed hybrid associates with other types of Nox isoforms, in particular, from Nox of immune cells membranes.

The above mentioned isoforms of O_2^--producing associates from BM cells, nucleus and mitochondria membranes were indicated high thermostability. The loss of O_2^-- producing

activity and changes of optical spectral characteristics practically were not indicated (to 7-10%) after its incubation in boiling water during 10-12 min. These associates can be storaged in lyophilised state in anaerobic conditions during more than a year. Thus, these associates can be used for the determination of the mechanism of the influence of O_2^- in various biosystems at a high temperature.

The mechanism of the production of O_2^- by these isoforms of O_2^--producing associates of BM membranes also connected with immediately transferring of the electrons from own NADPH in NLP to Fe(III) of the hem group of Nox, then to molecular oxygen, reducing its up to O_2^- [5]. The rate of the production of O_2^- by these associates from BM cell, nucleus and mitochondrial membranes at high temperature (90-95ºC) increases to 4-6 times. These associates are new natural O_2^--producing associates in homogeneous phase and gas phase and can be used for determination of the mechanism of the influence of these O_2^- in various biosystem in high temperatures, also.

What are the possible perspectives for the use of NLP-Nox, separated from BM cells, nucleus and mitochondrial membranes and NCL, separated from these associates?

It is known, that at inflammation the level of reactive oxygen species (ROS), in particular O_2^-, in cells formation of mammals tissues were increased, and antioxidants had a protective effect [15,16]. On the other hand, the O_2^- was influence as antimicrobial and antiviral agent, damaging its DNA [17-20]. It is supposed, that in first stage NLP acts as antioxidant (the reductive effect of NLP was observed) and can indicate the positive effect at inflammation diseases. On the second stage, after association with localized on the surfice of immune cells membranes Nox, NLP can stimulate the production of O_2^- by immune cells, particularly, at immunodeficiency [21]. In fact, these NLP possesses with dual effects: 1) as an antioxidant, 2) as a prooxidant (O_2^--producing component) after association with total fraction of therminal Nox1+Nox2, was localized on the cells membranes surface [22].

On the other hand, the produced O_2^- in gas phase, transfered with air or oxygen through silicone tube, especially at high temperature, can be used as antibacterial and antiviral agent at the infection diseases of the lung in experiment.

Thus, the isoforms of O_2^--producing associate of the NLP-Nox of white rats BM cells, nucleus and mitochondria membranes, in homogeneous phase and gas phase as a new, thermostable, natural, structural-functional component of these membranes and NLP as a activator of the Nox of biomembranes were indicated.

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