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Thermo-induced Irreversible Changes of the Properties of Rats NADPH Oxidase

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It is known that FAD is an important cofactor for Nox isoform, as an electron carrier from NADPH to Fe(III) of the heme group then to molecular oxygen reducing it up to O_2^-[1, 6]. On the other hand, the thermostable Nox is isolated from thermostable microorganisms [5]. However, the role of FAD as a factor in changing the conformation and thermostability of Nox isoform has not yet been determined.

The aim of the present work was to determine the corresponding conditions under which the thermostability and production of superoxide radicals are also observed in the absence of NADPH.

Material and Methods

Isolation and purification of the total fraction of the Nox isoforms (Nox1-Nox2)

The total fraction of highly purified and terminal Nox isoforms (Nox1+Nox2) from EM and LCM was isolated and purified by a licensed method using the process of complex formation of exogenous ferrihemoglobin from red blood cells of donor blood with Nox isoforms on the surface of these membranes and their release in the soluble phase [7]. Next, anion-exchange chromatography on cellulose DE-52 ("Whatman", England), gel filtration on G-100 sefadex ("Pharmacia", Sweden) and fractionation with ethanol-chloroform mixture (1.5 ml ethanol with 0,9 ml chloroform for 10 ml of Nox solution) to remove traces of ferrihemoglobin was carried out.

Determination of produced O_2^- by the isoforms of Nox

As a marker of O_2^- production of Nox isoforms, the process of adrenaline oxidation in adrenochrome (at 500 nm) by superoxide radicals was used, with the determination of the rate of an increased density of maximum optical absorption of adrenochrome in the absence and presence of Cu, Zn-SOD at varying temperature (25, 50 and 100 ^oC) [4]. Optical absorption spectra of Nox isoforms were recorded on a "Cary 60" spectrophotometer (USA) with an optical range of 1 cm. The results were statistically processed by Student's t-test; F-test (M ± m, n = 6).

Results and Discussion

The forms of optical absorption spectra of the isoforms of fNox from EM and LCM of the rats in 5-6 days after isolation and purification and at 8-10 min incubation at 25^{o}C (Fig. 1a-1) and 100^{o}C (Fig. 1a-2) differs sharply. After 8-10 min incubation of nfNox isoforms in boiling water bath at 25°C (Fig. 1-b1) and 100°C (Fig. 1-b2), the shape of the optical absorption spectra doesn't change, however, the optical absorption features slightly decrease.

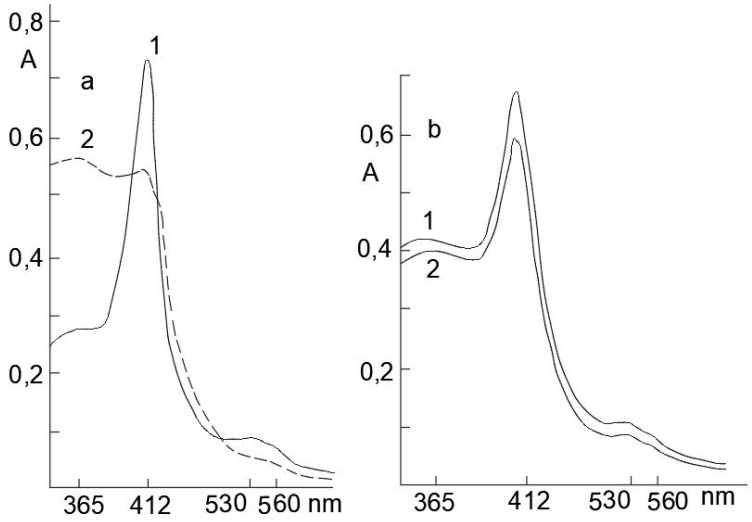

Fig. 1. Optical absorption spectra of the total fraction of isoforms of fNox ($6,10^{-5}$ M) from EM of rats blood during incubation at 25°C (a1) and 100°C (a2) for 8-10min. The observed parameters for nfNox from EM following incubation at 25°C (b1) and 100°C (b2). Similar results are obtained for the Nox isoforms from LCM and are not presented. Proteins are dissolved in 0.1 M potassium phosphate buffer at pH 7,4

An increase in the density of maximum optical absorption at 365nm, with a corresponding increase in the optical spectral index (A_{365}/A_{530}) of the nfNox was observed. This for fNox from EM and LCM is 3,2 ± 0,01 ($p<0,05$, n=6) and 3,6±0,03 ($p<0,05$, n=6) respectively. These indices for nfNox are 4,3±0,04 ($p<0,05$, n=6) and 4,5±0,03 ($p<0,05$, n=6) for nfNox from EM and LCM respectively.

This may be due to a certain advancement of the FAD group [3] in the nfNox molecule during storage under the above mentioned conditions, in general, causing irreversible conformational changes in the isoforms of the given Nox.

The second distinguishing feature between fNox and nfNox isoforms from EM and LCM is due to the fact, that the conformational change in nfNox isoforms caused by an increase in the A_{365}/A_{530} value leads to a sharp increase of the thermal stability of nfNox. The shape of the optical absorption spectra of nfNox practically does not change and the rate of production of O_2^- (tgα) increases exponentially during incubation of nfNox solution and adrenaline for 4-5 minutes at 25, 50 and 100°C (Fig. 2).

