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Conditions for a Sharp Increase in the Thermal Stability of NADPH Oxidase Isoforms and Production of Superoxide Radicals in the Absence of NADPH

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Abstract

During storage at 3-4 months in frozen at -10°C or lyophilized state of the isoforms of NADPH oxidase (Nox) from human donor blood Erythrocytes Membranes (EM) and from the Lung of Albino Rats Cells Membranes (LCM), the conformational changes, is conditioned with an increase in the maximal optical absorption was observed. This absorption is typical for the Flavin Adenine Dinucleotide (FAD) in composition of the Nox, with the corresponding increase in the optical spectral index (A365/A530). These indices for the fresh Nox (fNox) from EM and LCM are 3,2 ± 0,01 ($p<0,05$, n=6) and 3,6 ± 0,03 ($p<0,05$, n=6), and for the non fresh Nox (nfNox) from EM and LCM are 4,3±0,04 ($p<0,05$, n=6) and 4,5±0,03 ($p<0,05$, n=6), correspondingly. nfNox isoforms don't lose solubility and possess the ability for the production of O_{2^-} in the absence of NADPH, indicating a higher thermal stability. On the incubation of these nfNox in a boiling water bath for 10-15 min, a decrease in the maximal optical density at 412 nm and 530 nm only for 10-15% is observed. In these conditions, sharp changes in the optical spectral indices for the fNox and appreciable lose of the solubility and the absence of the ability for the production of O_2- in the presence of NADPH were presented.

It can be concluded that during storage of the NADPH oxidase (Nox) isoforms from EM and LCM at the above-mentioned conditions, the conformational changes, an increase in the index A365A530 and a sharp elevation of the thermal stability and the ability of the superoxide production by these Nox without NADPH were observed.

Keywords: NADPH oxidase;Tthermal stability;Superoxide radicals

Introduction

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, restoring it to O_{2^-} [1,2]. On the other hand, the thermostable Nox is isolated from thermostable microorganisms [3]. 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 conditions under which thermostability and autonomous production of superoxide radicals in the absence of NADPH dramatically increased.

Material and Methods

Isolation and purification of the total fraction of the Nox isoforms (Nox^{1-} Nox^2)

The total fraction of highly purified and terminal Nox isoforms ($Nox^{1+}Nox^2$) from EM and LCM was isolated and purified by a licensed method, using the process of complex formation of exogenous ferrihaemoglobin from red blood cells of donor blood with Nox isoforms on the surface of these membranes and their release in the soluble phase [4]. Next, anion-exchange chromatography on cellulose DE-52 ("Whatman", England), gel filtration on G-100 sefadex ("Pharmacia", Sweden) and fractionation with ethanol mixture with chloroform (1.5 ml ethanol with 0.9 ml chloroform, for 10 ml of Nox solution) to remove traces of ferrihemoglobin

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was carried out.

Determination of O_2- production 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˚C) [5].

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 shape of the optical absorption spectra of isoforms of fNox from EM and LCM of rats after direct isolation and purification, and at 10 minute-15 minute incubation at 25°C (FIG.1) and 100˚C (FIG. 1) differs sharply. However, after 10 minute-15 minutes of incubation of nfNox isoforms in a boiling water bath at 25°C (FIG.1-b1) and 100°C (FIG.1), the shape of the optical absorption spectra practically does not change, although the density of the characteristic maximum optical absorption γ-bands (absorption of the Nox heme group at 412 nm) and β-absorption bands (at 530 nm) slightly decreased.

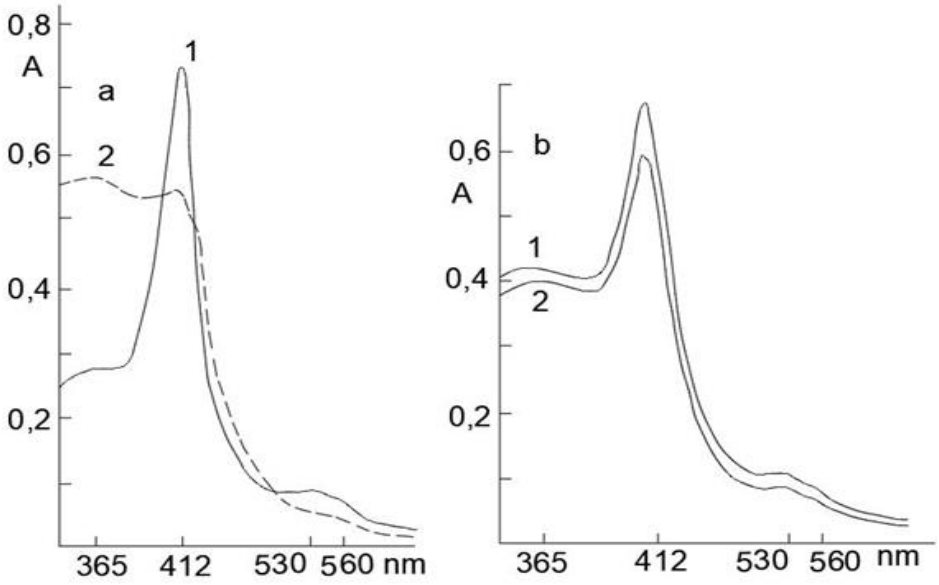

FIG.1. **Optical absorption spectra of the total fraction of isoforms of fNox (6.10- 5 M) from EM of donor human blood during incubation at 25°C (a1) and 100°C (a2) for 10-15 minutes. The given parameters for nfNox from EM following incubation at 25°C (b1) and 100°C (b2). Similar results are obtained for the Nox isoforms from LCM and are not presented. Proteins are dissolved in 0.1 M potassium phosphate buffer at pH 7.4.**

This increases of the density of maximum optical absorption at 365nm, with a corresponding increase in the optical spectral index (A365/A530). 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 data 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.