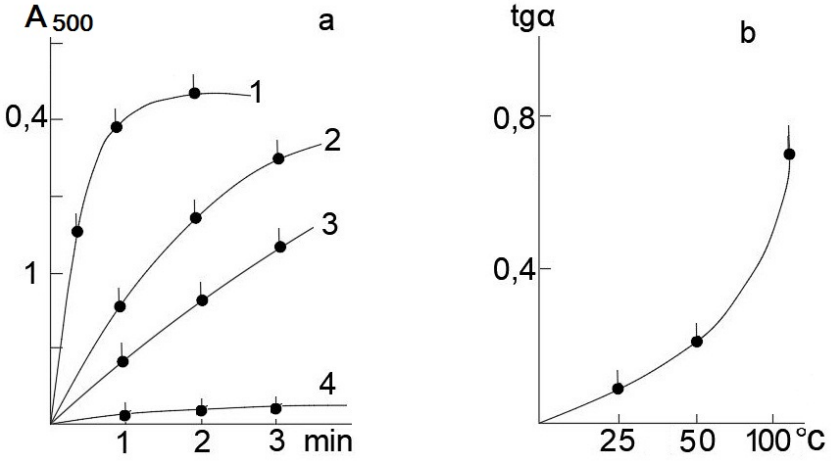

Fig. 2. A–Kinetic curves of adrenochrome formation (at 500 nm) as a result of oxidation of adrenaline (4.10^{-4}M) by superoxide radicals produced by nfNox ($2,10^{-5}$) isoforms from EM or LCM at 100°C (1), 50°C (2) and 25°C (3), in the absence and presence of $2,10^{-8}$M Cu, Zn-SOD at 25 and 50°C (4). At the same time, the Cu, Zn-SOD is thermally stable up to 60-65°C. b – The rate of adrenochrome formation (tg of the slope of the angle of kinetic curves) – A_{500}/min at 25, 50 and 100 °C. ($p<0,05$, n=6), in the absence of exogenous NADPH

The third characteristic feature between fNox and nfNox is that nfNox also produces O_2^- in the absence of exogenous NADPH. On the other hand, in the presence of exogenous NADPH the intensity of observed indices increases

to 25-30%. As a result of thermal influence, the nfNox exhibits both NADPH-dependent and NADPH- independent O_2^--producing activity.

In fact, under these conditions, the FAD plays a role in an electron carrier to the Fe(III) of heme group of the Nox, then to O_2, reducing it up to O_2^-. At the same time there is a sharp increase in thermal stability and the rate of production of O_2^- in the absence of NADPH. Molecular mechanisms of such thermal stabilization associated with a possible change in hydrophilic and hydrophobic bonds in the composition of the molecule of the given Nox isoforms are also not excluded [2, 8].

Thus, thermal stabilization of indicated above nfNox isoforms under storage conditions is a new phenomenon and makes it possible to use these nfNox at high temperatures in various fields of biomedicine and biotechnology as natural and energetic sources for monocomponent O_2^-production in the absence of NADPH.

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Термоиндуцированные необратимые изменения свойств НАДФН-оксидазы крыс

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При сравнении оптических спектральных показателей нетермостабильных изоформ свежей НАДФН-оксидазы (fNox) из мембран эритроцитов и легких белых крыс (через 5-6 дней после ее получения) с аналогичными показателями несвежей Nox (nfNox), после хранения этих Nox в течение 20-25 дней в лиофилизированном состоянии наблюдалась более высокая термостабилизация изоформ nfNox и продукция супероксидных радикалов (O_2^-) в отсутствие НАДФН. Можно заключить, что в этих условиях в nfNox наблюдались необратимые конформационные изменения, обусловленные увеличением оптического поглощения ФАД (при 365 нм) в составе nfNox.

Առնետների NADPH օքսիդազի հատկությունների՝ ջերմությամբ խթանված անդառնալի փոփոխություններ

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Սպիտակ առնետների էրիթրոցիտների և թոքերի թաղանթներից (նրա ստացումից 5-6 օր հետո) թարմ NADPH օքսիդազի (fNox) ոչ ջերմակայուն իզոֆորմների օպտիկական սպեկտրային պարամետրերի համեմատությունը Nox-ի պահպանումից հետո ոչ թարմ Nox-ի (nfNox) նմանատիպ պարամետրերի հետ, 20-25 օրվա ընթացքում լիոֆիլացված վիճակում նկատվել են nfNox իզոֆորմների ավելի բարձր ջերմային կայունացում և սուպերօքսիդ

ռադիկալների (O_2^-) արտադրություն NADPH-ի բացակայության դեպքում: Կարելի է եզրակացնել, որ այս պայմաններում nfNox-ում նկատվել են անդառնալի կոնֆորմացիոն փոփոխություններ՝ կապված nfNox-ի կազմի մեջ FAD-ի օպտիկական կլանման ավելացման հետ (365 նմ):

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