An increased A365/A530 ratio for nfNox is the first distinguishing feature between nfNox and nfNox. This may be due to a certain advancement of the FAD group [6] in the nfNox molecule during storage under the above conditions, causing, in general, a conformational change 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 A365/A530 value leads to a sharp increase in 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 10 minute-15 minutes at 25˚C, 50˚C and 100˚C (FIG. 2).

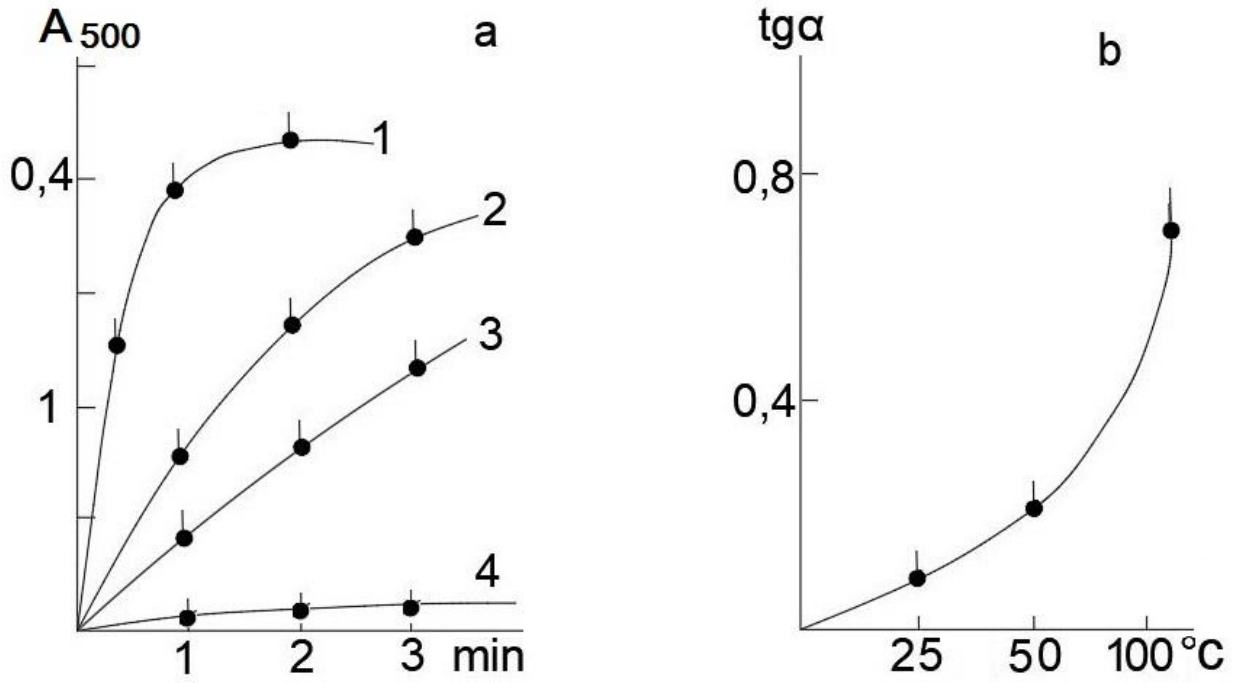

FIG.2. **A-Kinetic curves of adrenochrome formation (at 500 nm) as a result of oxidation of adrenaline (4.10-4M) by superoxide radicals produced by nfNox (2.10-5) isoforms from EM or LCM at 100°C (1), 50°C (2) and 25°C (3), in the absence and presence of 2.10-8M Cu,Zn-SOD at 25 and 50°C (4). At the same time, 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) - A500/ min at 25, 50 and 100°C. (p<0,05, n=6), in the absence of exogenous NADPH.**

A third characteristic feature between fNox and nfNox is that nfNox produces O_2- in the absence of NADPH. In fact, we are recording experimentally reliable results, on the basis of which we accept that this is a speculative conclusion. Under the conditions of the given (so far undetermined) changes in FAD in the composition of Nox isoforms during storage, thermal stabilization of Nox isoforms occurs. It is possible that under these conditions, FAD plays a role in an electron carrier mechanism from the corresponding reducing component in the Nox molecule to the O_2 molecule, 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 [7,8].

Thus, thermal stabilization of Nox isoforms of mammals 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, monocomponent and energetic sources of O_2. production in the absence of NADPH.

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